EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **119**: 232–241, 2022
doi: 10.14411/eje.2022.025
ORIGINAL ARTICLE

Effect of solvent extraction time on the hydrocarbon profile of *Drosophila suzukii* (Diptera: Drosophilidae) and behavioural effects of 9-pentacosene and dodecane

Maria BELENIOTI[1], Emmanouil RODITAKIS[2], Manolis SOFIADIS[3], Maria FOUSKAKI[1], Maria APOSTOLAKI[4] and Nikos CHANIOTAKIS[1]

[1] Laboratory of Analytical Chemistry, Department of Chemistry, University of Crete, Panepistimioupoli Vouton, 70013 Heraklion, Crete, Greece; e-mails: maria.belenioti@uoc.gr, fouskakm@uoc.gr, chaniotakis@uoc.gr

[2] Department of Agriculture, Hellenic Mediterranean University, Stavromenos PC 71410, Heraklion, Crete, Greece; e-mail: eroditakis@hmu.gr

[3] Laboratory of Organic Chemistry, Department of Chemistry, University of Crete, Panepistimioupoli Vouton, 70013 Heraklion, Crete, Greece; e-mail: msofiadis@gmail.com

[4] Laboratory of Environmental Chemistry, Department of Chemistry, University of Crete, Panepistimioupoli Vouton, 70013 Heraklion, Crete, Greece; e-mail: apostolm@uoc.gr

Key words. Cuticle hydrocarbons, bioassays, extraction steady state

Abstract. Hydrocarbons play a major role in the life cycle of insects. Their composition and concentration can be affected by several factors. Hydrocarbons are biosynthesized in oenocytes and subsequently transported to the cuticle of insects, such as *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). As the extraction procedure markedly affects the type and amount of hydrocarbon obtained we determined the association between the time taken to extract the maximum amounts of these compounds and the behaviour of *D. suzukii*. The required extraction time to reach a steady state is different for each hydrocarbon, which in most cases is more than one hour. On the other hand, if the entire hydrocarbon profile of *D. suzukii* needs to be investigated, extraction times significantly longer than one hour were required. By extending the extraction time 5 additional hydrocarbons were detected in *D. suzukii* for the first time. One of them, dodecane proved to be repulsive to *D. suzukii*. In addition, it took 3 h of extraction to determine the maximum value of 9-pentacosene, which is responsible for triggering mating behaviour in *D. suzukii*.

INTRODUCTION

The outermost layer of the insect cuticle, the epicuticle, is composed of a mixture of lipids. These lipids consist of branched and unbranched, saturated and unsaturated hydrocarbons, free fatty acids, sterols and aldehydes (Blomquist et al., 1987). Hydrocarbons are also present in the inner cuticle as well as in epidermal tissue, fat body, other organs and especially in lipophorin in the haemolymph (Blomquist et al., 2010a). Hydrocarbons are mainly biosynthesized in oenocytes and then transported by high-density lipoproteins in the haemolymph to the insect cuticle (cuticular hydrocarbons) via specialized pore canals (Haruhito & Haruo, 1982; Schal et al., 2001; Holze et al., 2021). Internal hydrocarbons in many insects, such as *Drosophila* spp. are qualitatively similar to cuticular hydrocarbons (Schal et al., 1998; Tillman et al., 1999; Everaerts et al., 2010; Choe et al., 2012). However, the cuticular hydrocarbon profiles may be quantitatively different from those of other tissues (Choe et al., 2012). Hydrocarbons occur in all insect life stages and their production can be affected by several factors, such as, reproductive status (Sledge et al., 2001; Howard & Blomquist, 2005), sex, age (Gibbs & Markow, 2001; Kuo et al., 2012), food (Carvalho et al., 2012; Fedina et al., 2012), temperature, social experience (Belenioti & Chaniotakis, 2020) and photoperiod (Gershman et al., 2014; Belenioti & Chaniotakis, 2020). Cuticular hydrocarbons not only protect insects against environmental factors such as desiccation (Gibbs et al., 2003) and entomopathogens (Blomquist et al., 1987), but also play a major role in chemical communication, including aggregation, mating, alarm and recognition signals (Howard, 1982; Yew & Chung, 2015).

Several studies have used analytical methods to determine the qualitative and quantitative aspects of insect hydrocarbons (Bagneres & Morgan, 1990; Cvacka et al., 2006; Dossey et al., 2006; Blomquist, 2010b; Yew et al., 2008, 2011a, b). Currently, chromatographic separation combined with mass spectrometry (GC-MS) is the main method used to identify hydrocarbons (Blomquist, 2010b). There is several extraction procedures for determining ei-

ther internal or cuticular hydrocarbons (Blomquist, 2010b; Colebiowski et al., 2011; Choe et al., 2012; Cerkowniak et al., 2013). Cuticular hydrocarbons are extracted using non-polar solvents, such as hexane or pentane. Using solvents with different polarities and extraction times, one can quantitatively measure the amounts of internal hydrocarbons (Cerkowniak et al., 2013). Solvent free extraction methods have also been proposed for isolating cuticular hydrocarbons. For example, solid phase micro extraction (SPME) (Everaerts et al., 2010; Al-Khshemawee et al., 2018) and silica gel-based methods are novel extraction techniques for the analysis of insect cuticular hydrocarbons (Choe et al., 2012).

Since solvent extraction is an equilibrium process, the amount extracted depends on the type and amount of the solvent used and the extraction duration (Blomquist, 2010a). Extraction times range from 1 min to 24 h (Drijfhout et al., 2009; Cerkowniak et al., 2013). It is expected that long extraction periods might influence the amounts of hydrocarbons extracted from internal body tissues, glands and haemolymph (Drijfhout et al., 2009), which may contaminate the cuticular hydrocarbons (Choe et al., 2012). The exact method used to extract hydrocarbons from the body of insects will subsequently determine both the amount and type of hydrocarbons measured. Since the data from such analyses could be the basis for the development of novel monitoring or pest control tools (Yew & Chung, 2015), the extraction efficacy is fundamental.

In this study, we used *D. suzukii* as a model, because this pest has recently spread throughout the world (Walsh et. al., 2011), is highly polyphagous (Lee et al., 2011a) and causes substantial economic damage (Knapp et al., 2020). We examined the effect of five different extraction periods on the levels of hydrocarbons and related the amount extracted of two of them (dodecane, C12 and 9-pentacosene, 9-C25) to the behaviour of *D. suzukii.*

MATERIALS AND METHODS

Insects

A colony of *D. suzukii* provided by the Beldade Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal was maintained under a 12L : 12D photoperiod (lights on at 07:00, 20–22°C and 65 ± 5% RH) (Revadi et al., 2015) in the quarantine facility at the Laboratory of Entomology, Department of Biology, University of Crete, Greece. Insects were reared in sterile vials containing a cornmeal diet (Belenioti & Chaniotakis, 2020).

Extraction of CHCs

1-day-old flies were sexed under ice anaesthesia (Barron, 2000) and same sex samples of *D. suzukii* kept in vials containing a diet. Then, the hydrocarbons in samples of 15 flies (4-day- old) were extracted by adding 500 µl n-hexane (organic trace analysis grade) at 24°C in 4 ml glass vials. Extraction times of 1, 3, 6, 12 and 24 h were used. Contents of each vial were mixed using a vortex mixer for 1 min at the end of each test period (Snellings et al., 2018). Preliminary experiments indicated that 15 flies were sufficient to provide enough hydrocarbons for analysis. The hexane extract was transferred to a new glass vial and 1050 ng of hexadecane-d_{34} was added as internal standard, which was based on its retention time and that it was not detected in *D. suzukii.* 100 µl of the resulting solution were transferred into a crimp top vial (Thermo Scientific) and stored at –20°C until the GC-MS analysis. Ten biological replicates of pools of 15 flies each were analysed for each extraction time and each resulting solution was measured three times.

GC-MS analysis

This was done using an Agilent 6890N gas chromatograph equipped with a cool on column injector and interfaced with an Agilent 5973 inert mass spectrometer operating in electron impact ionization (70 eV electron energy). Analytes were separated on a 30m DB-5MS capillary column (Agilent, phenyl arylene polymer, 0.25 mm. i.d., 0.25 µm film thickness) with helium (99.999%) as the carrier gas at a flow rate of 1 mL/min. A 1-µl aliquot of the 100 µl sample was injected on-column with an Agilent 7683 AutoSampler and scanning was performed from 50 to 550 amu. Temperature program was from 50°C to 150°C at a rate of 5°C/min, reaching a final temperature of 290°C at a rate of 15°C/min. The final temperature of 290°C was held for 12 min. Fragmentation patterns and ions of the components were compared with those in the NIST/EPA NIH Library and previously published work (Dekker et al., 2015; Snellings et al., 2018).

Bioassays

Based on data obtained from the extraction experiments, two hydrocarbons, C12 and 9-C25 were used in the behavioural assays. C12 is a volatile compound that was detectable only after 24 h of extraction, while the amount of 9-C25, which is non-volatile, showed a logarithmic increase with extraction time. C12 was obtained from Sigma Aldrich and 9-C25 was synthesized in the laboratory as described below. Three different types of bioassays were carried out based on the expected behavioural response of the flies. All bioassay experiments were carried out under well-controlled environmental conditions (temperature 21 ± 1°C and relative humidity 65 ± 5%), at the same time of the day two hours after the beginning of the photo phase (9:00–11:00 am), since the level of locomotion of 4-day-old *D. suzukii* is independent of photo phase (Belenioti & Chaniotakis, 2020).

Courtship assay (9-C25)

We investigated the effect of two different amounts of 9-C25 on the mating behaviour of 4-day-old unmated *D. suzukii.* The effect of a 1h extracted amount (50.9 ng) on courtship behaviour was compared with the effect of the mean maximum extracted amount (179.3 ng). The 1h extracted amount was evaluated by applying hexane solvent (control treatment) with a fine paintbrush (Snellings et al., 2018) onto the abdomen of 4-day-old unmated *D. suzukii* female, since 1h extracted amount of 9-C25 already exists in the insect cuticle. A 1 min extraction was used in order to confirm that 50.9 ng of 9-C25 already exists in the insect cuticle. The maximum extracted amount was tested by applying a hexane solution containing 179.3 ng of 9-C25 (9-C25 treated insects) onto the abdomen. Confirmation of final amount (already present + applied) was carried out using GC-MS. Females exposed to the scent of 1500 ng of 9-C23 were used as a positive control in order to evaluate its role in courtship, since it is reported that 9-C23 at high concentrations reduces mating and courtship behaviour in *D. suzukii* (Snellings et al., 2018).

A 4-day-old unmated female and unmated male was transferred using a pooter without anaesthesia into a watch glass courtship arena (4 cm internal diameter) (Vieillard & Cortot, 2016). Interactions between the two flies were video recorded for 60 min. Mating events were scored as 1 when there was copulation and 0 when there was no copulation (Hwee et al., 2014). Sixty females were observed and the percentage that mated was calculated. The courtship duration is defined as the time in minutes during which

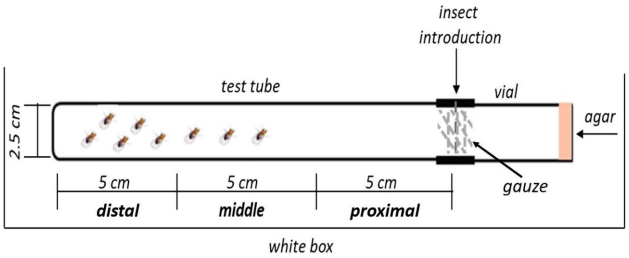

Fig. 1. Experimental setup for the repulsion test. Individuals were introduced into the test tube and the opening closed with a vial containing agar and a filter paper impregnated with the test stimulus (C12) separated by gauze from the test tube. Flies were gently positioned near the gauze and their position along the tube was video recorded over the next 20 min.

the male spends courting within the first 10 min of a test (n = 60) (Koemans et al., 2017).

Locomotion assay (C12)

A simple bioassay setup was designed to study the effect of C12 on the locomotion of *D. suzukii*. Approximately 1 ml of agar solution (5% w/v) was placed in a 5-cm diameter petri dish, covering its entire internal surface and then twenty male or female flies were installed in the dish via a small hole in the lid using a pooter. Each petri dish was placed in a white box for 30 min to avoid visual stimuli and acclimatize the insects and then a filter paper (1 × 1 cm) impregnated with 10 µl of C12 solution (1 ng/µl in hexane) was placed at the centre of the petri dish via a slit at the side. Each replication (n = 45) was video recorded for 2 min. The effect of C12 was evaluated by measuring the movement of the insects in terms of the percentage that changed position during the tests. Position changes were recorded when insects moved a distance of at least twice their body length.

Repulsion assay (C12)

Based on the results of the locomotion assay, the role of C12 as a possible repellent for *D. suzukii* was further examined using a test tube (Fig. 1) (Devaud, 2003; Vang et al., 2012). Twenty male or female flies were placed in a test tube (2.5 × 15 cm), which was plugged with cotton wool and then placed in a white box for 30 min to avoid visual stimuli and acclimatize the insects. Subsequently 1 ml of agar solution (5% w/v) was transferred into a 4 ml glass vial and then a filter paper (1 × 1 cm) impregnated with 10 µl of C12 solution (1 ng/µl in hexane) added. The vial was covered with gauze and then attached to the open end of the test tube. Gentle tapping of the side of the test tube resulted in the flies ending up on the gauze, at which time the recording was initiated. The tube was divided into three parts (proximal, middle, distal) by means of fine marks as shown in Fig. 1. Replicates (n = 45) were video recorded and the position of the individuals in the tube was recorded every minute for 20 min. A hexane-soaked filter paper was also added to the agar as a control, while a solution of benzaldehyde was used as a positive control (n = 45), since it is known to repell *Drosophila* species (Vang & Adler, 2016).

Synthesis of chemical compounds

9-C25: Nonyltriphenylphosphonium bromide

A tube containing 1-bromononane (1.91 mL, 10 mmol) and triphenylphosphine (2.62 g, 10 mmol) was sealed and the temperature raised to 120°C. The resulting homogenous mixture was stirred for 16 h at 120°C. Then, the mixture was cooled to room temperature and dichloromethane (20 mL) added and then the solution was transferred to a flask and the volatiles were removed in vacuo yielding a white solid. The solid was transferred to a fritted funnel and washed with hexane (10 mL). Then, the solid was vacuum dried for 1 h providing a highly hygroscopic white solid (4.58 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$): 7.63–7.67 (m, 9H), 7.53–7.58 (m, 6H), 3.46–3.41 (m, 2H), 1.43–1.49 (m, 4H), 1.01–1.07 (m, 10H), 0.65 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 134.3 (d, J_{C-P} = 2.74 Hz), 132.8 (d, J_{C-P} = 9.95 Hz), 129.8 (d, J_{C-P} = 12.6 Hz), 117.3 (d, J_{C-P} = 85.7 Hz), 30.9, 29.6, 28.3, 22.2, 21.8, 21.7, 13.3.

Hexadecanal (Palmitaldehyde)

To a solution of 1-hexadecanol (1.2 g, 5 mmol) in dichloromethane (100 mL, 0.05 M) pyridinium chlorochromate (PCC, 1.6 g, 1.5 mmol) was added at room temperature. After the complete consumption of the alcohol, as indicated by TLC analysis, Et_2O (20 mL) was added and then the heterogeneous mixture was filtered through Celite®. Volatiles were removed in vacuo and the crude residue was purified by flash column chromatography (Silica gel, Petroleum Ether : Ethyl Acetate = 60 : 1) affording Hexadecanal (950 mg, 80%). ^{1}H NMR (500MHz, $CDCl_3$): 9.76 (t, J = 1.9 Hz, 1H), 2.41 (td, J_1 = 7.4, J_2 = 1.9 Hz, 2H), 1.62 (qui, J = 7.4 Hz, 2H), 1.23–1.33 (m, 22H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 202.9, 43.9, 31.9, 29.7 (2C), 29.6 (4C), 29.4, 29.3 (2C), 29.2, 22.7, 22.1, 14.1 ppm.

(Z)-Pentacos-9-ene

To a suspension of Nonyltriphenylphosphonium bromide (1.05 g, 2.2 mmol) in dry THF (5 mL) a solution of Lithium Diisopropylamide (LDA, 2.2 mmol) was added at 0°C and stirred for 30 min at the same temperature, by which time the mixture became translucid orange. Then, a solution of Hexadecanal (528 mg, 2.2 mmol) in dry tetrahydrofuran (THF, 1 mL) was added at 0°C and the solution stirred for 30 min. After the complete consumption of the aldehyde, as indicated by TLC analysis, a saturated solution of $NH_4Cl_{(aq)}$ (2 mL) and Et_2O (10 mL) was added. The organic phase was washed with a saturated solution of $NH_4Cl_{(aq)}$ (2 × 2 mL) and Brine (1 × 2 mL). Volatiles were removed in vacuo and the crude residue was purified by flash column chromatography (Silica gel, Hexanes = 100%) affording (488 mg, 70%) the desired alkene as a mixture of stereoisomers (Cis : Trans = 68 : 32) as was indicated by GC-MS-analysis. ^{1}H NMR (500MHz, $CDCl_3$): 5.32–5.39 (m, 2H), 1.98–2.04 (m, 4H), 1.27–1.34 (m, 38H), 0.89 (t, J = 6.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 129.9, 31.9 (2C), 29.8, 29.7 (2C), 29.6 (2C), 29.4, 29.3, 27.2, 22.7, 14.1 ppm.

Statistical analysis

For the statistical analysis the software IBM SPSS Statistics 24 was used. Statistical difference for (%) mating events and (%) locomotion was evaluated using Chi square tests. Statistical difference of courtship duration and results of repulsion experiments was evaluated by independent sample T-test and One-Way ANOVA, respectively. p-value was set at 0.05 corresponding to 95% confidence limit.

RESULTS AND DISCUSSION

Hydrocarbon composition

Solvent soaking is an established method for studying the chemical ecology of insects (Howard & Blomquist, 2005). Reported soaking periods vary from 1 min to 24 h (Drijfhout et al., 2009; Gołębiowski et al., 2011; Cerkowniak et al., 2013). Initially, the effect of 5 different extraction times (1, 3, 6, 12 and 24 h) on *D. suzukii* hydrocarbons was investigated. During the analysis of the samples, 51 chromatographic peaks were detected, of which 47 were identified as either saturated or unsaturated hydrocarbons

Table 1. Hydrocarbons (ng/per insect) identified in female *D. suzukii*.

RT	Compound	Abbr.	M_r	1 h	3 h	6 h	12 h	24 h
10.62	undecane (N)	C11	156	Not detected	8.1 ± 0.31	19.8 ± 0.4	40.5 ± 4.6	78.1 ± 3.4
13.40	dodecane (N)	C12	170	Not detected	Not detected	Not detected	Not detected	10.7 ± 0.7
18.87	tetradecane	C14	198	Not detected	Not detected	Not detected	2.1 ± 0.4	5.3 ± 0.8
21.56	pentadecane (N)	C15	212	Not detected	Not detected	Not detected	8.8 ± 0.7	14.4 ± 0.8
24.17	hexadecane-d_{34}	C16-d34	260	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0
25.24	hexadecane (N)	C16	226	Not detected	Not detected	Not detected	10.1 ± 1.7	14.6 ± 1.9
30.75	heptadecane (N)	C17	240	Not detected	6.6 ± 0.1	11.0 ± 0.5	20.1 ± 1.3	22.9 ± 0.4
33.45	octadecane	C18	254	Not detected	Not detected	4.9 ± 1.1	9.7 ± 2.3	14.7 ± 6.6
34.84	nonadecane	C19	268	4.4 ± 0.5	6.2 ± 0.5	8.7 ± 0.6	12.3 ± 1.2	22.5 ± 2.1
34.92	unidentified 1			7.5 ± 0.9	14.3 ± 0.5	22.8 ± 0.8	31.5 ± 1.9	37.9 ± 2.4
35.41	unidentified 2			11.0 ± 0.9	24.1 ± 1.4	36.8 ± 1.8	48.0 ± 3.8	68.6 ± 1.1
35.90	eicosane	C20	282	6.6 ± 0.6	10.4 ± 0.3	12.6 ± 2.0	14.6 ± 1.6	20.6 ± 1.3
36.56	9-heneicosene	9-C21:1	294	2.6 ± 0.6	3.7 ± 0.1	4.4 ± 0.5	5.6 ± 0.3	5.9 ± 0.3
36.61	7-heneicosene	7-C21:1	294	22.0 ± 1.6	19.9 ± 1.3	23.2 ± 1.7	24.0 ± 1.7	22.2 ± 3.0
36.66	5-heneicosene	5-C21:1	294	13.7 ± 1.4	12.7 ± 0.5	11.9 ± 0.8	13.4 ± 1.4	13.9 ± 1.3
36.78	heneicosane	C21	296	867.1 ± 57.6	799.3 ± 27.33	799.3 ± 29.2	815.7 ± 38.1	1070.7 ± 78.5
36.92	2-methyleicosane (N)	2-MeC20	296	7.1 ± 0.5	19.4 ± 0.7	20.2 ± 0.9	22.3 ± 1.8	27.4 ± 9.5
37.24	2-methylheneicosane (N)	2-MeC21	310	9.1 ± 0.7	20.3 ± 0.7	20.3 ± 0.8	24.8 ± 0.9	24.8 ± 1.3
37.27	6.9-docosadiene	6.9-C22:2	306	2.6 ± 0.3	6.7 ± 1.0	20.1 ± 0.7	20.5 ± 3.7	20.9 ± 1.8
37.31	9-docosene	9-C22:1	308	14.7 ± 0.6	28.5 ± 0.8	31.4 ± 1.1	32.7 ± 3.1	33.2 ± 1.7
37.36	7-docosene	7-C22:1	308	95.6 ± 6.0	81.4 ± 3.7	81.2 ± 3.4	99.7 ± 9.1	124.5 ± 9.5
37.44	5-docosene	5-C22:1	308	7.3 ± 2.4	11.4 ± 3.9	13.2 ± 4.2	13.4 ± 3.6	17.1 ± 1.2
37.50	docosane	C22	310	39.4 ± 2.6	95.5 ± 0.1	101.9 ± 2.6	123.5 ± 7.0	130.4 ± 8.3
37.93	2-methyldocosane	2-MeC22	324	83.7 ± 5.6	80.4 ± 3.9	79.8 ± 5.9	84.8 ± 8.1	90.0 ± 6.1
37.97	6,9-tricosadiene	6,9-C23:2	320	96.4 ± 6.5	114.1 ± 3.1	175.1 ± 7.3	247.2 ± 27.1	179.5 ± 10.6
38.01	9-tricosene	9-C23:1	322	518.1 ± 36.4	899.4 ± 29.8	932.1 ± 32.2	938.1 ± 62.9	1270.1 ± 119.1
38.06	7-tricosene	7-C23:1	322	3297.1 ± 245.1	3369.5 ± 78.9	3352.4 ± 97.5	3332.2 ± 166.9	3596.8 ± 241.2
38.12	5-tricosene	5-C23:1	322	206.4 ± 18.4	126.4 ± 1.6	164.4 ± 11.6	172.8 ± 4.7	243.2 ± 14.3
38.17	tricosane	C23	324	995.1 ± 67.68	989.61 ± 11.8	985.4 ± 37.9	992.9 ± 119.9	1312.4 ± 102.7
38.38	unidentified 3			4.3 ± 2.3	15.8 ± 1.6	18.4 ± 1.4	16.1 ± 1.6	14.8 ± 0.2
38.62	3-methyltricosane	3-MeC23	338	8.4 ± 0.6	19.5 ± 0.6	27.8 ± 0.9	41.3 ± 4.4	43.1 ± 3.9
38.65	9-tetracosene	9-C24:1	336	9.4 ± 1.1	20.2 ± 1.3	22.9 ± 2.1	21.6 ± 3.4	24.2 ± 2.4
38.68	7-tetracosene	7-C24:1	336	33.1 ± 1.7	51.7 ± 1.3	52.1 ± 2.1	54.6 ± 5.6	60.9 ± 3.3
38.72	5-tetracosene	5-C24:1	336	22.14 ± 1.40	23.22 ± 0.70	29.15 ± 1.06	32.73 ± 5.13	25.78 ± 3.75
38.79	tetracosane	C24	338	20.20 ± 1.76	40.64 ± 5.03	46.92 ± 1.84	43.00 ± 4.21	44.70 ± 2.20
39.15	2-methyltetracosane	2-MeC24	352	20.08 ± 1.65	28.30 ± 0.79	22.90 ± 0.71	28.54 ± 2.75	22.42 ± 2.72
39.21	6,9-pentacosadiene	6,9-C25:2	348	40.65 ± 3.36	37.56 ± 1.93	30.96 ± 0.67	42.74 ± 3.99	50.57 ± 2.52
39.22	9-pentacosene	9-C25:1	350	50.9 ± 4.8	170.1 ± 4.7	168.9 ± 13.5	208.5 ± 27.8	169.9 ± 10.3
39.26	7-pentacosene	7-C25:1	350	193.9 ± 14.7	305.1 ± 7.3	317.1 ± 16.3	296.8 ± 53.1	299.5 ± 21.8
39.35	5-pentacosene	5-C25:1	350	19.9 ± 0.5	33.6 ± 0.56	31.5 ± 0.4	39.9 ± 1.4	31.7 ± 2.2
39.36	pentacosane	C25	352	215.9 ± 11.6	221.5 ± 13.9	216.6 ± 10.5	206.8 ± 36.6	251.4 ± 16.3
39.95	hexacosane	C26	366	19.3 ± 4.6	24.6 ± 3.5	22.9 ± 5.1	20.5 ± 3.7	23.5 ± 2.9
40.35	2-methylhexacosane	2-MeC26	380	33.9 ± 2.2	73.9 ± 1.6	64.5 ± 2.9	71.3 ± 32.6	44.6 ± 2.8
40.42	9-heptacosene	9-C27:1	378	10.7 ± 1.3	20.3 ± 1.5	16.9 ± 0.9	16.3 ± 7.3	14.1 ± 1.3
40.50	7-heptacosene	7-C27:1	378	27.2 ± 1.6	34.6 ± 1.1	22.7 ± 2.1	25.9 ± 2.5	23.3 ± 1.1
40.60	heptacosane	C27	380	132.9 ± 7.9	183.5 ± 5.6	167.5 ± 6.1	178.8 ± 31.8	233.1 ± 12.6
41.34	octacosane	C28	394	43.5 ± 3.7	152.8 ± 10.2	159.5 ± 22.6	161.8 ± 5.3	170.9 ± 8.9
41.47	unidentified 4			23.9 ± 2.1	116.7 ± 10.6	127.5 ± 13.4	162.1 ± 15.4	169.6 ± 4.9
41.88	2-methyloctacosane	2-MeC28	408	155.1 ± 9.9	383.1 ± 4.1	309.1 ± 18.1	309.6 ± 44.1	291.8 ± 18.3
42.09	7-nonacosene	7-C29:1	406	40.1 ± 3.9	57.9 ± 2.4	44.3 ± 7.1	48.5 ± 2.9	30.1 ± 2.3
42.22	nonacosane	C29	408	98.5 ± 14.6	129.9 ± 9.2	120.1 ± 4.4	143.6 ± 28.1	233.3 ± 50.5
44.00	2-methyltriacontane	2-MeC30	436	13.9 ± 1.6	86.8 ± 3.7	73.1 ± 13.3	81.4 ± 31.4	58.5 ± 5.6

Compounds recorded at different extraction times (1, 3, 6, 12 and 24 h) plus the extracted amounts (ng/individual, mean ± SEM). RT – retention times, Abbr. – abbreviation, M_r – molecular weight, N – newly detected compound.

and 4 remained unidentified (Table 1 and 2 for females and males, respectively). As a result of increasing the extraction time beyond 1 h, as reported (Bartelt & Jackson, 1984), we found 5 additional hydrocarbons (C11 undecane, C12 dodecane, C15 pentadecane, C16 hexadecane, C17 heptadecane), that were not previously reported for *D. suzukii* (Dekker et al., 2015; Snellings et al., 2018). Moreover, all the newly detected hydrocarbons are saturated with short carbon chains ranging between 11 and 17 C. Previous studies indicate that the longer an insect is exposed to a solvent the greater the amounts and number of hydrocarbons that are extracted from haemolymph, fat body and various glands (Vander et al., 1989; Schal et al., 1994; Cu et al., 1995; Drijfhout et al., 2009; Moris et al., 2021). It is suggested that the longer extraction period leads to the extraction of internal hydrocarbons that are typically not accessible to the olfactory or gustatory organs of other insects, since the components of most importance in chemical communication are likely to be on the surface of the cuticle (Choe et al., 2012). Potentially, the 5 additional hy-

Table 2. Hydrocarbons (ng/per insect) identified in male *D. suzukii*.

RT	Compound	Abbr.	M_r	1 h	3 h	6 h	12 h	24 h
10.62	undecane (N)	C11	156	Not detected	8.8 ± 1.4	21.6 ± 1.2	42.6 ± 1.3	90.9 ± 9.9
13.40	dodecane (N)	C12	170	Not detected	Not detected	Not detected	Not detected	10.7 ± 0.4
18.87	tetradecane	C14	198	Not detected	Not detected	Not detected	2.8 ± 0.9	6.2 ± 0.2
21.56	pentadecane (N)	C15	212	Not detected	Not detected	Not detected	11.3 ± 0.5	21.6 ± 2.8
24.17	hexadecane-d_{34}	C16-d34	260	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0	1050 ± 0.0
25.24	hexadecane (N)	C16	226	Not detected	Not detected	Not detected	6.9 ± 2.1	12.1 ± 1.1
30.75	heptadecane (N)	C17	240	Not detected	10.3 ± 1.5	15.0 ± 3.5	27.3 ± 2.0	43.8 ± 5.1
33.45	octadecane	C18	254	Not detected	Not detected	2.4 ± 0.2	5.3 ± 0.9	12.18 ± 0.86
34.84	nonadecane	C19	268	3.3 ± 0.4	5.8 ± 0.5	7.2 ± 0.5	10.2 ± 0.8	18.5 ± 2.5
34.92	unidentified 1			5.4 ± 0.2	11.0 ± 0.5	20.6 ± 0.2	26.8 ± 0.7	46.1 ± 1.5
35.41	unidentified 2			6.7 ± 1.2	19.8 ± 1.6	31.1 ± 0.6	42.8 ± 1.9	69.8 ± 0.3
35.90	eicosane	C20	282	5.6 ± 0.1	16.1 ± 0.6	30.8 ± 0.6	36.1 ± 1.4	43.3 ± 0.8
36.56	9-heneicosene	9-C21:1	294	3.6 ± 0.1	3.1 ± 0.1	2.9 ± 0.4	3.7 ± 0.1	3.4 ± 1.7
36.61	7-heneicosene	7-C21:1	294	13.2 ± 0.4	15.4 ± 0.2	17.0 ± 0.4	13.8 ± 0.3	15.4 ± 0.3
36.66	5-heneicosene	5-C21:1	294	3.9 ± 0.1	4.8 ± 0.3	4.3 ± 0.3	4.2 ± 1.7	4.5 ± 1.4
36.78	heneicosane	C21	296	637 ± 9.6	633.1 ± 20.4	616.9 ± 54.8	692.4 ± 14.4	657.3 ± 38.5
36.92	2-methyleicosane (N)	2-MeC20	296	4.8 ± 0.1	19.7 ± 0.6	16.9 ± 1.3	18.5 ± 1.2	20.0 ± 0.2
37.24	2-methylheneicosane (N)	2-MeC21	310	6.5 ± 0.2	16.4 ± 2.8	21.3 ± 0.8	21.12 ± 1.7	23.3 ± 2.1
37.27	6,9-docosadiene	6,9-C22:2	306	2.7 ± 0.3	9.2 ± 0.2	16.1 ± 0.4	14.6 ± 0.	16.2 ± 1.0
37.31	9-docosene	9-C22:1	308	9.1 ± 0.3	26.1 ± 3.9	23.9 ± 3.8	26.5 ± 0.3	31.2 ± 1.0
37.36	7-docosene	7-C22:1	308	70.9 ± 0.7	74.1 ± 6.0	77.4 ± 7.9	79.4 ± 4.1	108.1 ± 1.4
37.44	5-docosene	5-C22:1	308	2.55 ± 0.3	9.4 ± 0.3	6.3 ± 1.1	17.3 ± 5.8	28.4 ± 3.1
37.50	docosane	C22	310	33.3 ± 1.1	104.4 ± 4.7	108.2 ± 1.1	103.9 ± 17.1	107.8 ± 2.5
37.93	2-methyldocosane	2-MeC22	324	44.5 ± 1. 7	42. 6 ± 4.1	48.2 ± 1.8	44.9 ± 1.9	49.2 ± 3.4
37.97	6,9-tricosadiene	6,9-C23:2	320	73.8 ± 2.0	102.8 ± 7.4	105.3 ± 14.3	123.6 ± 18.5	142.2 ± 3.4
38.01	9-tricosene	9-C23:1	322	520.4 ± 12.5	606.6 ± 8.5	551.9 ± 155.2	681.71 ± 32.2	606.3 ± 75.2
38.06	7-tricosene	7-C23:1	322	691.6 ± 43.3	1700.4 ± 42.7	2697.9 ± 227.2 ±	2787.4 ± 82.1	2702.1 ± 95.2
38.12	5-tricosene	5-C23:1	322	68.1 ± 0.9	150.5 ± 3.5	137.7 ± 13.9	127.3 ± 5.8	131.3 ± 3.4
38.17	tricosane	C23	324	413.4 ± 9.3	814.4 ± 27.5	788.8 ± 95.7	872.7 ± 54.7	778.7 ± 25.8
38.38	unidentified 3			7.6 ± 0.1	14.1 ± 2.3	14.1 ± 1.2	29.2 ± 4.5	28.2 ± 2.7
38.62	3-methyltricosane	3-MeC23	338	4.8 ± 0.1	14.7 ± 0.1	18.4 ± 0.4	16.0 ± 2.0	17.8 ± 0.5
38.65	9-tetracosene	9-C24:1	336	9.4 ± 0.1	22.7 ± 0.9	26.4 ± 4.3	23.7 ± 3.1	29.3 ± 0.8
38.68	7-tetracosene	7-C24:1	336	2.9 ± 0.1	27.6 ± 0.9	33.6 ± 5.1	32.4 ± 2.8	37.8 ± 0.2
38.72	5-tetracosene	5-C24:1	336	1.4 ± 0.1	3.6 ± 0.2	9.8 ± 5.4	11.1 ± 1.8	11.3 ± 0.2
38.79	tetracosane	C24	338	7.6 ± 0.4	39.3 ± 1.5	51.5 ± 0.6	52.3 ± 6.3	56.7 ± 0.9
39.15	2-methyltetracosane	2-MeC24	352	7.7 ± 0.1	5.4 ± 0.2	11.4 ± 5.1	15.8 ± 2.1	16.5 ± 4.2
39.21	6,9-pentacosadiene	6,9-C25:2	348	31.1 ± 2.0	45.8 ± 1.5	42.7 ± 7.1	47.8 ± 2.2	47.8 ± 0.7
39.22	9-pentacosene	9-C25:1	350	23.9 ± 1.8	77.3 ± 3.7	86.3 ± 7.4	82.9 ± 1.7	82.8 ± 1.6
39.26	7-pentacosene	7-C25:1	350	111.1 ± 6.4	205.1 ± 7.1	194.2 ± 54.3	217.3 ± 18.5	212.9 ± 5.8
39.35	5-pentacosene	5-C25:1	350	6.5 ± 0.1	38.8 ± 0.7	37.8 ± 7.8	34.3 ± 3.3	33.2 ± 3.7
39.36	pentacosane	C25	352	167.1 ± 0.7	174.6 ± 7.2	191.3 ± 5.2	180.6 ± 9.9	187.7 ± 9.2
39.95	hexacosane	C26	366	30.4 ± 1.3	38.7 ± 16.6	32.5 ± 5.6	44.2 ± 0.2	55.5 ± 0.7
40.35	2-methylhexacosane	2-MeC26	380	19.1 ± 0.1	13.5 ± 0.5	30.7 ± 8.1	51.5 ± 2.2	39.3 ± 3.6
40.42	9-heptacosene	9-C27:1	378	8.9 ± 0.9	6.7 ± 0.1	10.6 ± 0.1	11.7 ± 1.4	10.6 ± 0.1
40.50	7-heptacosene	7-C27:1	378	17.1 ± 0.6	12.2 ± 1.2	13.8 ± 2.8	17.8 ± 4.2	11.8 ± 1.7
40.60	heptacosane	C27	380	58.3 ± 0.9	160.3 ± 7.7	183.1 ± 9.7	158.1 ± 3.7	165.9 ± 3.9
41.34	octacosane	C28	394	28.9 ± 0.6	62.1 ± 3.7	58.5 ± 9.7	61.4 ± 2.5	63.6 ± 3.9
41.47	unidentified 4			18.6 ± 0.5	86.4 ± 2.9	96.0 ± 1.9	91.9 ± 2.7	97.1 ± 2.5
41.88	2-methyloctacosane	2-MeC28	408	123.9 ± 1.2	191.8 ± 6.5	200.3 ± 8.1	197.9 ± 11.4	191.4 ± 1.4
42.09	7-nonacosene	7-C29:1	406	24.2 ± 0.6	23.5 ± 1.4	26.3 ± 0.4	24.0 ± 2.6	24.4 ± 4.9
42.22	nonacosane	C29	408	65.2 ± 2.8	194.2 ± 13.2	210.4 ± 4.7	189.3 ± 3.0	204.1 ± 6.9
44.00	2-methyltriacontane	2-MeC30	436	13.2 ± 2.1	36.9 ± 1.9	37.6 ± 5.3	36.2 ± 1.2	35.4 ± 1.0

Compounds recorded at different extraction times (1, 3, 6, 12 and 24 h) plus the extracted amounts (ng/individual, mean ± SEM). RT – retention times, Abbr. – abbreviation, M_r – molecular weight, N – newly detected compound.

drocarbons were extracted from internal tissues since the integument is not the only tissue that contains hydrocarbons (Schal et al., 1998) and different times are required for their extraction. It was also observed that for both sexes of *D. suzukii* the relative amounts of volatile hydrocarbons with $M_r \leq 282$ increases linearly with the extraction time and does not reach the equilibrium point even after 24 h (Fig. 2A). In contrast, for both sexes, the amount of the non-volatile hydrocarbons with $M_r > 282$ either remained constant or increased logarithmically as the extraction time increased, reaching an equilibrium after 1h or 3h of extraction (Fig. 2B). Similar results are reported for n-heptacosane ($M_r = 380$), one of the most abundant hydrocarbons in *Solenopsis invicta* (Buren, 1972), which increases logarithmically after 3 min, 10 min and 24 h extraction periods (Vander et al., 1989). With long extraction periods it is difficult to determine if an extracted hydrocarbon came from the cuticle or internal tissues. Volatility could be used to differentiate between internal and cuticular hydrocarbons, since those that vaporize quickly, such as C12, are unlikely

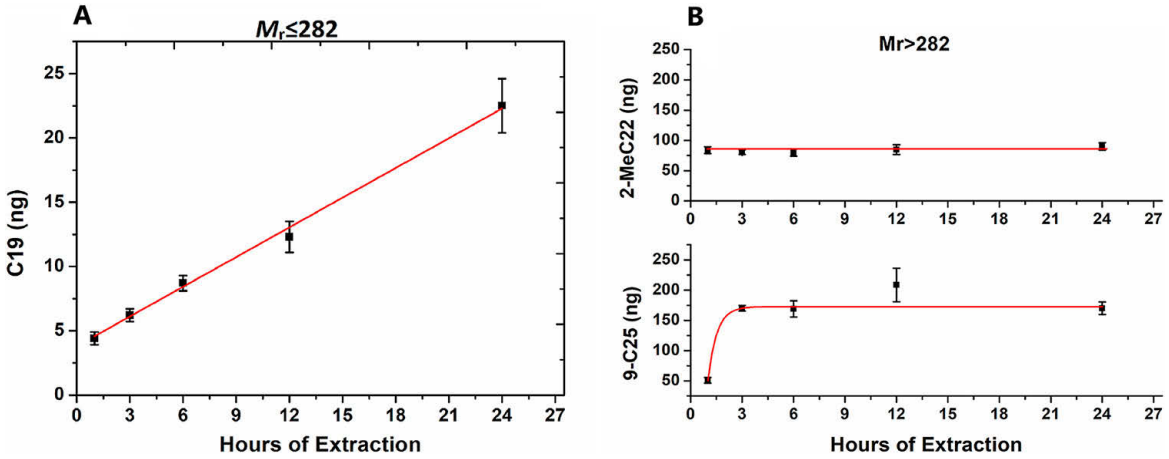

Fig. 2. Amount of (A) nonadecane (C19) and (B) 9-pentacosene (9-C25) and 2-methyldocosane (2-MeC22) extracted from *D. suzukii* females at different times after being placed in n-hexane. The lines represent linear and logarithmic regression curves (R^2 = 0.99).

to be present in the cuticle. Less volatile hydrocarbons, such as 9-C25, can also be present in the cuticle. This can be confirmed using a solvent-free extraction method (Choe et al., 2012). In addition, it was observed that in both sexes there are alkenes, such as 9-C21, 7-C21 and 5-C21 (M_r = 294), whose amount was the same at all of the extraction times, while there are others, such as 9-C25, 7-C25 and 5-C25 (M_r = 350), whose amount increased logarithmically with extraction time (Fig. 3A, B).

We conclude that the time required to reach equilibrium varies for the different types of hydrocarbons and an overall trend in extraction cannot be established. This could be attributed to the fact that increasing the extraction period beyond 1 h results in hydrocarbons being extracted either from various glands, internal tissues, or haemolymph, which serves not only as a transport medium but also as hydrocarbon reservoir (Schal et al., 1998; Holze et al., 2021). This could indicate that certain hydrocarbons are present in 'storage vesicles', since haemolymph contains approximately as many hydrocarbons as the cuticle and also serves not only as a transport medium but also as a hydrocarbon reservoir (Schal et al., 1998).

Behavioural effect of hydrocarbons

Based on the above results, two hydrocarbons were selected to study their effect on the behaviour of *D. suzukii*, C12 and 9-C25, since the first is a volatile hydrocarbon that is detected only after a 24 h extraction while the latter is less volatile and increased logarithmically over the extraction period.

Courtship assay (9-C25)

Mating behaviour of many species of *Drosophila* is enhanced by 9-C25 (Siwicki et al., 2005). Also, non-volatile hydrocarbons, such as 9-C25, act over short distances and their role in chemical communication is contact and gustation dependent (Montell, 2009; Vang et al., 2012). Two different amounts were investigated, 1 h extracted amount (50.9 ng, control treatment) and the maximum extracted amount (179.3 ng is the mean amount of plateau). Initially, we confirmed that the 1-h 9-C25 extraction amount (50.9 ng) is already present in the insect cuticle after 1 min of extraction (mean ± SEM, 49.9 ± 0.6 ng, N = 10). In addition, the total amount applied was measured as 173.7 ± 6.4 ng (mean ± SEM, N = 10). Since the control, 9-C23, re-

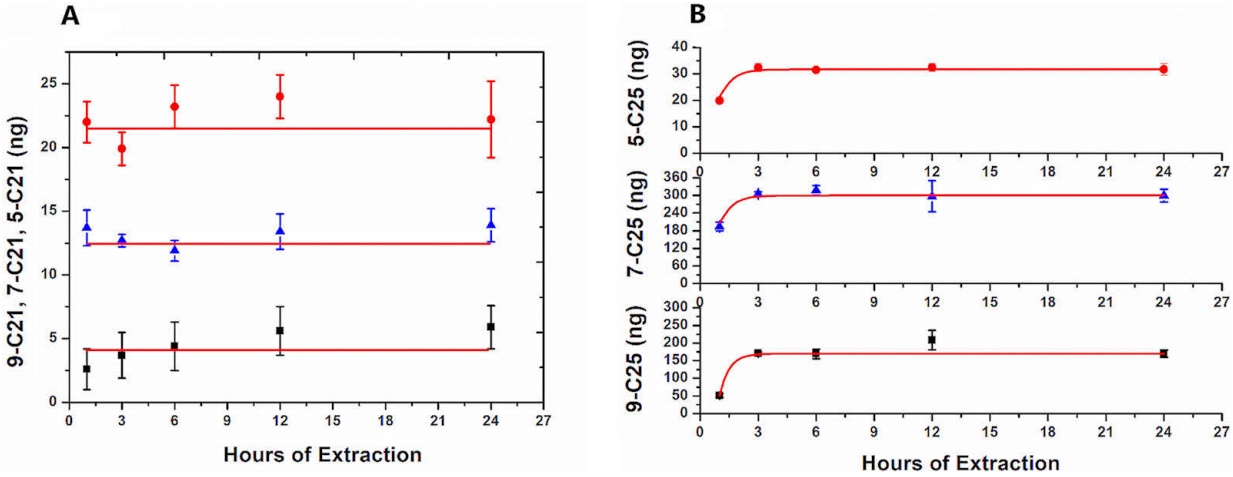

Fig. 3. Amount of (A) 9-heneicosene, 7-heneicosene, 5-heneicosene and (B) 9-pentacosne, 7-pentacosene, 5-pentacosene extracted from *D. suzukii* females at different times after being placed in the solvent n-hexane. The lines represent linear and logarithmic regression curves (R^2 = 0.99).

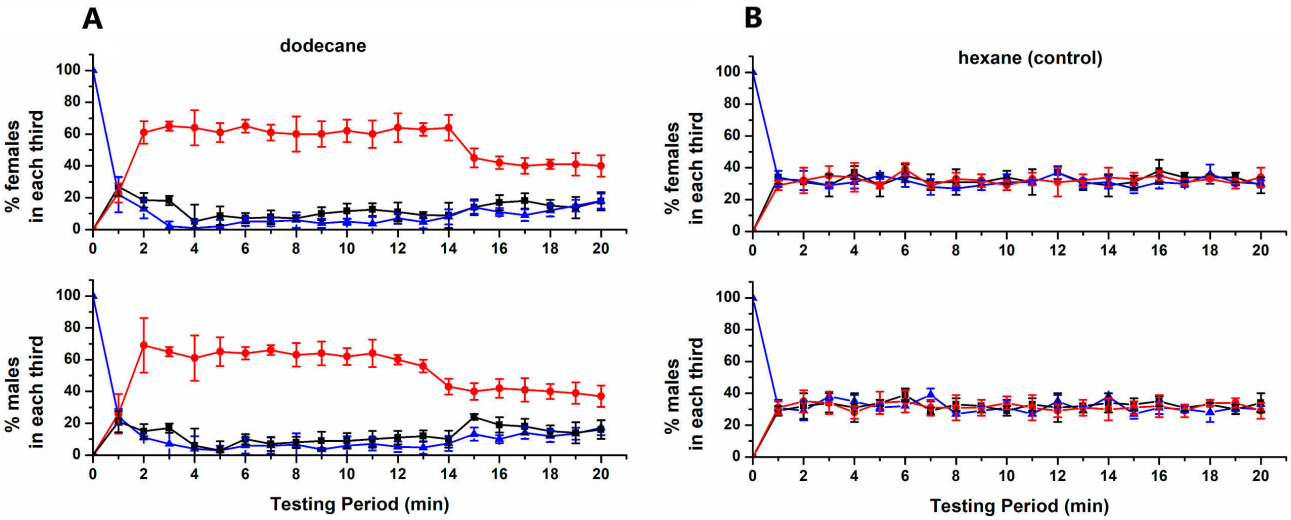

Fig. 4. Effect of time on the distribution of *D. suzukii* in a test-tube arena relative to an area treated with C12. (▲ – proximal, ■ – middle, ● – distal).

sulted in a significant decrease in mating events (9 ± 2%, x^2 (1,120) = 9.75; $p < 0.05$) it is concluded that the experimental setup was appropriate. 9-C25 treated females had significantly more mating events than control females (mean ± SEM, 82 ± 2%, 30 ± 13%, x^2 (1, 120) = 34.47; $p < 0.05$). The enhancement of mating behaviour by the application of 9-C25 at the maximum extracted amount was also associated with courtship duration. It was found that courtship duration was significantly longer (t (118) = –24.872; $p < 0.05$) in 9-C25 treated *D. suzukii* females (6.4 ± 1.2 min, mean ± SEM) than in the control treatment (3.7 ± 0.9 min, mean ± SEM).

This agrees with the fact that olfactory or gustatory responses of *Drosophila* can change dramatically even with a slight change in the stimulus concentrations (Devaud, 2003). The above findings agree with published data suggesting that a high dose of 9-C25 stimulates courting males to attempt to copulate (Siwicki et al., 2005).

Locomotion assay (C12)

C12 is a volatile compound considered to be an alarm pheromone in other insects, such as ants (Lofqvist, 1976; Welzel et al., 2018) and beetles (Kou et al., 1989), but there is limited data on its role in species of *Drosophila*. There are procedures for measuring the behavioural responses of fruit flies to volatile compounds (Devaud, 2003). Volatile chemicals are largely detected by odour receptors (Hallem & Carlson, 2006). In general, the method for determining their biological role is determined by their degree of volatility (Vang et al., 2012). In our locomotion assays, 46.0 ± 4.7% (mean ± SEM) of the females and 42.0 ± 3.9% (mean ± SEM) of the males moved when n-hexane was used. In contrast, locomotion increased significantly to 93.7 ± 8.5% (mean ± SEM) for females and to 92.1 ± 9.6% (mean ± SEM) for males when C12 was used (Female: (x^2(1120) = 51.42; $p < 0.05$, Male: (x^2(1120) = 57.77; $p < 0.05$).

Repulsion assay (C12)

Following the locomotion assays, we examined the potential repulsion effect of C12 on the behaviour of *D. suzukii* (Devaud, 2003; Vang et al., 2012). In order to quantify the response of the flies to C12, individuals were distributed equally in the three parts (proximal, middle and distal third) of the test tube during the test. When C12 is applied, both sexes moved to the distal section of the test tube (Fig. 4A). The percentage of flies at the end of the tube was significantly higher than in the proximal or middle sections (One-Way ANOVA results are presented in Table 3 for males and females). These findings are in accordance with previous results (Vang et al., 2016), in which *D. melanogaster* moved to the distal part of a test

Table 3. One-Way ANOVA results of repulsion assay showing the effect of C12 on the percentage of male and female *D. suzukii* in the different sections of the test tube.

Minute	Males		Females	
	$F_{(2,123)}$	*P*	$F_{(2,123)}$	*P*
1	0.127	0.877	2.171	0.118
2	13279.016	0.001	7339.939	0.001
3	7310.381	0.001	9006.601	0.001
4	12027.006	0.001	10495.070	0.001
5	13876.474	0.001	10714.803	0.001
6	11573.698	0.001	13154.959	0.001
7	7260.624	0.001	14773.050	0.001
8	9627.205	0.001	12931.995	0.001
9	9280.554	0.001	11942.262	0.001
10	4598.424	0.001	10355.094	0.001
11	7924.405	0.001	9324.988	0.001
12	6192.660	0.001	10465.257	0.001
13	8485.034	0.001	12586.992	0.001
14	1312.552	0.001	10716.677	0.001
15	2302.669	0.001	1508.849	0.001
16	2344.443	0.001	2385.231	0.001
17	2202.253	0.001	4664.034	0.001
18	1853.749	0.001	3889.756	0.001
19	1494.846	0.001	4050.199	0.001
20	1561.171	0.001	3173.402	0.001

Table 4. One-Way ANOVA results of repulsion assay showing the effect of hexane (control) on the percentage of male and female *D. suzukii* in the different sections of the test tube.

Minute	Males		Females	
	$F_{(2,123)}$	P	$F_{(2,123)}$	P
1	0.488	0.615	0.205	0.815
2	0.003	0.997	0.213	0.808
3	1.672	0.192	1.423	0.245
4	0.248	0.781	0.457	0.634
5	0.077	0.926	2.160	0.119
6	2.137	0.103	0.935	0.395
7	0.412	0.663	0.141	0.865
8	1.543	0.198	0.401	0.670
9	2.362	0.138	0.020	0.980
10	0.054	0.948	1.187	0.308
11	0.564	0.570	0.821	0.442
12	0.284	0.753	2.609	0.077
13	0.751	0.454	0.102	0.903
14	0.804	0.450	2.618	0.077
15	1.629	0.200	13.27	0.269
16	0.126	0.882	2.153	0.120
17	0.528	0.591	0.029	0.971
18	1.696	0.187	0.454	0.636
19	1.228	0.296	1.350	0.179
20	0.045	0.956	0.160	0.852

tube when benzaldehyde, a known repellent, was applied. Similar behaviour was also observed when benzaldehyde was used as positive control for *D. suzukii* in this study (Fig. S1). Finally, we observed a decrease in repulsion after 14 min. A plausible explanation for this behaviour could be a decrease in the partial pressure of the C12 due to diffusion (Vang et al., 2012). It is also reported that non-social insects, such as *Drosophila*, avoid harmful stimuli, when alarm pheromones are applied (Enjin & Bae Suh, 2013). In contrast, there was an even distribution of flies in the three parts of the test tube when hexane (control) was applied (Fig. 4B) (One-Way ANOVA results are shown in Table 4 for both males and females). Based on this data, C12 repels *D. suzukii* at the concentration tested.

The optimization of the hydrocarbon extraction method showed that depending on the type of compound and its distribution in the body, different extraction periods are required. A one-hour extraction time is not sufficient for the analysis of the entire hydrocarbon profile of *D. suzukii*. Thus, the extraction times should be determined for specific compounds. Here we show the repulsive effect of C12, which occurs inside the body since it was detected after a 24 h extraction period, even though it is usually assumed that the primary communication compounds are mainly on the outer surface of an insect (Choe et al., 2012). On the other hand, the maximum extracted amount of the 9-C25, which is an attractant for *D. suzukii* is optimally measured by a 3 h extraction period. Further studies with a more selective analytical technique, such as Solid Phase Micro Extraction (SPME) coupled to GC-MS, may be used to confirm if specific hydrocarbons are indeed internal or cuticular (Everaerts et al., 2010; Farine et al., 2012; Levi-Zada et al., 2012; Al-Khshemawee et al., 2018). Finally, further field experiments are warranted to confirm the behavioural effect of C12 and 9-C25 to help develop new control methods for this pest.

ACKNOWLEDGEMENTS. This research was co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme "Human Resources Development, Education and Lifelong Learning" in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

REFERENCES

Al-Khshemawee H., Du X., Agarwal M., Yang J.O. & Ren Y.L. 2018: Application of direct immersion solid-phase microextraction (DI-SPME) for understanding biological changes of Mediterranean fruit fly (*Ceratitis capitata*) during mating procedures. — *Molecules* **23**: 2951, 13 pp.

Bagneres A.G. & Morgan E.D. 1990: A simple method for analysis of insect cuticular hydrocarbons. — *J. Chem. Ecol.* **12**: 3263–3276.

Barron A.B. 2000: Anaesthetizing *Drosophila* for behavioural studies. — *J. Insect Physiol.* **46**: 439–442.

Bartelt R.J. & Jackson L.L. 1984: Hydrocarbon component of the *Drosophila virilis* (Diptera: Drosophilidae) aggregation pheromone: (Z)-10-Heneicosene. — *Ann. Entomol. Soc. Am.* **77**: 364–371.

Belenioti M. & Chaniotakis N. 2020: Aggressive behaviour of *Drosophila suzukii* in relation to environmental and social factors. — *Sci. Rep.* **10**: 7898, 10 pp.

Blomquist G. 2010a: Biosynthesis of cuticular hydrocarbons. In Blomquist G.J. & Bagnères A.G. (eds): *Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology*. Cambridge University Press, UK, pp. 35–52.

Blomquist G. 2010b: Structure and analysis of insect hydrocarbons. In Blomquist G.J. & Bagnères A.G. (eds): *Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology*. Cambridge University Press, UK, pp. 19–34.

Blomquist G., Nelson D. & Derenobales M. 1987: Chemistry, biochemistry, and physiology of insect cuticular lipids. — *Arch. Insect Biochem. Physiol.* **6**: 227–265.

Carvalho M., Sampaio J.L., Palm W., Brankatschk M., Eaton S. & Shevchenk A. 2012: Effects of diet and development on the *Drosophila* lipidome. — *Mol. Syst. Biol*. **8**: 600, 17 pp.

Cerkowniak M., Puckowski A., Stepnowski P. & Gołębiowski M. 2013: The use of chromatographic techniques for the separation and the identification of insect lipids. — *J. Chromatogr. (B, Analyt. Technol. Biomed. Life Sci.)* **937**: 67–78.

Choe D.H., Ramírez S.R. & Tsutsui N.D. 2012: A silica gel based method for extracting insect surface hydrocarbons. — *J. Chem. Ecol.* **2**: 176–187.

Cvacka J., Jiros P., Sobotnik J., Hanus R.V. & Svatos A. 2006: Analysis of insect cuticular hydrocarbons using matrix-assisted laser desorption/ionization mass spectrometry. — *J. Chem. Ecol.* **32**: 409–434.

Gołębiowski M., Boguś M.I., Paszkiewicz M. & Stepnowski P. 2011: Cuticular lipids of insects as potential biofungicides: methods of lipid composition analysis. — *Anal. Bioanal. Chem*. **399**: 3177–3191.

Dekker T., Revadi S., Mansourian S., Ramasamy S., Lebreton S., Paul G., Becher Angeli S., Rota-Stabell O. & Anfora G. 2015: Loss of *Drosophila* pheromone reverses its role in sexual communication in *Drosophila suzukii*. — *Proc. Biol. Sci*. **282**(1804): 20143018, 9 pp.

Devaud J.M. 2003: Experimental studies of adult *Drosophila* chemosensory behavior. — *Behav. Processes* **64**: 177–196.

Dossey A.T., Walse S.S., Rocca J.R. & Edison A.S. 2006: Single-insect NMR: a new tool to probe chemical biodiversity. — *ACS Chem. Biol.* **1**: 511–514.

Drijfhout F.P., Kather R. & Martin S.J. 2009: The role of cuticular hydrocarbons in insects. In Zhang W. & Liu H. (eds): *Behavioral and Chemical Ecology*. Nova Science, Hauppauge, pp. 91–114.

Enjin A. & Bae Suh G.S. 2013: Neural mechanisms of alarm pheromone signaling. — *Mol. Cells* **35**: 177–181.

Everaerts C., Farine J.P., Cobb M. & Ferveur J.F. 2010: *Drosophila* cuticular hydrocarbons revisited: mating status alters cuticular profiles. — *PLoS ONE* **5**(3): e9607, 12 pp.

Farine J.P., Ferveur J.F. & Everaerts C. 2012: Volatile *Drosophila* cuticular pheromones are affected by social but not sexual experience. — *PLoS ONE* **7**(7): e40396, 11 pp.

Fedina T.Y., Kuo T-H., Dreisewerd K., Dierick H.A., Yew J.Y. & Pletcher S.D. 2012: Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. — *PLoS ONE* **7**(12): e49799, 11 pp.

Gershman S.N., Toumishey E. & Rundle H.D. 2014: Time flies: time of day and social environment affect cuticular hydrocarbon sexual displays in *Drosophila serrata*. — *Proc. R. Soc. (B)* **281**: 20140821, 8 pp.

Gibbs A.G. & Markow T.A. 2001: Effects of age on water balance in *Drosophila* species. — *Physiol. Biochem. Zool.* **74**: 520–530.

Gibbs A.G., Fukuzato F. & Matzkin L.M. 2003: Evolution of water conservation mechanisms in *Drosophila*. — *J. Exp. Biol.* **206**: 1183–1192.

Hallem E.A & Carlson J.R. 2006: Coding of odors by a receptor repertoire. — *Cell* **125**: 143–160.

Haruhito K. & Haruo C. 1982: Transport of hydrocarbons by the lipophorin of insect hemolymph. — *Biochim. Biophys. Acta* **710**: 341–348.

Holze H., Schrader L. & Buellesbach J. 2021: Advances in deciphering the genetic basis of insect cuticular hydrocarbon biosynthesis and variation. — *J. Hered.* **126**: 219–234.

Howard R.W. 1982: Chemical ecology and biochemistry of insect hydrocarbons. — *Annu. Rev. Entomol.* **27**: 149–172.

Howard R.W. & Blomquist G.J. 2005: Ecological, behavioral, and biochemical aspects of insect hydrocarbons. — *Annu. Rev. Entomol*. **50**: 371–393.

Hwee S.N., Shankara S., Shikichic Y., Akasakad K., Moric K. & Yewa Y.J. 2014: Pheromone evolution and sexual behavior in *Drosophila* are shaped by male sensory exploitation of other males. — *Proc. Natn. Acad. Sci. U.S.A*. **25**: 3056–3061.

Koemans T.S., Oppitz C., Donders R.A., Bokhoven H., Schenck A., Keleman K. & Kramer J.M. 2017: *Drosophila* courtship conditioning as a measure of learning and memory. — *J. Vis. Exp*. **124**: e55808, 11 pp.

Kou R., Tang D.S. & Chow Y.S. 1989: Alarm pheromone of pentatomid bug, *Erthesina fullo* Thunberg (Hemiptera: Pentatomidae). — *J. Chem. Ecol.* **15**: 2695–2702.

Kuo T.H., Yew J.Y., Fedina T.Y., Dreisewerd K., Dierick H.A. & Pletcher S.D. 2012: Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. — *J. Exp. Biol*. **5**: 814–821.

Knapp L., Mazzi D. & Finger R. 2020: The economic impact of *Drosophila suzukii*: perceived costs and revenue losses of Swiss cherry, plum and grape growers. — *Pest Manag. Sci*. **77**: 3597–3597.

Lee J.C., Bruck D.J., Curry H., Edwards D., Haviland D.R., Van Steenwyk R.A. & Yorgey B.M. 2011a: The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. — *Pest Manag. Sci*. **67**: 1358–1367.

Levi-Zada A., Nestel D., Fefer D., Nemni-Lavy E., Deloya-Kahane I. & Maayan D. 2012: Analyzing diurnal and age-related pheromone emission of the olive fruit fly, *Bactrocera oleae* by sequential SPME-GCMS analysis. — *J. Chem. Ecol.* **38**: 1036–1041.

Lofqvist J. 1976: Formic acid and saturated hydrocarbons as alarm pheromones for the ant *Formica rufa*. — *J. Insest Physiol.* **22**: 1331–1346.

Montell C. 2009: A taste of the *Drosophila* gustatory receptors. — *Curr. Opin. Neurobiol.* **19**: 345–353.

Moris V.C., Christmann K., Wirtgen A., Belokobylskij S.A., Berg A., Liebig W., Soon V., Baur H., Schmitt T. & Niehuis O. 2021: Cuticular hydrocarbons on old museum specimens of the spiny mason wasp, *Odynerus spinipes* (Hymenoptera: Vespidae: Eumeninae), shed light on the distribution and on regional frequencies of distinct chemotypes. — *Chemoecology* **31**: 311–322.

Revadi S., Lebreton S., Witzgall P., Anfora G., Dekker T. & Becher P.G. 2015: Sexual behavior of *Drosophila suzukii*. — *Insects* **6**: 183–196.

Schal C., Sevala V., Young H. & Bachmann J.A.S. 1998: Sites of synthesis and transport pathways of insect hydrocarbons: cuticle and ovary as target tissues. — *Am. Zool*. **38**: 382–393.

Schal C., Sevala V., Capurro M.L., Snyder T.E., Blomquist G.J. & Bagnères A.G. 2001: Tissue distribution and lipophorin transport of hydrocarbons and sex pheromones in the house fly, *Musca domestica*. — *J. Insect Sci.* **1**: 12, 11 pp.

Siwicki K.K., Riccio P., Ladewski L., Marcillac F., Dartevelle L., Cross S.A. & Ferveur J.F. 2005: The role of cuticular pheromones in courtship conditioning of *Drosophila* males. — *Learn. Mem.* **12**: 636–645.

Sledge M.F., Boscaro F. & Turillazzi S. 2001: Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. — *Behav. Ecol. Sociobiol*. **49**: 401–409.

Snellings Y., Herrera B., Wildermann B., Beelen M., Zwarts L., Wenseleers T. & Callaerts P. 2018: The role of cuticular hydrocarbons in mate recognition in *Drosophila suzukii*. — *Sci. Rep.* **8**: 4996, 11 pp.

Tillman J.A., Seybold S.J., Jurenka R.A. & Blomquist G.J. 1999: Insect pheromones – an overview of biosynthesis and endocrine regulation. — *Insect Biochem. Mol. Biol.* **29**: 481–514.

Vander Meer R.K., Saliwanchik D. & Lavine B. 1989: Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*. Implications for nestmate recognition. — *J. Chem. Ecol.* **15**: 2115–2125.

Vang L. & Adler J. 2016: *Drosophila* mutants that are motile but respond poorly to all stimuli tested. — *bioRχiv* 045062, 17 pp.

Vang L.L., Medvedev A.V. & Adler J. 2012: Simple ways to measure behavioral responses of *Drosophila* to stimuli and use of these methods to characterize a novel mutant. — *PLoS ONE* **7**(5): e37495, 11 pp.

Vieillard D.A. & Cortot J. 2016: When choice makes sense: menthol influence on mating, oviposition and fecundity in *Drosophila melanogaster. — Front. Integr. Neurosci.* **10**: 5, 11 pp.

Walsh D.B., Bolda M.P., Goodhow R.E, Dreves A.J., Lee J., Bruck D.V., Walton M., O'Neal S.D. & Zalom F.G. 2011: *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. — *J. Integr. Pest Manag.* **106**: 289–295.

Welzel K.F., Lee S.H., Dossey A.T., Chauhan K.R. & Choe D.H. 2018: Verification of argentine ant defensive compounds and

their behavioral effects on heterospecific competitors and conspecific nestmates. — *Sci. Rep.* **8**: 1477, 15 pp.

Yew J.Y. & Chung H. 2015: Insect pheromones: an overview of function, form and discovery. — *Prog. Lipid Res.* **59**: 88–105.

Yew J.Y., Cody R.B. & Kravitz E.A. 2008: Cuticular hydrocarbon analysis of an awake behaving fly using direct analysis in real-time-of-flight mass spectrometry. — *Proc. Natl. Acad. Sci. U.S.A.* **105**: 7135–7140.

Yew J.Y., Dreisewerd K., Oliveira C.C. de & Etges W.J. 2011a: Male-specific transfer and fine scale spatial differences of newly identified cuticular hydrocarbons and triacylglycerides in a *Drosophila* species pair. — *PLoS ONE* **6**(9): e16898, 15 pp.

Yew J.Y., Soltwisch J., Pirkl A. & Dreisewerd K. 2011b: Direct laser desorption ionization of endogenous and exogenous compounds from insect cuticles: practical and methodologic aspects. — *J. Am. Soc. Mass Spectrom.* **22**: 1273–1284.

Received August 31, 2021; revised and accepted June 24, 2022
Published online July 26, 2022

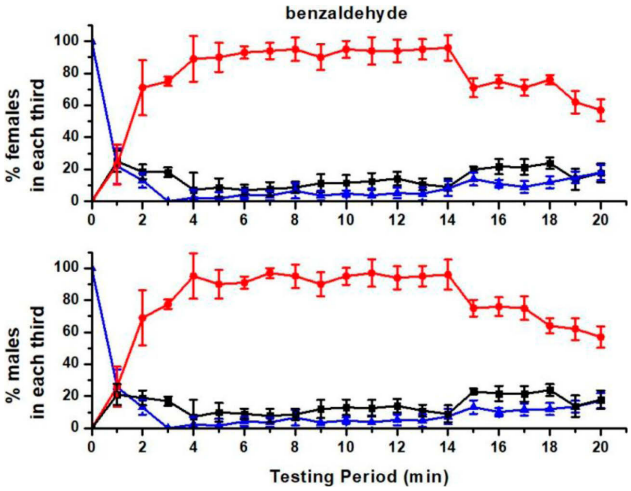

Fig. S1. Effect of time on the distribution of *D. suzukii* relative to an area treated with benzaldehyde. (▲ – proximal, ■ – middle, ● – distal).