Evaluation of Stable Isotope $^{13}$C$_6$-glucose on Volatile Organic Compounds in Different Stages of Mediterranean Fruit Fly (Medfly) Ceratitis Capitata (Diptera: Tephritidae)

Hasan AL-Khshemawee$^{1,2}$, Manjree Agarwal$^1$ and Yonglin Ren$^1$

$^1$School of Veterinary and Life Science, Murdoch University, WA 6150, Australia
$^2$College of Agriculture, Wasit University, Wasit, Iraq

Corresponding author: Hasan AL-Khshemawee, School of Veterinary and Life Science, Murdoch University, Murdoch, WA 6150, Australia; Tel: 61893601397; E-mail: hasan_hadi1984@yahoo.com

Received date: May 23, 2017; Accepted date: July 04, 2017; Published date: July 11, 2017

Abstract

The Mediterranean fruit fly (Medfly) Ceratitis capitata (Diptera: Tephritidae), as most of the Tephritidae species, is a pest of great economic importance around the world. For Integrated Pest Management (IPM) and biological pest management purposes, this research will investigate characterization of the broadest possible range of volatile organic compounds and the possible changing trends of volatile biological emissions during development stages of insect. During the last years, many types of research have been done to understand chemical communications between pest-pest interactions and about the insect responses to specific volatile organic compounds. An early comparison of the VOCs emitted from larvae, pupae and adult was performed. Our research focuses on the comparison of volatile compounds emitted from a different stage of Medfly using stable isotope $^{13}$C$_6$-glucose. Gas Chromatography (GC) technique coupled with Flame Ionization Detection (FID) and gas chromatography with mass spectrometry (GC-MS) for identification of VOCs was employed. Head Space-Solid Phase Micro Extraction HS-SPME method with Three-phase fiber 50/30 µm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) was used. The results showed that there are different chemicals emitted in a different stage of Medfly (Larvae, pupae, and adults M/F) especially in the adult stage. GC-MS detected 27 compounds from larvae, 23 compounds from pupae and 29 compounds from adults. These different VOCs emitted in different stages of Medfly were clearly displayed, and a broad range of emitted volatile compounds was successfully described. The characterization of release patterns could be useful tool for the selection of compounds and for further investigated in biological studies to understand of the key semi-chemicals involved in medfly behaviour.

Keywords: Mediterranean fruit fly; VOCs; Stable isotope; SPME-GC-MS; $^{13}$C$_6$-glucose

Introduction

Mediterranean fruit fly Ceratitis capitata (Medfly) is an invasive agriculture pest species that impact fruit production and export worldwide. C. capitata attacks around 250 different types of fruit around the world [1]. The United States spends $57 million per year on Medfly risk management [2]. Furthermore, over the period 2003-2008, Australian industry and government invested around $128 million in the control of Medfly C. capitata [3]. Currently, fruit fly management is almost exclusively carried out with chemicals that are harmful to human health and the environment [4]. In organic fruit production, the problem is more dangerous, since the law regarding organic farming prevents the use of synthetic materials that include pesticides [5]. For this reason, farmers are trying to limit these issues by avoiding infection by Mediterranean fruit fly. Results obtained in field and laboratory tests explain different susceptibilities to Medfly damage [6]. Identification of the VOCs released by different stages of Medfly can help us to understand the chemical communications between insect-insect interactions in different stages of Medfly [7]. The potential detection method is to analyse the Volatile Organic Compounds (VOCs) released by different stages of medfly (Larvae, pupae and adults) and understanding the insect response to stable isotope. Currently, Head Space-Solid Phase Micro Extraction (HS-SPME) method coupled with Flame Ionization Detection (FID) and Gas Chromatography with Mass Spectrometry (GC-MS) for identification of VOCs has been used successfully to examine volatile compounds [8,9]. Volatile organic compounds have been widely used to detect stored grain insects and aggregation pheromone in pest [10,11]. HS-SPME has been used to detect ongoing spoilage and fruit damage based on the production of volatile organic compounds by insects to communicate with their own species [12,13]. Baker et al. [14] reported nine components released by males of Medfly, like cyclic imine 3,4-dihydro-2H-pyrrrole (1-pyrrole) as a key for sexual period. Jang et al. [15] reported 69 different compounds from male of Medfly, but the females showed small number of short-chain aldehydes compounds. Jacobson et al. [16] found sex pheromone of Medfly as a mixture of 15 substances, these were carboxylic acids and other compounds including methyl (E)-6-nonen-1-ol. Based on these studies, we need to develop a precise pest-detection technique, detect tools and management strategies for Medfly and investigation of their evolutionary relationships as well as the characterisation of different stages of Medfly [17-19]. The main goal of this research was to provide the qualitative description of volatile profiles at different stages of Medfly and characterize of compounds according to their emission pattern by using stable isotope $^{13}$C$_6$-glucose. Also, to understand the communication signals between males and females during mating time in adult stage.
### Materials and Methods

#### Insects

Medfly colony were obtained from the Department of Agriculture and Food, Western Australia (DAFWA) and reared in the Murdoch University Laboratory, in Perth Australia. The flies were reared under conditions: 23 ± 2°C and 75 ± 5% RH, and 12:12-h (L:D) [1]. Adults were placed in screen cages (40 cm length × 40 cm height × 40 cm depth) and each cage contained medfly food made from crystalline sugar (Bidvest, Australia), yeast hydrolysate (Australian Biosearch) in the ratio of 4:1 and water 50 ml. About 10-12 days after adult emergence from pupae and mating of adult flies, eggs were collected every day in a water tray kept adjacent to the cage as they drop from the cage after being deposited.

#### Volatile Organic Compounds (VOC) collection

We placed 100 eggs in 9 mm sterile Petri dishes (Thomas Scientific, Australia) with 25 g of carrot media. After hatching, 0.2 g of D-glucose-13C6, 99 atom % 13C (Sigma-Aldrich; St. Louis, MO 63178, USA) was added to carrot media. The similar way was for normal glucose and control. The cultures were then incubated for 2-5 days at 23 ± 1°C and 75% ± 5 Relative Humidity (RH) for rearing the larvae stage while for pupae stage, they kept in 26 ± 2°C and 65 ± 5% Relative Humidity (RH). Then, when all adults emerged, the placed in screen cages (40 cm length × 40 cm height × 40 cm depth), 0.2 g of D-glucose-13C6 was incorporated with 1 g of sucrose 99.5% (Sigma-Aldrich; Castle Hill, NSW, AU) in 15 ml of water. The samples prepared for assay were 20 larvae, 20 pupae in a 4 ml flask and 20 adults 5M/5F in 36 ml jar. Each sample was replicated 3 times and all samples were conditioned for 4 h at a constant temperature 26°C.

#### Respiration rate

Respiration rate (O2 and CO2) was measured by Oxybaby 6.0 (Witt Gaasetechnik, VIC), serial No. 908911 for different stages of Medfly (Figure 1). Three replicate were taking from each treatment.

#### Equipment for collected VOCs

VOCs were collected by solid phase microextraction (SPME) fibre with 50/30 μm Carboxen/DVB/PDMS (2 cm) (Sigma-Aldrich, Bellefonte, USA) coating. The samples were collected by inserting the SPME fibre into the jar and exposed to the headspace. Sample's extraction and desorption time was 4 h. The profile of chemicals extracted by HS-SPME with an Agilent Technologies gas chromatograph 7892A (serial number CN14272038) fitted with an HP-5MS column non-polar (30 m × 0.25 mm, film thickness 0.25 µm, RESTEK, catalogue number 13423), with a flame ionization detector (FID) was used in this research. The carrier gas was Helium (HE) at 1.1 ml/min constant flow, and FID temperatures of 290°C, injection temperature 250°C, and the GC-FID instrument was operated in a splitless mode. Total run was 45 min and each flask was sampled three times. For identification of chemicals, Gas Chromatography Mass Spectrometry (GC-MS) was equipped with a mass spectrometer detector 5977E (Agilent Technologies, USA), a HP-5MS column non-polar (30 m × 0.25 mm, film thickness 0.25 μm, catalogue number 59051) (Santa Clara, CA 95051, USA) was used. The carrier gas 99.999% was helium supplied by (BOC, gas, Sydney, AU). The GC-MS operation conditions were as follows: temperature for injector port was 250°C. The initial oven temperature was 50°C which increased to 250°C by (5°C/min). The column Flow rate was 0.7 ml/min, and splitless was 20 ml/min at 1.5 min. The total run time was 45 min. The volatile organic compounds were identified by comparison of the mass spectrum with the NIST 2014 database (the US National Institute of Standards and Technology) with retention index RI confirmation samples.

### Statistical analysis

Metaboanalyst 3.0 (a comprehensive tool suite for metabolomics data analysis) (Online) was used for data analysis while the variations (standard deviations of means) of VOCs concentrations by (ANOVA) test. Three samples were analysed from each treatment and stage. The results were compared by using the least significant differences test (LSD P ≤ 0.05).

### Results and Discussion

#### GC-FID compounds and analysis

In general, Figure 1 shows the representative chromatograms gained from different stages of Medfly (larvae, pupae and adults). The results showed that the chromatograms of larvae were different with pupae and adult stages. The adults of Medfly gave the highest number of chemicals compared with other stages. Therefore, GC-FID can distinguish between different stages of Medfly under these experimental conditions that were used. The first aim of this study was to identify whether the volatile organic compounds can be used to evaluate the behaviour of fruit fly and stable isotope 13C6-glucose on different stages of volatile compounds of Medfly. Previous researches employed analytical techniques based on thermal desorption, solvent extraction and headspace, which were explained the differences in medfly volatile profiles [20]. This study showed that the fruit fly emitted different VOCs when treated by a stable isotope, especially with adult treatment [21]. The techniques applied in this current research (headspace collection of insect volatiles, GC-FID and GC-MS) allowed for the identification of volatiles produces by different stages of Medfly. SPME fibre technique could be used for the qualitative analyses of the fruit fly emanation (Table 1) [18].

#### Compounds identified by GC-MS

Volatile organic compounds identified by GC-MS used the same conditions with GC-FID. We used pure chromatography (Restek, EZGC method translator) to set up the method from GC-FID to GC-MS. GC-MS detected 27 compounds from larvae, 23 compounds from pupae and 29 compounds from adults. Some of the previous studies worked on headspace, solvent extraction and analytical techniques, which explained the differences in Medfly volatile profile [22]. Some compounds in this study have been described previously as being part of volatile constituents of Medfly and some were not described before. These include acetophenone, eicosene [23]. In larvae stage, control treatment detected 22 chemicals, but labelled treatment detected 13 chemicals only and 21 in unlabelled treatment (Figure 2). This is because the stable isotope has been changed by some chemicals compared with others (Figure 3). There is no reference available to describe the VOCs from larvae and pupae stage treated by stable isotope 13C6-glucose.
Table 1: Compounds were significant differences between treatments in adult stage.

| RT  | Compound           | RI  | Treatments | P-value |
|-----|--------------------|-----|------------|---------|
|     |                    |     |            |         |
| 3.61| Acetoin            | 717 | Labelled   | 97.830* |
|     |                    |     | Unlabelled | 8.56    |
|     |                    |     | Control    | 7.815   |
| 4.99| 2,3-Hexanediione   | 757 | Labelled   | N.D     |
|     |                    |     | Unlabelled | N.D     |
|     |                    |     | Control    | 0.946   |
| 5.54| Hexaldehyde        | 769 | Labelled   | 9.270*  |
|     |                    |     | Unlabelled | N.D     |
|     |                    |     | Control    | 2.023   |
| 7.88| o-Dimethylbenzene  | 862 | Labelled   | N.D     |
|     |                    |     | Unlabelled | 5.312   |
|     |                    |     | Control    | N.D     |
| 8.35| Nonane             | 900 | Labelled   | N.D     |
|     |                    |     | Unlabelled | 4.095   |
|     |                    |     | Control    | N.D     |
| 9.67| Butanoic acid, 4-hydroxy | 933 | Labelled   | 8.433   |
|     |                    |     | Unlabelled | 13.864* |
|     |                    |     | Control    | 5.425   |
| 11.29 | 2,3,4-Trithiapentane | 943 | Labelled   | N.D     |
|     |                    |     | Unlabelled | N.D     |
|     |                    |     | Control    | 1.765   |
| 12.19 | Octane, 2,7-dimethyl- | 964 | Labelled   | 46.140* |
|     |                    |     | Unlabelled | 6.095   |
|     |                    |     | Control    | N.D     |
| 12.79 | Octanal           | 982 | Labelled   | N.D     |
|     |                    |     | Unlabelled | 2.035   |
|     |                    |     | Control    | 2.883   |

a: Retention time (min)
b: Compounds detected
c: Retention index
d: Treatments (labelled, unlabelled and control); N.D: Not Detected
e: P-value (P ≤ 0.05)

*High significant differences

Identification of volatile constituents from larvae stage

Three main compounds were detected in control compared with labelled and unlabelled treatments. These compounds were myrcene, 1-tetradecanol, palmitolic acid (Figure 3). Some of the compounds were not detected in labelled and unlabelled like 1-h-1,2,3-triazole, L-caryophyllene and ethyl palamitate (Figure 3). The quantity of 27 compounds recorded in control with unlabelled were almost same, but the differences was with labelled larvae. This suggests that the labelled larvae little effect on the VOCs of larvae and their behaviour [22]. Some compounds identified in the present research have not been previously described as being part of the volatile constituents of Medfly larvae. Total of 32 VOCs were detected from Bactrocera oleae L. Larvae stage [23]. The main compounds were compounds were α-pinene, limonene, sabinen, β-pinene, myrcene and careen.
Identification of volatile constituents from adult stage

Nine special compounds showed significant differences between treatments; these were 2,3-Hexanedione; o-dimethylbenzene; nonane; octane, 2,7-dimethyl-; butanoic acid, 4-hydroxy; 2,3,4-trithiapentane; hexaldehyde; octanal and acetoin. Most of these compounds were detected in control treatment (Figure 5). Twenty-nine compounds detected in the adult stage, some of these compounds have been reported in similar works aimed at detecting VOCs from male Medflies [24]. Jang et al. [15] reported that some short-chain aldehydes at trace levels were released the female Medfly. The current research did not detect some compounds reported by other studies; however, most of these chemicals are minor chemicals. The reason could be the use of different techniques to collect the volatile compounds or other experimental factors like time of taking samples, air flow, and GC temperature as mentioned by other authors [25]. Acetophenone group have been described before released by females of Dendroctonus spp. [22]. The main constituents of male volatile compounds include geranyl acetate, (E,E)-R-farnesene, and ethyl (3E)-3-octenoate, which is consistent with the results of similar studies [26]. Relationships between compounds structures and emission patterns were the similar positions in some compounds like nonane and octanal [27]. In fact, this kind of experiment was carried out with C. capitata and the conditions (age, RH, temperature, light) could also be an impact on the detected VOCs.

Conclusion

Twenty seven compounds emitted by larvae, 23 compounds emitted by pupae and 29 main compounds emitted by adults were identified using HS-SPME fibre coupled with GC-MS technique. GC-MS detected 1-h-1,2,3-triazole, l-caryophyllene and ethyl palmitate compounds from larvae stage; acetophenone, undecane, undecane, 2,6-methyl-, heptadecane, eicosane and hexane,3,3-dimethyl compared with other treatments (Figure 4). To identify the main peaks, the value of each compound with respect to the total integrated peaks was calculated to compare the emission between compounds in the same treatment. The main chemicals include ethyl acetate and ethyl (E)-3-hexenoate which recorded by Cavalli et al. [20]. The application of an experimental design with three treatments (labelled, unlabelled and control) is reported in this study for the first time in the study of C. capitata volatile organic compounds. Each detected compounds were comparison column shows the comparisons between different levels that are significant given the p value threshold. Bold points mean significant differences with light points. Significant compounds were acetoin; 2,3-hexanedione; hexaldehyde; o-dimethylbenzene; nonane; butanoic acid, 4-hydroxy; 2,3,4-trithiapentane and octanal.

Citation: AL-Khshemawee H, Agarwal M, Ren Y (2017) Evaluation of Stable Isotope 13C6-glucose on Volatile Organic Compounds in Different Stages of Mediterranean Fruit Fly (Medfly) Ceratitis Capitata (Diptera: Tephritidae). Entomol Ornithol Herpetol 6: 195. doi: 10.4172/2161-0983.1000195
characterized of emission pattern according to NIST library 2014. From these results, we analysed the chemical composition of the volatile organic compounds emitted by different stages of Medfly and effected stable isotope $^{13}$C$_6$-glucose on these chemicals. Also, to understand the communication signals during mating time using stable isotope. This technology can be used for explain the chemicals emitted by Medfly and select these compounds to be existing tools for understanding oviposition, repellence, and attraction of Medfly.

Acknowledgements

We thank Iraqi government (HCED) and Murdoch University for support. We would also like to acknowledge the support of Murdoch University Laboratories for providing technical assistance.

References

1. Duyck PF, Quilici S (2002) Survival and development of different life stages of three Ceratitis spp. (Diptera: Tephritidae) reared at five constant temperatures. Bull Entomol Res 92: 461-469.
2. USDA-APHIS (2006) exotic fruit fly strategic plan FY 2006–2010. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Plant Protection and Quarantine.
3. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/fruit-flies/ct_fruit_flies_home
4. National Fruit Fly Strategy (NFFS) (2010) Prepared by the NFFS Implementation Committee.
5. Bolognesi C, Merlo FD (2011) Pesticides: Human health effects. In: Journal of Njrugu (ed.), Encyclopedia of environmental health, Burlington: Elsevier pp: 438-453.
6. Wang YC, Yang SLi, Yang L, Wang Y, Zhao J (2009) Volatile characteristics of 30 peaches and nectarines evaluated by HS-SPME with GC-MS. Food Chem 116: 356-364.
7. Horvat RJ, Chapman GW, Robertson JA, Meredith Fl, Scorza R, et al. (1990) Comparison of the volatile compounds from several peach cultivars. J Agr F Ch 36: 234-237.
8. Gadi VPR, Angel G (2004) Interactions of insect pheromones and plant semiochemicals. Trends Plant Sci 9: 253-261.
9. Seitz LM, Ram MS (2004) Metabolites of lesser grain Borer in grains. J Agric Food Chem 52: 898-908.
10. Arnaud L, Lognay M, Verscheure L, Leenaers C, Gaspar H (2002) Is dimethyldecanal a common aggregation pheromone of Tribolium flourbeetles? J Chem Ecol 28: 523-532.
11. Villaverde ML, Juarez MP (2007) Detection of Tribolium castaneum (Herbst) volatile defensive secretions by solid phase microextraction-capsillary gas chromatography (SPME-CGC). J Stored Prod Res 43: 540-545.
12. Campbell JF, Toews MD, Arthur FH, Arbogast RT (2010) Long-term monitoring of Tribolium castaneum in two flour mills: seasonal patterns and impact of fumigation. J Econ Entomol 103: 991-1001.
13. Weston PA, Rattingouind PL (2000) Progeny production by Tribolium castaneum (Coleoptera: Tenebrionidae) and Orzyaephalus surinamensis (Coleoptera: Silvanidae) on maize previously infested by Sitotrogacerealella (Lepidoptera: Gelechiidae). J Econ Entomol 93: 533-536.
14. Baker R, Herbert RH, Grant GG (1985) Isolation and identification of the sex-pheromone of the Mediterranean fruit fly, Ceratitis capitata (Wied.). Chem Commun pp: 824-825.
15. Jang EB, Light DM, Flath RA, Nagata JT, Mon TR (1989) Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata to identified volatile constituents from calling males. Entomol Exp Appl 50: 7-19.
16. Jacobson M, Ohinata K, Chambers DL, JonesWA, Fujimoto MS (1973) Insect sex attractants. Isolation, identification, and synthesis of sex pheromones of male mediterranean fruit fly. J Med Chem 16: 248-251.
17. De Meyer M, Freidberg A (2006) Revision of the subgenus Ceratitis (Pierandrus) Bezzi (Diptera:Tephritidae). Isr J Entomol 35: 197-315.
18. Al-khshemawee H, Manjee A, Xin D Yonglin R (2017) Detection of mediterranean fruit fly larvae Ceratitis capitata (Diptera: Tephritidae) in different types of fruit by hs-SPME GC-MS method. J Biosci Med 5: 154-169.
19. Delatte H, Virgilio M, Simiand C, Quilici S, Nzogela YB, et al. (2013) Isolation and characterisation of sixteen microsatellite markers amplifying an African agricultural pest, Ceratitis cosyra (Walker) (Diptera: Tephritidae). Conservation Genetics Resources.
20. Cavalli J, Fernandez X, Lizzani-Cuvelier L, Loiseau A (2003) Comparison of static headspace, headspace-solid phase microextraction, headspace sorptive extraction, and direct thermal desorption techniques on chemical composition of french olive oils. J Agric Food Chem 51: 7709-7716.
21. Virgilio M, Delatte H, Quilici S, Backeljau T, Meyer MD (2013) Cryptic diversity and gene flow among three African agricultural pests: Ceratitis rosa, Ceratitis fasciventris and Ceratitis ananae (Diptera, Tephritidae). Mol Ecol 22: 2526-2539.
22. Aluja M, Norrbom AL (2001) Fruit flies (Tephritidae) phylogeny and evolution of behavior. CRC Press LLC, Boca Raton, pp: 794-809.
23. Alfaro C, Vacas S, Zarzio M, Navarro-Llopis V, Primo J (2011) Solid phase microextraction of volatile emission of Ceratitis capitata (Wiedemann) (Diptera: Tephritidae): Influence of fly sex, age, and mating status. J Agric Food Chem 59: 298-306.
24. Puriswaran D, Borden JH (2004) New repellent semiochemicals for three species of Dendroctonus (Coleoptera: Scolytidae). Chemoecology 14: 67-75.
25. Baratelia V (2009) Development of a continuous small-rearing technique on natural host for the olive fruit fly Bactrocera oleae (Rossi) and study on volatile organic compounds (VOCs) emitted by Olea europaea L. as potential host allelochemics. PhD thesis. Department of Plant Protection, University of Tuscia, Italy.
26. Mavraganis VG, Liaropoulos C, Papadopoulos NT, Kouloussis NA, Broumas T (2008) Whole body extract of Mediterranean fruit fly males elicits high attraction in virgin females. Entomol Exp Appl 127: 20-29.
27. Flath RA, Jang EB, Light DM, Mon TR, Carvalho LA, et al. (1993) Volatile pheromonal emissions from the male Mediterranean fruit fly: effects of fly age and time of day. J Agric Food Chem 41: 830-837.

Citation: AL-Khshemawee H, Agarwal M, Ren Y (2017) Evaluation of Stable Isotope $^{13}$C$_6$-glucose on Volatile Organic Compounds in Different Stages of Mediterranean Fruit Fly (Medfly) Ceratitis Capitata (Diptera: Tephritidae). Entomol Ornithol Herpetol 6: 195. doi: 10.4172/2161-0983.1000195