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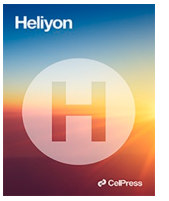

Research article

Unveil the sugar diet and associated environmental compounds in the crop of the mosquito *Culex pipiens*

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A R T I C L E I N F O

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A B S T R A C T

Culex pipiens (Linnaeus, 1758) mosquitoes search plant sources of sugars to cope with the energetic demand of various physiological processes. The crop as part of the digestive system is devoted to the storage of sugar-based meal obtained from various nectars sources. The profiling of sugars and metabolites in the *Culex pipiens*' crop is scarce, and only few studies used Liquid Chromatography – Mass Spectrometry (LC-MS), which provides broad detection for biomonitoring environmental substances and even contaminants in the sugar diet of mosquitoes populations.

Therefore, sugar and metabolite profiling were performed on crops obtained from mosquitoes exposed to plant nectar under laboratory or natural conditions by Ultra High-Performance LC-MS (UHPLC-MS). This method allowed us a precise quantitative and qualitative identification of sugar diet and associated environmental compounds in the crop of the mosquito *C. pipiens*. Under laboratory condition, mosquitoes were allowed to feed on either glucose solution, commercially-available flowers or field collected flowers. In addition, we collected mosquitoes from the field to compare those crop metabolomes with metabolome patterns occurring after nectar feeding in the lab.

The sugar quantities and quality obtained from the crops of mosquitoes collected in the field were similar to those crops obtained from mosquitoes that fed on commercially-available flowers and from field collected flowers with a limit of detection of 10 μg/L for sucrose, glucose and sucrose. Next to sugar compounds, we identified 2 types of amino acids, 12 natural products, and 9 pesticides.

Next to the diversity of sugar compounds, we could confirm that secondary metabolites and environmental pollutants are typically up taken from floral nectar sources by *C. pipiens*. The in-depth knowledge on mosquito–plant interactions may inspire the development and further optimization of mosquito trap systems and arboviral surveillance systems.

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1. Introduction

Mosquito-borne diseases and biodiversity are strongly interconnected given the diversity of pathogens of human and veterinary medical significance and the involvement of a myriad of species and genetic backgrounds of vectors and hosts. The enormous complexity in biodiversity-health-environment interactions is a major challenge when designing vector control strategies at local and global level. However, biodiversity can also provide inspiration for new surveillance tools such as FTA-card based arboviral surveillance [1] and vector control tools such as attractive toxic sugar bait traps [2]. The efficiency of those systems needs to be optimized [3] in terms of sugar source that reliably attracts the mosquitoes, because sugar sources from natural vegetation are likely to outcompete the sugar-based trap systems.

Mosquitoes rely on plants as a source of sugars, amino acids and other nutrients found in the floral nectars, honeydew and decayed fruits [4]. Sugar-based meals uptaken by mosquitoes contain a mix of different sugars such as sucrose, fructose and glucose, which are used as their principal source of energy [5]. After ingestion, the sugar-based meal is stored in the crop (*ventral diverticulum*), and subsequently sugars are metabolized to hexose phosphates and enter to catabolism or anabolism pathways [6].

These macronutrients are fundamental for survival, reproduction and others physiological activities of the mosquito [7]. For example, nectar diet is needed for the synthesis of sugars to trehalose, which accumulates in the hemolymph for the subsequent glycogen synthesis and storage in the muscle as an energy reserve [6]. Blood diet, which is restricted only to female mosquitoes, is essential for ovarian and egg development [6], but the blood is stored in the midgut (medium intestinal section), a different anatomical compartment.

Plant characteristics such as the diversity of floral nectar compounds, the quantity of pollen, and the presence of extrafloral nectar and honeydew has proved to be important for the insect physiology of moths, bees, butterflies, mosquitoes and others insects [8,9]. In addition, plant characteristics such as the presence or absence of floral nectar, deeply impact the attractiveness of insects to the plants [9]. A recent study aimed to investigate the attraction of *Culex pipiens* Linnaeus, 1758 to fruit-based sugar baits shows significantly differences against the 5% of sucrose [10]. *Culex* mosquitoes play a key role in transmission of zoonotic arboviruses such as West Nile virus (WNV - Flaviviridae; Flavivirus) [11] and Rift Valley fever phlebovirus (RVF – Phenuiviridae; Phlebovirus) [12] around the globe [13,14]. Despite the major importance of *C. pipiens* for public health [15], there isn't so far an in depth understanding about its sugar diet, the interaction of mosquitoes with plant biodiversity via sugar feeding and its role for public health [16].

In the present study, we investigate the sugar diet and metabolomic profile of field-collected mosquitoes and mosquitoes artificially fed under laboratory condition by analysing their crop content using Ultra High-Performance Liquid Chromatography – Mass Spectrometry (UHPLC-MS). We hypothesize, that i) the floral sugar source determines the sugar quantity and sugar metabolome in the mosquito crop and differs between lab fed mosquitoes and field collected mosquitoes; and ii) next to sugar compounds, secondary metabolites and environmental pollutants are up taken from nectar sources as well.

To test our hypothesis, we analysed the chemical compounds present in the crop of *C. pipiens* after artificial feeding on six different

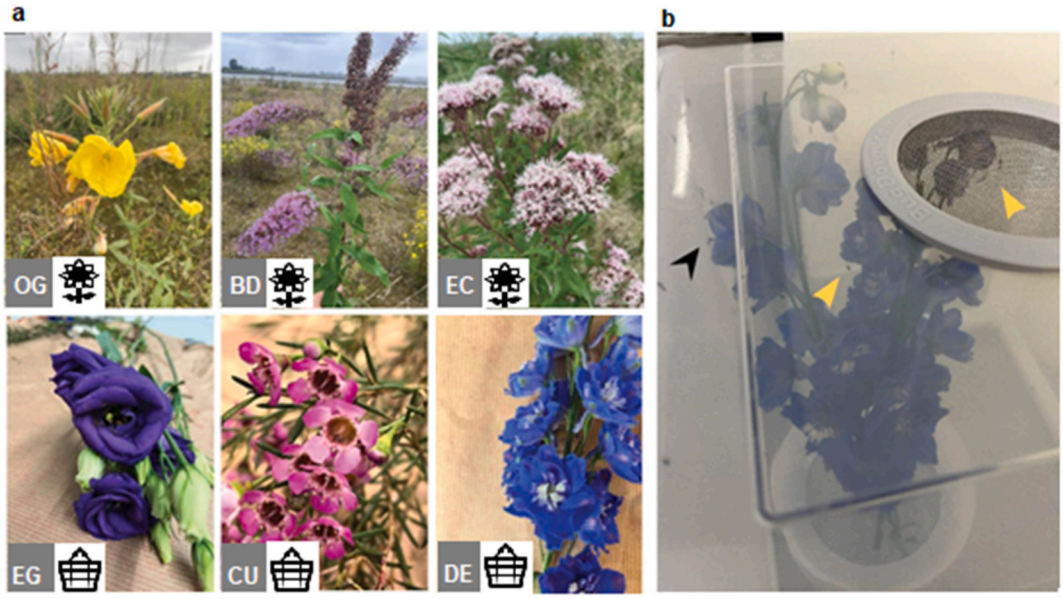

Fig. 1. Flower plants used in the artificial feeding bioassays with *Culex pipiens*. a) Field collected flower *Oenothera glazioviana* (OG), *Buddleja davidii* (BD) and *Eupatorium cannabinum* (EC) from Antwerp Zuidand ornamental flowers; *Eupatorium grandiflores* (EG), *Chamelaucium uncinatum* (CU) and *Delphinium elatum* (DE) bought at the flower shop. All flowers were chosen based on the presence of floral and extra floral nectars, pollen, inflorescences, night-time opening and freshness. b) Experimental setup where flowers were placed into BugDorm-1 Insect cages and fifty mosquitoes were allowed to feed on each plant species for 24 h. Yellow and black arrowheads indicate female and male mosquitoes, respectively, during feeding.

plants in the laboratory or from field collected *C. pipiens*. Our result demonstrates the significance of different sugar sources for the quantity and quality of sugar compounds and the presence of amino acids, natural products and pollutants in the mosquito's crop; obtaining a database will help to future research focused on the mosquito's food preference and to develop attractive sugar traps [17].

2. Materials and methods

2.1. Artificial feeding assays on floral nectar from six plant species

Culex pipiens were raised in climatic chambers (Rumed; Germany) in the Merian insectary of the Institute of Tropical Medicine of Antwerp (Belgium) under the following conditions: 23 °C, 80% relative humidity and a light cycle of 12 h light: 12 h darkness.

A selection of six different plants was used for artificial feeding assay on floral nectar: *Oenothera glazioviana* (OG), *Buddleja davidii* (BD) and *Eupatorium Cannabinum* (EC) were collected from the field in Antwerp Zuid (51°12′21.9″N 4°22′46.9″E) during the last week of August 2021; whereas *Eustoma grandiflores* (EG), *Chamelaucium uncinatum* (CU) and *Delphinium elatum* (DE) were purchased at a local flower shop (Fig. 1a). The selection of plants was done based on the following criteria of mosquito-attractive flowers [16,18,19]: 1) presence of floral and extra floral nectars, 2) presence of pollen, 3) with inflorescences, 4) opened at night and 5) freshness. The plants selected and collected from the field in Antwerp Zuid were chosen due to their higher abundance in the riverbank area, and following the aforementioned floral criteria.

The plants were transported to the ITM insectary and used as sugar-source in the artificial feeding assay. Each experimental plant was placed into a separate mosquito cage. The flowering plants were placed in a plastic jar filled with water and then transferred to an individual mosquito cage (30 cm × 30 cm x 30 cm) (Bugdorm, Taiwan) (Fig. 1b). Jar mouths were closed with parafilm to prevent the mosquitoes to access the water during the experiment. Female mosquitoes were starved for 24 h before the start of the experiment to avoid possible interference with the normal sugar diet of the colony (10% glucose, 0.1% methylparaben).

After starvation, fifty 5–7 days old female mosquitoes were added to each cage. Mosquitoes were allowed to feed on the floral nectar for 24 h. Afterwards, the mosquito crops were dissected as previously described [20]. Each crop was placed into an individual Eppendorf tube filled with 100 μl of 100% ethanol molecular grade and stored at −20 °C. Mosquitoes fed with 10% of glucose solution were used as quality control group (QC; n = 10). The samples of three experimental groups: 1) mosquitoes fed with 10% of sucrose are quality control (QC); 2) mosquitos fed with ornamental plants purchased at local flower shop *Eustoma grandiflores* (EG), *Chamelaucium uncinatum* (CU) and *Delphinium elatum* (DE) and 3) plants collected in the field *Oenothera glazioviana* (OG), *Buddleja davidii* (BD), *Eupatorium cannabinum* (EC) were sent to the Universidad Peruana Cayetano Heredia (Lima, Peru) for further downstream analysis (Fig. 2).

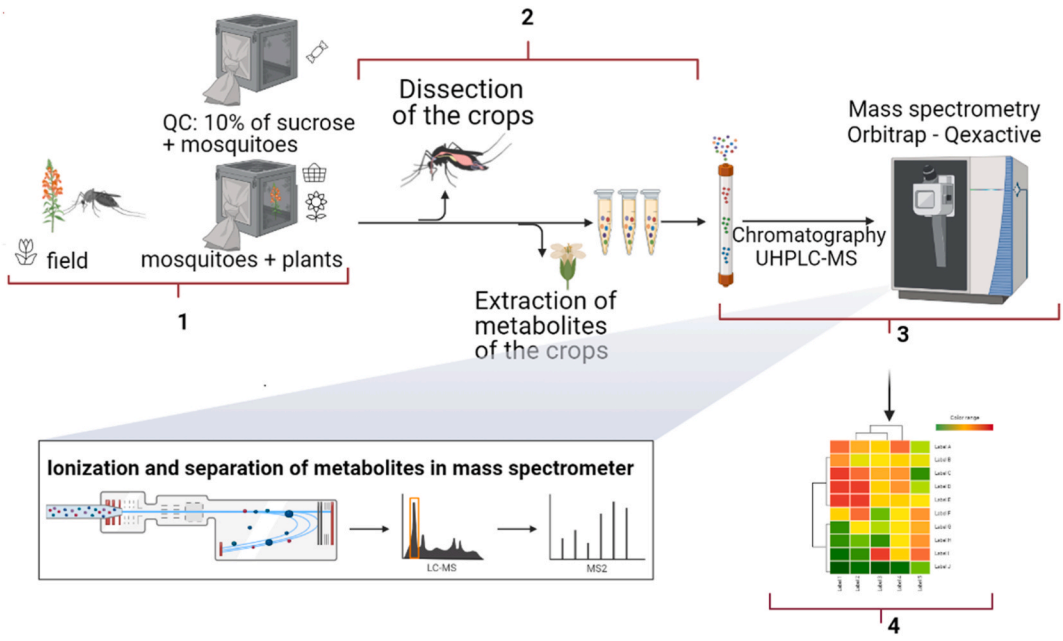

Fig. 2. Flowchart of metabolomic analysis. The process consists of four parts: 1) sample collection in the field and artificial feeding bioassay with three different feeding sources, which are mosquitos fed with plants collected in the field, mosquitoes fed with ornamentals plants purchased at local flowers shop and mosquitoes fed with 10% of sucrose to quality control, 2) preparation of sample, dissection of the mosquitoes' crops and chemical extraction with solvents of the metabolites' crop, 3) chemical analysis by UHPLC-MS technique with NH2 amine column and 4) statistical analyses of data. Mosquito internal organs related to feeding are coloured in (2) only for representation.

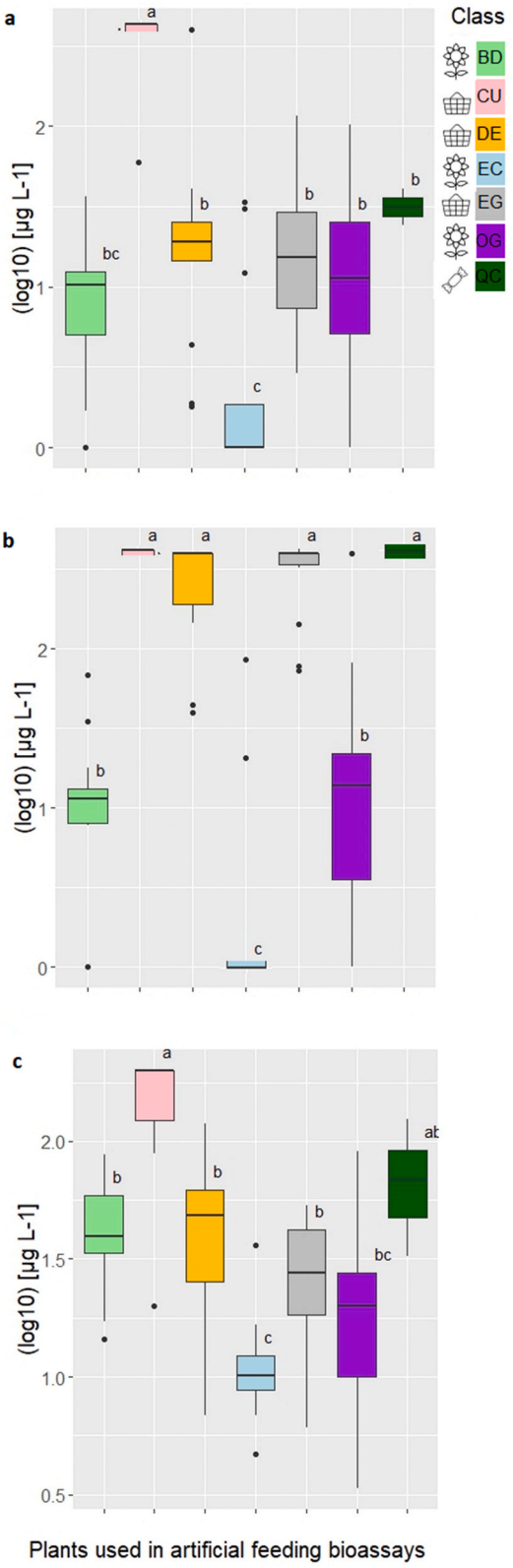

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Fig. 3. Quantity of a) fructose, b) glucose and c) sucrose measured in the crop of *Culex pipiens* after artificial feeding bioassays via UHPLC-MS. Plants used in the bioassays were either collected in the field *Oenothera glazioviana* (OG), *Buddleja davidii* (BD), *Eupatorium cannabium* (EC); or ornamental plants purchased at the local flower shop *Eustoma grandiflores* (EG), *Chamelaucium uncinatum* (CU), *Delphinium elatum* (DE). The crops dissected from mosquitoes fed with 10% of glucose have been used as quality control (QC). The concentration is expressed as the $\log_{10}$ of [$\mu g\ L^{-1}$]. Different letters show significant differences between the experimental groups via ANOVA and Tukey post-hoc test (Supplementary Tables 1–3).

2.2. Field collected mosquito samples

Culex pipiens mosquitoes were collected in a different area of Antwerpen (Belgium) using BG-Sentinel trap (Biogents, Germany) equipped with BG-Lure. The trap was placed at 6:00 p.m. and harvested at 6:00 a.m. The mosquito crop was dissected from 4 male and 6 female *C. pipiens* as described in Ref. [20]. Crop samples were prepared and transported as described above.

2.3. Sample preparation for ultra-high performance liquid chromatography

Sixteen crops by each experimental group were analysed by quantitative to sugar compounds analysis and eight crops by each experimental group for qualitative (metabolomic profile) analysis of and nine crops obtained from field-collected mosquitoes for both analysis (qualitative and quantitative). The samples were place into individual 0.5 ml reaction tubes with 30 μl of acetonitrile hyper grade solvent for LC-MS (Merck Reag Ph, Europe) and centrifuged to 14000 rpm for 15 min. The supernatant was transferred into a 0.5 ml reaction tube with 300 μl of methanol grade LC-MS (Merck)-ultrapure water (1:2) v/v. The samples were filtered with poly-vinylidene fluorid filter and each transferred to an insert of 200 μl.

2.4. Mass spectrometry measurement, spectral data processing

Ultra-high performance liquid chromatography Orbitrap Exactive – Thermo Scientific (UHPLC-MS) was performed using a NH_4 column apHera 15 cm × 4.6 mm (Supelco Analytical– Sigma Aldrich). UHPLC parameters were: 40 °C temperature column, 10 μl injection volume, 0.3 ml/min flow injection, isocratic mode (ACN: H2O/70:30) and 19 min total retention time. Mass spectrometer parameters were: 2.5 KV spray voltage, 10 eV collision energy, negative mode ESI, 100 °C capillary temperature, 250 °C aux gas heater temperature, and a resolution of 70000.

The limit of quantification for the instrument was established with glucose, sucrose and fructose standards (Sigma Aldrich – Merck) and calibration curves for each of the main sugars. The range of calibration curve was from 25 to 200 μg/L for sucrose and from 50 to 400 μg/L for glucose and fructose, respectively. The lower limit of quantification was 9.82 μg/L glucose, 8.05 μg/L fructose and 10.69 μg/L sucrose, and the lower limit of detection was 2.95 μg/L glucose, 2.42 μg/L fructose and 3.2 μg/L sucrose.

2.5. Statistical analysis

Quantitative analysis of sugars such as sucrose, fructose and glucose were performed using Quan Browser of Excalibur (version 3.1) (Thermo Fisher Scientific, 2013). The sugar content values found in the crop of the mosquitoes from artificial feeding bioassays was normalized as following: (1) row wise normalization: Normalization to sample median; 2) data transformation: log 10 normalization; 3) data scaling: autoscaling (mean-centered and divided by standard deviation of each variable) [21] and compared between experimental groups by means of univariate one – way ANOVA.

Qualitative analysis, which includes identification of peak detection, retention time alignment, peak matching, signal filtering, noise removal (intensity), gap filter of raw data was evaluated with XCMS (version 3.7.1). The identification of chemical compounds was based on the spectra comparison of each chromatogram peak found for each metabolite identified using the Metlin database as reference for known metabolites. We detected 258 chemical compounds to which we applied a 1,000,000-intensity threshold, and the remaining were identified after filter processing with databases. Hierarchical clustering and heat map was used for cluster analysis between experimental groups and their chemical compounds identified, results shown as heatmap using Euclidean distance, and Ward's linkage were evaluated with MetaboAnalyst (version 5.0). The relationship analysis of sugar detected in plants with those found in the crop of the mosquitoes has been analysed by a principal components analysis (PCA), and the relationship of metabolomic profiles between plants and chemical compounds found in the crop of the mosquitoes has been analysed also by PCA. ANOVA and PCA statistics were run with R studio (version 4.2.0; R core packages) and graphics plotted with package ggplot2 version 3.3.6.

3. Results

3.1. Sugar content in the crop of Culex pipiens

The amount of sugar in the crop of *C. pipiens* ranges between 4.67 and >400 μg/L. The principal difference between experimental groups is the sugar-source feeding assays, the amount of glucose is highest in the crops after feeding on commercially purchased CU, DE and EG. The poorest sugar diet was associated with feeding on EC. The richest sugar diet was associated to the feeding on CU, which is cultivated widely for its large lasting attractive flowers. Specifically, the amount of fructose and sucrose is highest in the crops after feeding on commercially purchased CU (Fig. 3a and c). Fructose shows a significant difference between experimental groups feeding

on either EC and CU ($p < 0.001$) (Fig. 3a). Glucose shows a significant difference among BD, EC and OG ($p < 0.001$) (Fig. 3b) and sucrose shows significant difference among CU and EC ($p < 0.001$) (Fig. 3c–Supplementary Tables 1–3). We observed that FS had a higher proportion of glucose, 2.5-fold higher than fructose and 5-fold higher than sucrose, similar pattern is only observed on OG crop samples (Table 2).

Sugar amounts up taken during the artificial feeding bioassays resemble the sugar quantities observed in field collected mosquitoes, though a proper statistical comparison of data collected in the laboratory and from field-collected mosquitoes cannot be made due to unknown time exposure of metabolic processes from the field collected mosquitoes.

3.2. Crop content of *Culex pipiens* after feeding on various plant nectar sources

The PCA plot of quantitative analysis shows the relationship between ornamental plants for glucose between DE, EG and field sample (FS) while the other sugars don't present this relationship with single plants (Fig. 4). Crop contents of the majority of mosquitoes fed with ornamental plants do group with the crop contents of field sample (FS).

The PCA for metabolomic profile shows a wider range of values for the FS group over both axes, partially overlapping its ellipse with the CU and DE groups, while BD and OG group tightly together and the rest groups partially together based on the range of their ellipses (Fig. 5).

The clustering for the metabolomic profile found in the mosquito's crops demonstrate two distinct clusters: the first including DE, EG and CU with field samples FS and the second clustering together OG and BD, same as in PCA; and with close clustering to field samples due to a group of chemical compounds with high intensity (Fig. 6).

3.3. Secondary metabolites uptaken during sugar meal

In the crops of *C. pipiens*, we found a number of sugar compounds (n = 11), natural compounds of the plants (n = 12), amino acids

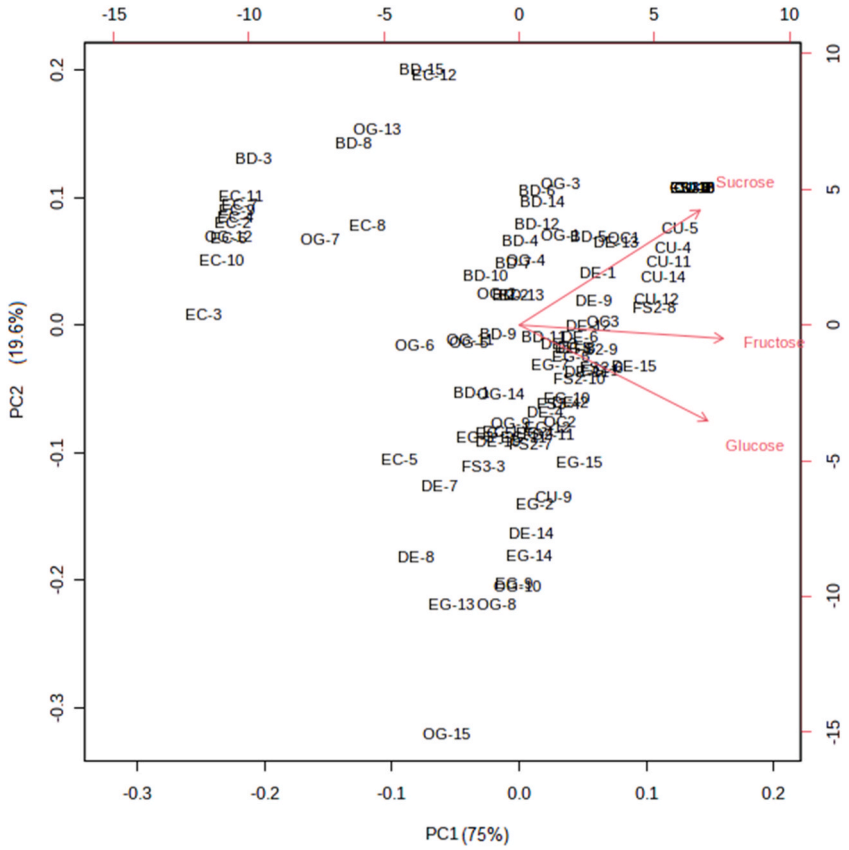

Fig. 4. Relationship between three types of sugars in the crops of *Culex pipiens* afterfeeding on different plant nectar sources. Principal Component Analysis biplot calculated from amount of sucrose, fructose and glucose as measured in the crop of *Culex pipiens* after feeding on plants used in plants the artificial feeding bioassays and the crops from field collected mosquitoes FS. Mosquitoes fed with plants collected in the field *Oenothera glazioviana* (OG), *Buddleja davidii* (BD), *Eupatorium cannabium* (EC) and mosquitoes fed with ornamental plants purchased at local flower shop *Eustoma grandiflores* (EG), *Chamelaucium uncinatum* (CU), *Delphinium elatum* (DE) and quality control QC mosquito fed with 10% of sucrose. Red arrows show vector contribution of each variable.

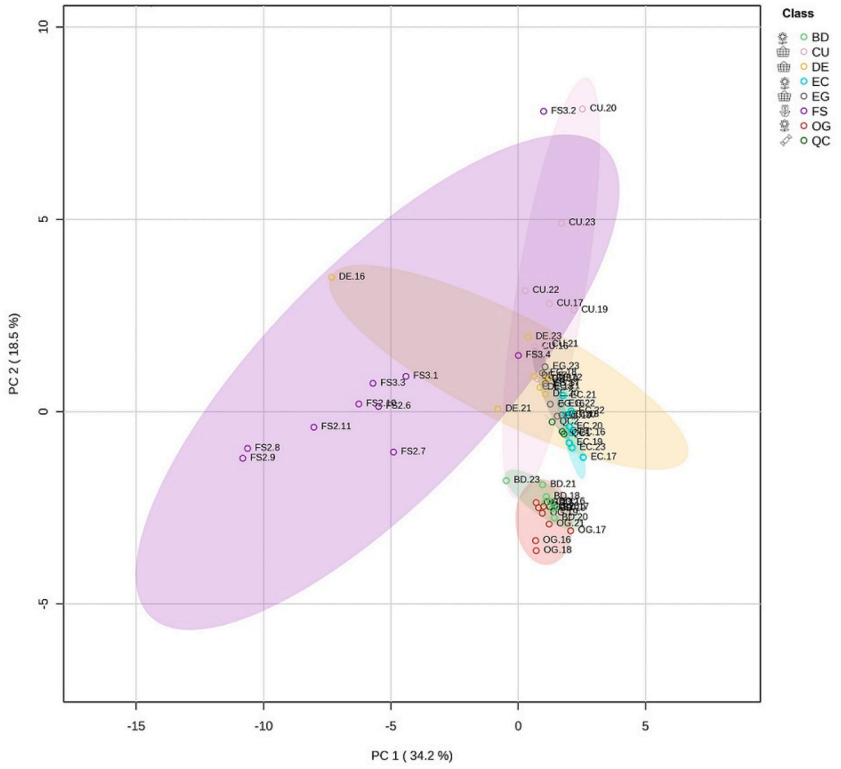

Fig. 5. Relationship between all metabolites found in the mosquito crops after feeding on different plant nectar sources. Principal component analysis calculated from the 62181 *m/z* of chemical compounds present in the crop of *Culex pipiens* after feeding on experimental plants and the crops from field collected mosquitoes (FS). Mosquitoes that fed on plants collected in the field *Oenothera glazioviana* (OG), *Buddleja davidii* (BD), *Eupatorium cannabium* (EC) or fed on ornamental plants purchased at local flower shop *Eustoma grandiflores* (EG), *Chamelaucium uncinatum* (CU), *Delphinium elatum* (DE). Ellipses represent 95% range of the sample group range.

(n = 2) and environmental pollutants (pesticides; n = 9) (Table 1). The sample size for this experiment was 8 samples for each experimental group. The metabolic profiles of the crops of *C. pipiens* show a broad chemical diversity of ingested compounds, however there is similitude found among the sugars when fed with CU and EG and those found in the mosquito's crops collected in the field (Fig. 6). Both, the natural compounds and pesticides have been detected in every of the experimental groups, that means in the crops after feeding of adult mosquitoes to either plants that have been collected in the field, purchased in the flower shop or to wild floral nectar from a field. The amino acid L-tyrosine has been solely detected in the commercially purchased CU and the amino acid L-glutamine solely in the commercially purchased DE and the field collected samples FS.

4. Discussion

State-of-the-art methodology ensuring a reliable low detection limit and high resolution for sugar compounds via UHPLC-MS allowed us to dissect the diet component by analysing the crops of *C. pipiens*. In previous studies, the total carbohydrates of the mosquito diets were analysed via cold anthrone test [22–24], whereas proteins, lipids, and other nutrients also including carbohydrates were analysed by gas chromatography - mass spectrometry (GC-MS) through a derivatization process [25–28]. In other studies, a number of environmental pollutants such as pesticides were detected in the crop and midgut of the mosquitoes [29]; as well as in fruits and plants [30,31] by liquid chromatography tandem mass spectrometry (LC-MS/MS). Considering the studies mentioned above and the simplest and most convenient method for sample preparation [32], LC-MS is a most appropriate technique for determining altogether sugars and other chemical compounds in mosquito' crops.

Here we show that the amount of sugars present in the crop of *C. pipiens* ranges from 4.67 to >400 μg/L, enhancing the limit of detection in comparison to what previous described by Muller and Schlein (5.29–47.72 μg/μl) [8]. The amount and composition of glucose compounds harvested from mosquito crops of the artificial feeding assays may vary according to the characteristics of the plants as well as the availability of floral nectars.

The mosquitoes are attracted by a diversity of plants and their flower characteristics such as floral nectar, honeydew excretions, nectaries on leaves and stems, damaged fruit [16] and other volatile chemical compounds that mosquitoes can perceive with their sensitive chemoreceptors [18,33,34]. The availability in quantity and quality of sugars and other compounds depends on the plant species [8]. In our study the metabolomic profiles identified in the mosquito's crops derived from artificial feeding assays is

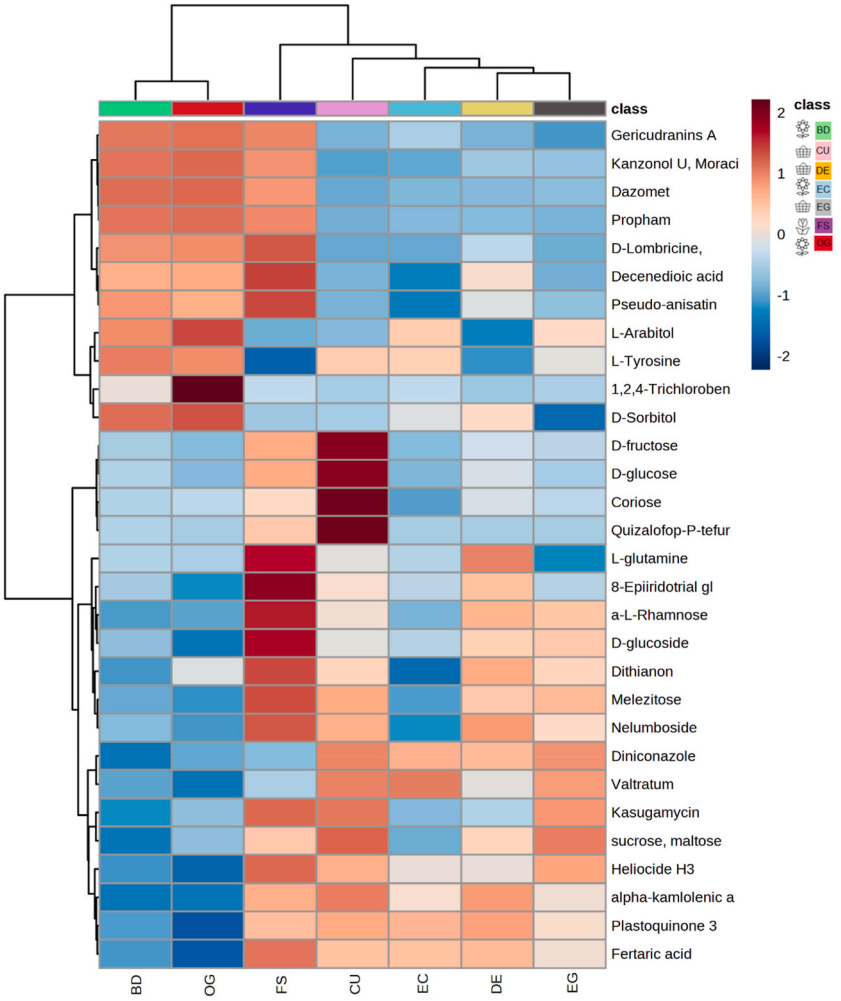

Fig. 6. Cluster analysis of a metabolomic profiles identified in the crops of *Culex pipiens* after feeding on plant nectar. The higher relative intensity was 141,996,256 for crop content harvested from female mosquitoes after feeding on experimental plants or from field collected mosquitoes. Cluster analysis allows us to evaluate the intensity of metabolites found in each experimental group. The range [-2; 2] represents the intensity of metabolites. The cluster analysis by metabolites reveals 2 groups with low and high intensity metabolites. In the first group we have BD and OG which have a common cluster of compounds with high intensity by heat map analysis that are also shared with FS; this shows a relationship in chemical compounds which are gericudranins A, kanzonol, dazomet, propham, D-lombricine, decenediodic acid, pseudo-anisatin, L-arabitol and L-tyrosine. The second samples group presents an inverted relation of high and low intensity compounds by heat map analysis; it shows a relationship between CU and FS for sugars D-fructose, D-glucose, coriose and quizalofop-P tefur; and a relationship between FS and DE for L-glutamine, 8 epi-ridotrial glucoside, a-L rhamnose, D-glucoside, dithianon, melezitose and nelumnoside.

comparable with those obtained from the crop of field collected mosquitoes. The results demonstrated that the artificial feeding assay in the laboratory coupled with our analytical method build a powerful toolbox to accurately replicate and analyse feeding behaviour of mosquitoes found in nature.

The metabolomic profile of mosquito crops shares several similarities after feeding on commercially purchased *Chamelaucium uncinatum* and *Delphinium elatum,* or on unknown field vegetation in an urban setting in Belgium. According to our results, we recommend these three plants as proxies for future studies about selection or feeding behaviour feeding and/or design of mosquito's trap bait: *Eustoma grandiflores* with high concentration of glucose, *Chamelaucium uncinatum* and *Delphinium elatum* with high concentration of sucrose and glucose. These plants were purchased for the bioassay, and they were selected because of the presence of floral and extrafloral nectar in their stem, leaves and flowers.

Previous studies about sugar feeding preferences and ratio (6:4:4) in mosquitoes conclude that sugars such as sucrose, glucose, fructose are common sugars which attract *Anopheles gambiae* and *Culex pipiens* mosquitoes [8,35]. Nonetheless, the field sample group (FS) showed a large variation in sucrose but a rather narrower range for fructose and glucose, which will suggest a feeding behaviour trend of *C. pipiens* for these two sugars in a 0.25:0.61 ratio (Table 2).

Two amino acids L-tyrosine and L-glutamine have been detected in the crops of mosquitoes fed with *Chamelaucium uncinatum*,

Table 1
Main secondary metabolites detected in *Culex pipiens*' crop.

Name of compound	ID Metlin	Mosquito sugar source								Commercial classification
		OG	BD	EC	EG	CU	DE	FS	QC	
D- glucose	133				x	x		x	x	Sugars
D-fructose	135				x	x		x	x	Sugars
Melezitose	43984							x		Sugars
D-galactose	134					x				Sugars
Coriose	73263					x				Sugars
a-L-Rhamnose	1672				x	x	x	x		Sugars
D-sorbitol	143					x		x		Sugars
L-Arabitol	141	x						x		Sugars
Ribitol	316					x				Sugars
D-glucoside	6958	x						x		Sugars
Sucrose	137				x	x			x	Sugars
Mulberrofuran C	67277			x	x	x	x	x		Natural compound
Pseudo-Anisatin	985609							x		Natural compound
Fertaric acid	86082			x				x		Natural compound
alpha-kamlolenic acid	35492				x	x	x			Natural compound
Decenedioic acid	5578	x	x					x		Natural compound
Valtratum	41160			x	x	x	x			Natural compound
Nelumboside	93152	x	x	x	x	x	x	x		Natural compound
Moracin D	89166	x	x					x		Natural compound
Gericudranins A	53177	x	x					x		Natural compound
Ikarisoside A	50126	x	x					x		Natural compound
Plastoquinone 3	89533			x	x	x	x	x		Natural compound
8-Epiiridotrial glucoside	41168							x		Natural compound
Heliocide H3	87167				x	x	x	x		Natural compound
L-Tyrosine	34					x				Amino acid
L-glutamine	18						x	x		Amino acid
Kasugamycin	71964				x	x	x			Pesticide
Propham	72648	x	x					x		Pesticide
1,2,4-Trichlorobenzene	66446	x		x						Pesticide
D-Lombricine	63423	x	x					x		Pesticide
Dithianon	72377	x	x			x	x	x		Pesticide
Quizalofop-P-tefuryl	72536					x				Pesticide
Dazomet	72263	x	x					x		Pesticide
Diniconazole	72496			x	x	x	x			Pesticide

Table 2
Ratio of three sugars found in the crop of the mosquito's crop. Ratio of fructose (Fru), glucose (Glu) and sucrose (Suc) in the crop of the mosquitoes fed with plants purchased in the flower shop (EG, CU. DE), crops of the mosquitoes collected in the field (FS) and crops fed with plants collected in the field.

		Fru	Glu	Suc
Eustoma grandiflores		0.07	0.85	0.08
Chamelaucium uncinatum		0.40	0.43	0.17
Delphinium elatum		0.11	0.77	0.12
Field sample		0.25	0.61	0.13
Oenethera glazioviana		0.18	0.58	0.24
Buddleja davidii		0.16	0.20	0.64
Eupatorium cannnabinnum		0.23	0.32	0.45

Delphinium elatum and collected from field sample (FS). These amino acids are necessary and critical for the production and maturation of egg chorion in mosquitoes [36]. L-tyrosine and L-glutamine are also crucial for the synthesis of protein, and L-tyrosine is an intermediary compound for melanin synthesis which is essential for egg melanization and increase the hatching rate [37]. The presence of these identified amino acids suggests a positive/attractive characteristic in the mosquito's choice of flowers, that should be considered for future experiments such as testing reproductive fitness [38].

Nonetheless, further examination of optimal sugar ratios and the possible role of secondary compounds for the ecophysiology and attractiveness of sugar sources is required for future applications in vector control strategies. To test the attraction of the plants, Y-maze assays should be performed with the pumped volatiles from the whole plants and/or flowers, and profiling and testing for the sugar ratios and secondary metabolites with supplemented Eppendorf tubes or 3D printed mock flowers [16].

We identified various pesticides in the mosquito crops. Recently, the insecticide resistance of Belgian *C. pipiens* have been investigated [39]. In this study, the authors showed that *C. pipiens* developed resistance against pyrethroids deltamethrin and permethrin, and possibly against carbamate Bendiocarb. The authors also suggested that the exposition to those or other agricultural pesticides during a sugar meal can be one of the possible drivers of insecticide resistance development. The detection of multiple pesticides in the crops of mosquitoes we obtained in this study support this hypothesis and this pathway for development of insecticide resistance should be further investigated. The pesticides found in our analysis may serve as targets for future studies, due to their relation to resistance in mosquitoes. Thus, it will be convenient to evaluate the concentration of pesticides and its attraction with mixes of secondary compounds (Table 1) from *Eustoma grandiflores*, *Chamelaucium uncinatum* and *Delphinium elatum* plants which shown high concentration of sugars.

To further explore the importance of mosquito-plant interactions, an adequate database on plant nectar profiles is needed. The implementation of this database would provide a powerful tool to identify the diversity of chemical compounds in field collected mosquitoes and would allow to better understand the interaction between medically relevant biodiversity and plant diversity (environment-vector interface) and the potential role of mosquitoes as pollinators. It will also enable the creation of artificial nectar sources to be used in behavioural studies or to utilize the knowledge for a more applicative approach, as for example the construction of attractive toxic-sugar bait traps. Through the glasses of a vector control manager, the impact of sugar diets on the development of insecticide resistance and the variation of vector competence should be further assessed.

In future studies, it will be interesting to address how broad and persistent is the presence of insecticides and other chemo-industry derived compounds.

Culex pipiens mosquitoes were used as model for the identification of sugars and other metabolites to link their source feeding and chemical compounds present in the mosquito's crop. These results will contribute to future research focused on mosquito's food selection or feeding behaviour, its application and the effects of environmental pollution on vector diseases.

5. Conclusion

We successfully quantified and qualified the sugar diet in the crops of the arboviral vector *C. pipiens* providing a baseline for further research on sugar diets of mosquitoes and environmental pollutants. This new field of research will support a better understanding of environment-vector interactions, and provide new insights about the link between biodiversity and health. The crop metabolome of mosquitoes feeding on the commercially purchased plant species *Chamelaucium uncinatum* resembles the crop metabolome of field collected mosquitoes and thus could be used as an ecological sugar source in functional behavioural studies. Next to the diversity of sugar compounds, we could confirm that secondary metabolites and environmental pollutants are typically up taken from floral nectar sources by *C. pipiens*. The in-depth knowledge on mosquito–plant interactions may inspire the development and further optimization of mosquito trap systems and arboviral surveillance systems.

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CRediT authorship contribution statement

Balvina Leyva: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marco Brustolin:** Writing – review & editing, Validation, Supervision, Investigation. **Ruth Müller:** Writing – review & editing, Supervision, Conceptualization. **Felipe Yon:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26565.

ID Metlin is the code of compounds in the METLIN - Metabolite and Chemical Entity Database. X indicates the presence of the respective metabolite in a given experimental feeding group. Mosquito sugar source:  experimental plants purchased in the flower shop (*Eustoma grandifloras* (EG), *Chamelaucium uncinatum* (CU), *Delphinium elatum* (DE)),  field-collected plants ((*Oenothera glazioviana* (OG), *Buddleja davidii* (BD), *Eupatorium cannabium* (EC)),  field-collected mosquitoes with unknown sugar source (FS), or  from the quality control (QC).

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