Entomopathogenic nematodes impact the volatile profile of their insect hosts with a cascading effect on the behaviour of uninfected hosts

Auteur : Hotermans, Pierre
Promoteur(s) : Verheggen, François
Faculté : Gembloux Agro-Bio Tech (GxABT)
Diplôme : Master en bioingénieur : gestion des forêts et des espaces naturels, à finalité spécialisée
Année académique : 2023-2024
URI/URL : http://hdl.handle.net/2268.2/21449

LIÈGE université
Gembloux
Agro-Bio Tech

Entomopathogenic nematodes impact the volatile profile of their insect hosts with a cascading effect on the behaviour of uninfected hosts

HOTERMANS PIERRE

TRAVAIL DE FIN D'ÉTUDES PRÉSENTÉ EN VUE DE L'OBTENTION DU DIPLÔME DE BIOINGÉNIEUR EN GESTION DES FORÊTS ET ESPACES NATURELS

ANNÉE ACADÉMIQUE 2023 - 2024

PROMOTEUR : François Verheggen (Gembloux Agro-Bio Tech)

LIÈGE université
Gembloux
Agro-Bio Tech

Entomopathogenic nematodes impact the volatile profile of their insect hosts with a cascading effect on the behaviour of uninfected hosts

HOTERMANS PIERRE

TRAVAIL DE FIN D'ÉTUDES PRÉSENTÉ EN VUE DE L'OBTENTION DU DIPLÔME DE BIOINGÉNIEUR EN GESTION DES FORÊTS ET ESPACES NATURELS

ANNÉE ACADÉMIQUE 2023 - 2024

PROMOTEUR : François Verheggen (Gembloux Agro-Bio Tech)

Preface

This master thesis was carried out in collaboration between Gembloux Agro-Bio Tech, University of Liège (Sustainable Pest Management Division), and the TERRA research unit (Chemical and Behavioural Ecology). This master thesis is presented in article format. The formatting rules applied follow those of the Journal of Chemical Ecology.

Acknowledgments

With this writing, my journey within the old walls of Gembloux comes to an end. Each year spent here has been filled with emotions, pleasures, and discoveries. For no reason could I imagine pursuing bioengineering studies anywhere other than at Gembloux Agro-Bio Tech. I am grateful for everything I have learned.

First and foremost, I would like to thank Professor François Verheggen for allowing me to carry out my final year project in the Chemical and Behavioural Ecology Laboratory. His valuable advice has been instrumental throughout this period. Next, I would also like to thank the entire laboratory team, especially Clément Martin and Fanny Ruhland, for their support, guidance, and encouragement throughout this journey, which culminates in a well-rounded and satisfying piece of work, largely thanks to you. Thank you also to Elise; I'm very happy we were both TFE students at the same time.

A huge thank you to Thomas Jouant for supporting me throughout this period. Whether it was a listening ear, advice, ideas, or reviews, know that you played a significant role in the completion of this TFE. Thanks also to Laura, without whom the electronics classes and volleyball would have been less exciting.

To you, Dorian, Maxime, Charline, Louise, Amélie, with whom I shared incredible slides and unforgettable moments. From the first year to now, from the first exams to the final project, we're finishing together, and THAT is beautiful.

To Anaïs, Justine, Émilien, Sarah, with whom I spent wonderful moments full of joy and laughter both at the Ag and beyond (special mention to all the Van MeMe!) as well as with Babeth Cabaraux, my dear "cokotteuse"!

I would also like to thank the Ag 2023 committee. Experiencing student life from the other side was incredible, and I'm glad I shared it with you! To our discussions and our weekend! How can we talk about student life without mentioning the grand Bar de l'Ag and everyone who is part of it? Each artistic figure was crafted with precision (e.g., Dark Doudou). When the pig and the bird met, anything could happen. "Team Soft, ohé ého…"

Thank you also to you, Lola; your presence and help were instrumental in reaching the finish line. That Italian breath of fresh air will forever be etched in my memory. A Focaccia?

Finally, I would like to end these acknowledgments with a special mention to my family (with a warm thought for Lo and Jean-Marc) and especially to my parents, without whom living these years at Gembloux would not have been possible. Saying thank you would be an understatement compared to all the support and trust you have given me.

Résumé

Les nématodes entomopathogènes (EPN) sont de plus en plus utilisés comme alternatives biologiques aux insecticides pour la lutte contre les ravageurs. Les juvéniles infectieux libèrent des bactéries mutualistes qui se multiplient rapidement et tuent l'insecte hôte. Malgré de nombreuses recherches sur les interactions entre les EPN et les insectes, il reste incertain si les infections par les EPN modifient les composés organiques volatils (COV) émis par l'hôte, pouvant potentiellement influencer le comportement des insectes non infectés. Cette étude a testé deux hypothèses : (1) les nématodes entomopathogènes modifient les émissions de composés organiques volatils de leur hôte insecte, et (2) ces émissions volatiles attirent les insectes non infectés, augmentant ainsi la dispersion des EPN.

En utilisant des larves de *Galleria mellonella* et deux espèces d'EPN (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*), les COV ont été collectés post-mortem en utilisant la méthodologie Hisorb et analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse. Des tests comportementaux, incluant des essais de pièges, de préférence et de temps d'évasion, ont évalué les réponses des insectes non infectés aux larves infectées.

Les résultats ont montré que les larves infectées par les EPN émettent des profils de COV significativement différents par rapport aux larves tuées par d'autres moyens, avec un taux de réussite de 100 % pour distinguer ces profils. Les tests comportementaux n'ont pas révélé d'effet d'attraction mais ont montré une réduction significative du temps d'évasion lorsque les insectes non infectés étaient exposés à des larves infectées au début du cycle de vie des EPN. Des composés sulfurés, typiques de la décomposition, ont été détectés dans les profils de COV. La variation des COV au cours du cycle reproductif des EPN pourrait être due aux bactéries symbiotiques. Une corrélation a été trouvée entre les profils de COV et les réponses comportementales, soutenant les hypothèses de l'étude.

Mots-clés : Nématode entomopathogène, *Steinernema*, *Heterorhabditis*, *G. mellonella*, VOC, comportement.

Table of contents

Figure List

Tables List

Acronyms

AZ	Liquid nitrogen modality
EPN	Entomopathogenic Nematodes
Hb	*Heterorhabditis bacteriophora* modality
PMI	Post-Mortem Interval
Sc	*Steinernema carpocapsae* modality
VOC	Volatile Organics Compounds

Entomopathogenic nematodes impact the volatile profile of their insect hosts with a cascading effect on the behaviour of uninfected hosts

Pierre Hotermans
Sustainable Pest Management Division
Gembloux Agro-Bio Tech
University of Liege
Gembloux, Belgique

Abstract

Entomopathogenic nematodes (EPNs) are increasingly utilized as biological alternatives to insecticides for controlling pests. Infective juveniles release mutualistic bacteria that rapidly multiply and kill the host insect. Despite extensive research on EPN-insect interactions, it remains unclear if EPN infections alter the volatile organic compounds (VOC) emitted by the host, potentially influencing the behaviour of uninfected insects. This study tested two hypotheses: (1) entomopathogenic nematodes modify the emissions of volatile organic compounds of their insect host, and (2) these volatile emissions attract uninfected insects, therefore increasing EPN dispersal.

Using *Galleria mellonella* larvae and two EPN species (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*), VOC were collected post-mortem using Hisorb methodology and analysed via gas chromatography-mass spectrometry. Behavioural tests, including pitfall, preference, and escape time assays, assessed the responses of uninfected insects to infected larvae.

The results showed that EPN-infected larvae emit significantly different VOC profiles compared to larvae killed by other means, with a 100% success rate in distinguishing these profiles. Behavioural tests didn't reveal an attraction effect but a significant reduction in escape time when uninfected insects were exposed to larvae infected early in the EPN life cycle. Sulphur compounds, typical of decomposition, were detected in the VOC profiles. The variation in VOC during the EPN reproductive cycle may stem from symbiotic bacteria. A correlation was found between the VOC profiles and the behavioural responses, supporting the study's hypotheses.

Key Words: Entomopathogenic Nematode, *Steinernema, Heterorhabditis, G. mellonella*, VOC, Behavioural.

Introduction

For over a century, synthetic pesticides have been widely used to control plant pests. With the recent ban on neonicotinoids, alternative methods are needed to control several insect species (Hurtado et al., 2023; Larsen et al., 2016) including soil-dwelling insects, which are

among the most challenging to control (P.S.Grewal, 2005). Biological alternatives, such as the use of entomopathogenic nematodes (EPNs) is considered one of the most promising (Barsics et al., 2013; Poggi et al., 2021). Although these microorganisms naturally inhabit the soil, their use in agricultural areas is challenging due to their sensitivity to temperature, UV radiation, and desiccation (Hiltpold et al., 2012). To overcome these limitations, EPNs can be formulated in biodegradable materials (Shapiro-Ilan & Gaugler, 2002), with or without specific attractants (Hurtado et al., 2023).

Entomopathogenic nematodes penetrate their insect hosts as infective juveniles (IJs) through natural openings (*e.g.*, spiracles, mouths) or by breaking through the integument (Eidt & Thurston, 1995). Then, IJs release symbiotic bacteria into the haemocoel. These bacteria produce toxins that kill the host within a few days(Ogier et al., 2015.; Shapiro-Ilan and Gaugler, 2002; Stock et al, 2019). Bacteria digest tissues into nutrients that EPNs feed on before initiating their reproduction. Finally, EPNs reproduce, undergoing multiple generations inside the insect body before releasing new IJs into the soil that seek new hosts (Stock et al. 2019).

Approximately 0.021% of the identified entomopathogenic nematode species exhibit pathogenicity towards insects (Grewal and al., 2005). *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are among the most efficient EPNs in killing insects (Boemaret A N, 1988; Ogier et al., 2015; La Forgia et al.,2021). These species are in symbiosis with bacteria such as *Photorhabdus spp.* and *Xenorhabdus spp.*(Boemare et al., 1993), which play a crucial role by excreting toxins leading to insects' death. These bacteria also secrete secondary metabolites, including those involved in inter-species communication and antimicrobial protection, preventing the development of saprophytes (*e.g.*, fungi), slowing down the decomposition process, and maintaining insect integrity (Chacón-Orozco et al., 2020; Mollah & Kim, 2020; Tarasco et al., 2023a; Ulug, 2023; Wollenberg et al., 2016). As a result, EPNs have a slow reproductive cycle that lasts about 10 to 14 days (Erdem et al., 2024; Schelkens, 2020).

Despite the extensive literature on EPN-insect interactions, it remains unknown whether the infection process modifies the volatile organic compounds (VOC) released by the insect body during entomopathogenic nematode infection. Usually after death, a complex process involving a wide range of chemical reactions leads to the decomposition of the animal, whether vertebrate or invertebrate. As a result, putrid odours are emitted from the remains (Verheggen et al., 2017). These by-products are released by the breakdown of the macromolecules constituting the insect. Bacterial activity during putrefaction decomposes proteins, lipids, and carbohydrates, resulting in the production of sulphur- and nitrogen containing compounds, aromatics, ketones, alcohols, and carboxylic acids (Gabriela Ioan et al., 2017). The presence of the symbiotic bacteria of the EPNs could impact the volatile profile, as they preserve the insect's integrity after death. Moreover, according to Baiome et al. (2022), each entomopathogenic bacterium produces distinct VOC. These compounds are typically oxygenated compounds like

alcohols, ketones, and aldehydes (Leroy et al., 2011).

Materials and Methods

Insect and entomopathogenic nematode rearing

Insects_ Galleria mellonella larvae were reared in plastic boxes with a lid containing a metal grid (28x19x18 cm) to facilitate gas exchange. Their diet consisted of a mixture of honey (11% w/v), glycerine (11% w/v), dog food pellets (39% w/v), water (8% w/v), and wheat bran ("Ferme Baré", 31% w/v). The containers were initially half-filled with the diet and continuously supplied over time. Only L5 and L6 larvae were selected for experiments (Jorjão et al., 2018). *G. mellonella* were kept in a storage box (⌀ = 7.5 cm, h = 3.5 cm) containing wood shavings (Pet's own choice Houtvezel; 2,5kg/35L) in an incubator at 13°C until use.

*Nematodes_* Entomopathogenic nematodes (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*) were bred on *Galleria mellonella* larvae placed on filter paper inside Petri dishes (Ø = 5.5 cm). In each Petri dish, 300 µl of a solution containing entomopathogenic nematodes (834 EPN/ml) was applied per group of five *G. mellonella* larvae. Once the larvae were dead, a White trap system (White, 1927) was used to collect the emerging infective juvenile entomopathogenic nematodes (IJs). The IJs were stored in 100 mL of tap water in 250 ml ventilated culture flasks (75 cm² Falcon Brand Products, New-York, United-States) at 13°C to ensure their viability until the experiments.

Volatolomic analyses

*Insect infestation_* We analysed the volatile organic compounds released by *Galleria mellonella* previously killed by entomopathogenic nematodes, either *Steinernema carpocapsae* or *Heterorhabditis bacteriophora*. Five *G. mellonella* larvae were placed per Petri dish (d = 5.5 cm with a wet filter), and 300 µl of a concentrated suspension of IJs (C = 834 IJs/mL) were spread on the larvae. The Petri dishes were then kept in the dark (T = 21 ± 1°C) until the larvae died. The two entomopathogenic nematode strains differ in their infestation times: larvae died after 48 hours with *S. carpocapsae* and after 72 hours with *H. bacteriophora*. Upon death, the larvae were kept in the same dark conditions, with the filter paper humidity maintained, until the targeted post-mortem intervals (PMI) were reached.

*VOC collection_* The volatile organic compounds (VOC) emitted by dead *G. mellonella* larvae were collected using a novel passive sampling method, namely HiSorb® extraction probes (DVB/CWR/PDMS, Markes, Llantrisant, United Kingdom). These probes are passive extraction devices composed of adsorbent materials designed to capture VOC. They were directly introduced into the headspace of 20 ml vials containing seven dead *G. mellonella* larvae. The crimped vials were then placed in a water bath set at 30 ± 1°C to enhance VOC volatility. Empty vials were also sampled as controls.

To cover the entire entomopathogenic nematode reproduction cycle for the two strains (*S. carpocapsae* and *H. bacteriophora*), samples were collected at four distinct PMIs: the 1st day after death

(PMI1), the 3rd day (PMI3), the 7th day (PMI7), and the 11th day (PMI11). The control consisted of *G. mellonella* larvae killed with liquid nitrogen. Each modality (four PMIs, three killing agents: *S. carpocapsae*, *H. bacteriophora*, and liquid nitrogen) was tested in five replicates.

*Chromatographic analyses*_ The Hisorb® probes were analysed using a thermal desorption gas chromatograph coupled with a mass spectrometer and a flame ionisation detector (TD-GC-MS/FID, Shimadzu, Kyoto, Japan). Compounds were first desorbed at 280°C for 8 minutes in a thermodesorber (TD30R, Shimadzu, Kyoto, Japan) and then cryo-focussed by Peltier effect in a glass tube set at −20°C. The trap was then warmed up to 270°C and injected onto the column head in spitless mode.

Compounds separation was performed on a HP-5 ms capillary column (30 m × 0.25 mm × 0.50 µm; Agilent technology; Santa Clara, California, USA). The oven temperature was initially set to 40°C, then increased to 200°C at a rate of 5 °C/min, and finally to 300°C at a rate of 10°C/min. Helium was used as the carrier gas in constant pressure mode set at 200 kPa. The mass spectrometer interface temperature was maintained at 230°C, with the ion source set at 200°C. Mass spectra were collected from 35 to 300 *m*/*z* with a data acquisition time of 0.2 seconds. The detector voltage was set at 0,1 kV during the mass spectrometer tuning.

The volatile compounds were identified by comparing their mass spectra with those in available databases (NIST, FFNAC). Identifications were confirmed using the retention indices determined with a series of n-alkane (from C7 to C30, 10 mg/ml in n-hexane) and compared with retention indices provided in the libraries. The methods used were previously tested to ensure accurate adsorption of VOC without carryover observation.

Chromatograms were integrated using GCms Solution software (Shimadzu, Kyoto, Japan). Compounds identified in the blank samples were removed using a predictive analysis (Partial Least Squares Discriminant Analysis, PLS-DA). The PLS-DA determined which compounds belonged to the blank by providing a confidence coefficient for each compound. Removed compounds had to reach a confidence coefficient above 15, were present in more than half of the blank replicates and were considered as non-trace (trace compounds were those with a mean area less than 1% of the mean area of compounds across all modalities).

Behavioural test

We determined whether the odour profile impacts the behaviour of uninfected *Galleria mellonella* using three different methodologies. These three behavioural experiments aimed to assess the response of *G. mellonella* to dead *G. mellonella* killed either by entomopathogenic nematodes or liquid nitrogen at different post-mortem intervals (PMIs: PMI1, PMI3, PMI7 and PMI11). These behavioural tests were conducted using *H. bacteriophora* since this entomopathogenic nematode species induces a reddish colour to infested insects, which simplifies the selection of infected larvae for the experiments.

*Test 1 - Preference Test*_ A single healthy *G. mellonella* larva was placed at the centre of a round glass dish with a humidified filter paper (Ø = 18.5 cm) (Figure 1a). The arena was mentally divided into two

distinct zones: one containing a dead *G. mellonella* larva (zone A) and the other one was kept empty (zone B) (Figure 1 a). After the introduction of the healthy larva in the arena, we noted the larva's position (Empty zone or Dead zone) during the five-minute test, as well as its position at the beginning and end of the test. Each dead larva was used for three consecutive replicates. Thirty replicates were conducted.

*Test 2 - Pitfall Test_* We used a glass cylinder tube (Figure 1b), with each side connected to a Schott glass bottle (250 ml). A dead larva was placed in one of the Schott glass bottles, while the other one remained empty. A living *G. mellonella* larva was then placed at the centre of the tube. The test lasted for ten minutes. At the end of that period, the larva's choice was recorded: whether it moved to the side with the dead larva (choice A), the empty side (choice B), or it remained at the centre (choice C; non-responding). Each dead larva was used for three consecutive replicates. Thirty replicates were conducted.

*Test 3 - Escape Time Test_* In a round glass dish with filter paper (Ø = 18.5 cm), a circle with a 5 cm radius was drawn at the centre of the dish. A dead larva and a healthy one were placed side by side within this central zone, with the infested larva placed first (Figure 1c). The timer was started when the living larva was introduced, and the time it took to exit the circle was recorded. Each dead larva was used for three consecutive replicates. Thirty replicates were conducted.

Statistical analyses

Volatolomic analyses_* Chromatograms were aligned using GCAligner 1.0 software. Pie charts were created for each PMI versus death type modalities to highlight major compounds of their volatile profiles using Excel software. To evaluate potential differences among the complete VOC profile released by larvae depending on the type of death (EPNs_*S. carpocapsae, EPNs_*H. bacteriophora*, and liquid nitrogen), volatile profiles were compared using permutational multivariate analyses of variance (perMANOVA) with a Euclidean distance matrix and 999 permutations ('adonis' command, R-package vegan, (Jari Oksanen et al., 2020). *P-values* were adjusted using Bonferroni's correction to mitigate type I error inflation due to multiple testing. Homoscedasticity was assessed using the 'betadisper' function. Finally, we used a partial least squares discriminant analysis (PLS-DA) to construct models for distinguishing samples at each PMI and death type based on their volatile composition. PLS-DA was chosen due to the correlation among some peaks. PLS-DA aims to establish a model that segregates observations into classes using the X matrix, comprising linear combinations of volatile composition called factors, and the Y matrix, containing dummy variables describing class membership. This analysis identifies a discriminant plan where the projected observations on the components are effectively separated according to class. From the PLS-DA model, confidence coefficients for each VOC were extracted to assess their importance in the predictive volatile profile released by entomopathogenic nematode-infested larvae at each PMI. A spider chart was then plotted to visually assess the number of VOC important in the predictive profile of each modality, using Excel software.

*Behavioural analyses*_ To compare each PMI condition with the larvae killed by liquid nitrogen (AZ condition)-, Rstudio software (Version 4.2.2) was used. Chi-square tests of independence were performed for the Pitfall Test for the Zone Preference Test. Statistical significance was considered when the *p*-value was less than 0.05. For the Escape Time Test, modalities for each PMI were compared using the Kruskal-Wallis test, followed by a post-hoc Dunn test with a Bonferroni correction (packages "PSA" and "Dunn.test").

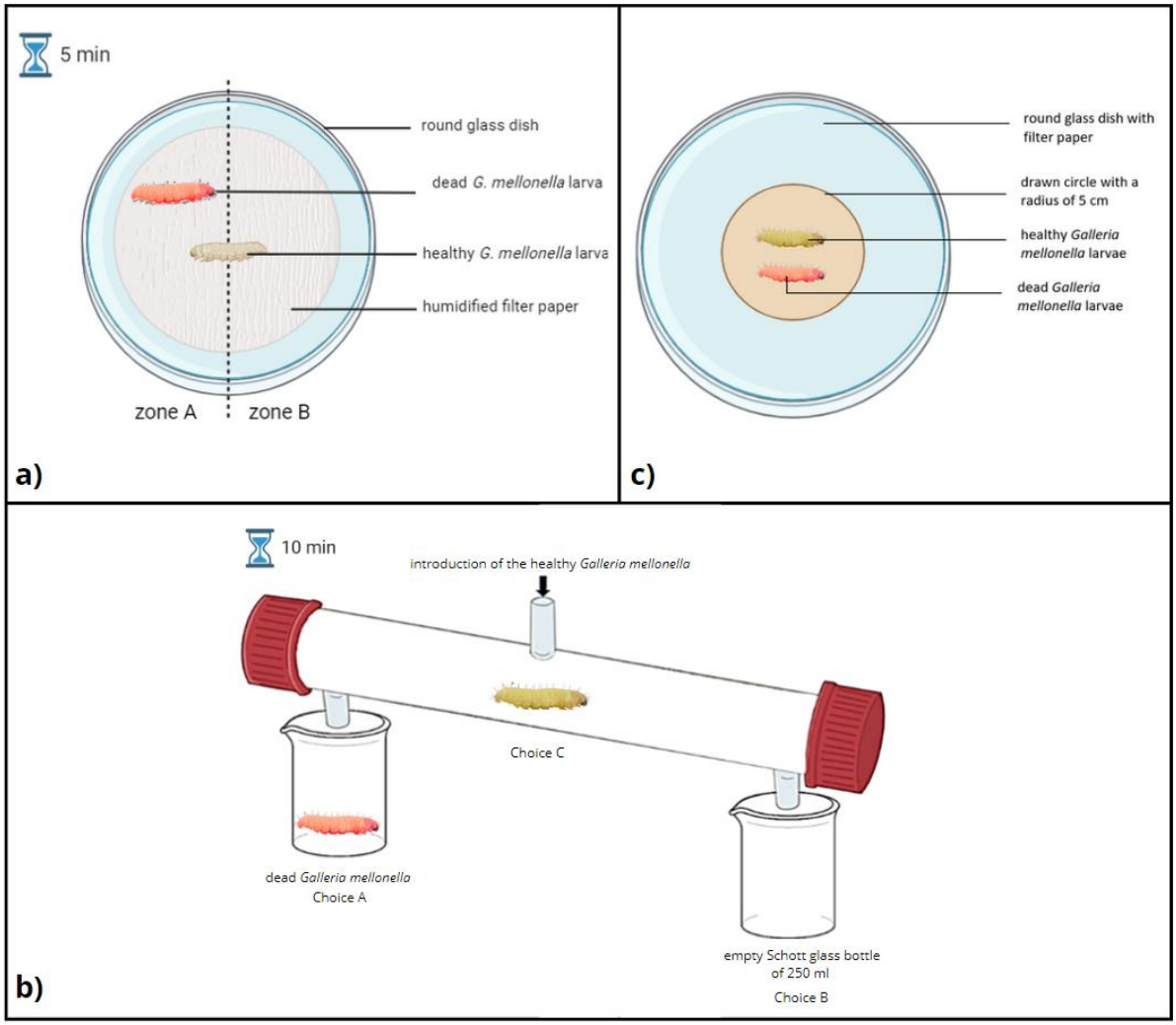

Figure 1 Experimental set up for the Preference test (a), Pitfall test (b) and Escape time test (c).

Results

Volatolomic Analyses

The analyses of all the 60 odour samples revealed a complex blend of 183 volatile organic compounds (VOC) across all conditions: larvae killed by liquid nitrogen (AZ, n=183), larvae killed by *Heterorhabditis bacteriophora* (Hb, n=179), and larvae killed by *Steinernema carpocapsae* (Sc, n=175) (Table 1). Eleven chemical classes were identified, with the most significant being alkanes (n=32), alcohols (n=34), ketones (n=61), alkenes (n=23), amines (n=9), and sulphur compounds (n=7).

Larvae killed by liquid nitrogen (AZ) displayed a gradual increase in the number of compounds in the odour profile depending on the post-mortem interval (PMI) (Figure 2). At PMI1 and PMI3, the profile is characterised by two major compounds: 2,3-butanedione and acetoin (55% for PMI1 and 50% for PMI3). Then, at PMI7, the predominant compounds shifted to 2-nonanone and 2-heptanone (20%), along with the appearance of sulphide compounds (dimethyl disulfide and trimethyl disulfide) as major components. The odour profile at PMI11 was more complex, with 10 compounds representing half of the total odour profile, where methyl disulfide was the most abundant (6%).

Regarding larvae killed by *H. bacteriophora* (Hb)*:* PMI1 was marked by five major compounds (carbon disulfide, dimethyl disulfide, dimethyl trisulfide, 2,5-dimethyl pyrazine, and prenol) which together represented nearly half of the emitted blend. At MPI3, the major compounds identified are 2-butenal, 3-methyl among the 12 compounds making up 50% of the profile. At PMI7, a greater diversity of compounds is present, with isoprene as the major compound. This diversity decreases at PMI11, where dimethyl disulfide (24%) and acetophenone (14%) were the two dominant compounds.

The volatile profile of larvae killed by *S. carpocapsae* (Sc) was similar to that of larvae killed by *H. bacteriophora* (Hb). Indeed, the same three compounds were identified in nearly the same proportions: carbon disulfide (16%), dimethyl disulfide (12%), and dimethyl trisulfide (7%). The number of major compounds then slightly increases up to PMI7, with PMI3 being predominantly composed of cyclohexene 3-butyl and 1-butanol, 2-methyl. At PMI7, half of the odour profile is predominantly composed of cyclohexene, 3-butyl, 1-butanol, 2-methyl, and propyl alcohol. The presence of similar compounds between PMI3 and PMI7 is noted, namely cyclohexene 3-butyl, 2-methyl-butanol, 1-ethenyl-3-ethyl-benzene, 2-heptanone, and heptan-2-ol. Compounds at PMI11 differ from the other two conditions by the predominant presence of propyl tetradecyl ether, propyl alcohol, and 2(3H)-furanone, dihydro-5-methyl-5-(2-methylpropyl) (54%).

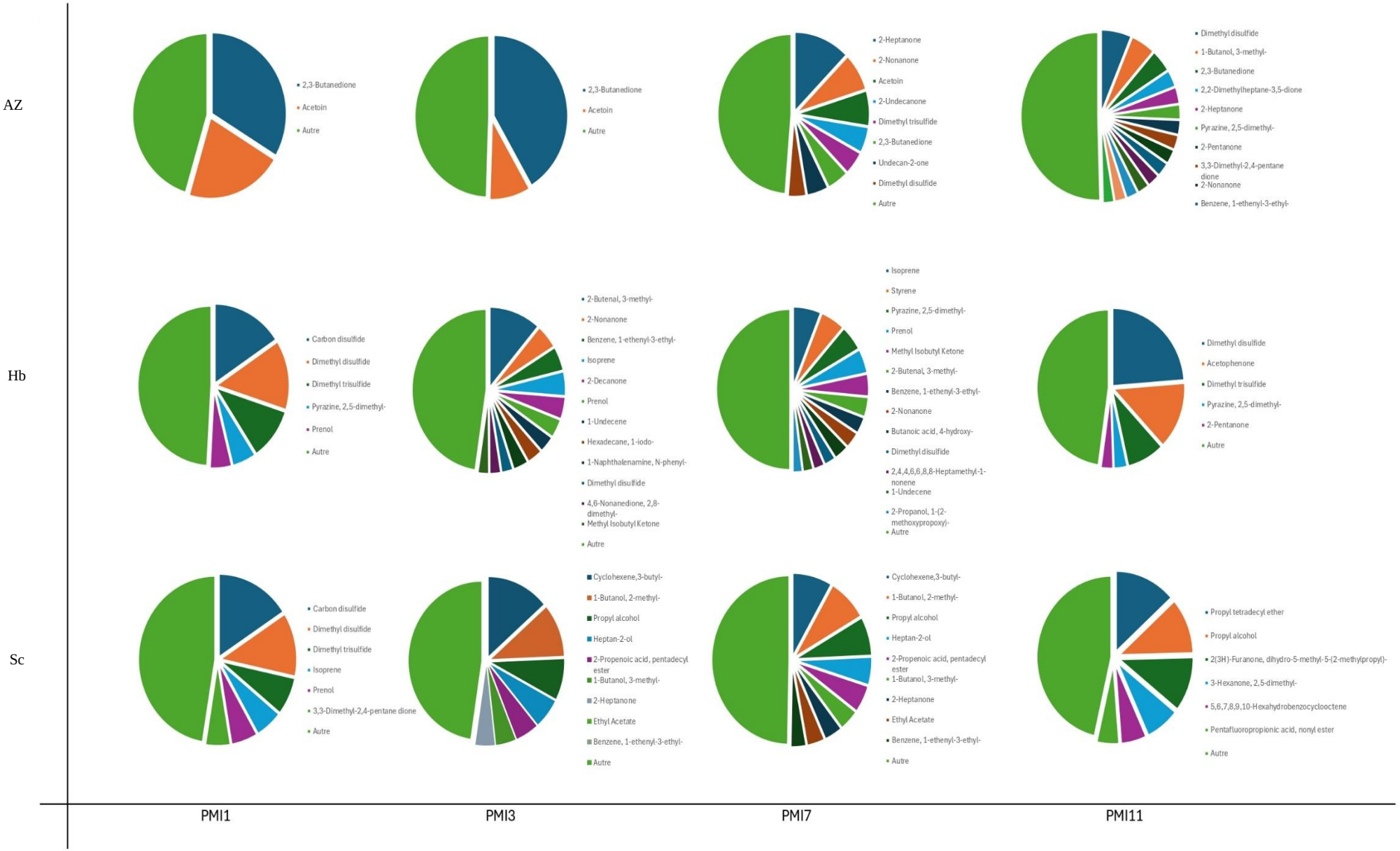

Figure 2 Relative proportions of the VOC contributing to each modality: killing method (liquid nitrogen (AZ), *Heterorhabditis bacteriophora* (Hb), and *Steinernema carpocapsae* (Sc)) and post-mortem intervals in days (PMI1, PMI3, PMI7 and PMI11). The x-axis represents the different moments of odour sampling covering the duration of a complete entomopathogenic nematode reproductive cycle. Less abundant compounds are grouped under the label "Others" (green).

Table 1 Volatile organic compounds (VOC) profile of each modality. The specificity highlights the importance of 183 the compounds in the predicted profile provide by the partial least square analyses (dark green: high specificity; light green: low specificity). The occurrence represented by four squared highlight the number of times the VOC was identified in the replicates. The relative mean (± standard deviation) is represented by n'±σ.

	AZ									Hb									Sc								
	PMI1			PMI3			PMI7			PMI11			PMI1			PMI3			PMI7			PMI11			PMI1			PMI3			PMI7			PMI11		
	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ	Specificity	Occurrence	n'±σ
Alkene- Alkyne																																				
2,4,4,6,6,8,8-Heptamethyl-1-nonene	4,06	0,01±0,02		4,14	0,779±0,759		10,4	0,214±0,24		5,51	0,503±0,433		11,9	0,019±0,022		7,91	0,52±0,693		9,33	0,632±0,41		9,97	0,303±0,3		13,8	0		6,87	0,137±0,227		14	0,098±0,142		7,24	0,075±0,081	
Styrene	7,12	0,00E+00		5,66	0		7,67	0,027±0,027		10,2	0,042±0,084		3,26	0,035±0,042		4,45	0,193±0,077		1,51	0,26±0,233		25	0,147±0,068		6,05	0,005±0,009		3,49	0		7,83	0		8,22	0,039±0,064	
D-Limonene	14,1	0,00E+00		15,7	0,001		13,8	0		11,5	0,001±0,001		23,6	0,001±0,002		17,1	0,269±0,119		85,5	0,424±0,21		27,9	0,005±0,012		14,5	0,003±0,005		30,5	0,001		11	0		14,2	0,001±0,002	
Cyclohexene, 4-methyl-	7,37	0		8,46	0		0,7	0,001		10,2	0		7,34	0,013±0,018		11,8	0,06±0,023		15,5	0,643±0,676		24,7	0,408±0,44		15,1	0		9,28	0		9,11	0		7,97	0,013±0,03	
1,3-Nonadiene, (E)-	10,5	1,00E-03		12,8	0		0,57	0		14,8	0		7	0,101±0,19		16	2,101±0,616		5,73	1,44±0,734		29	0,851±0,306		16,2	0		6,89	0		8,6	0		7,56	0,321±0,717	
1-Nonen-4-yne	17,6	0,025±0,025		4,44	0		3	0,048±0,05		12,6	0,174±0,362		8,73	0,064±0,128		7,34	2,512±0,718		10,1	2,081±1,083		30	1,714±0,368		11,1	0		6,18	0		6,11	0,385±0,86		13,1	0,798±1,29	
2-Ethyl-3,6-dimethylpyrazine	13,7	0,003±0,006		5,68	0,537±0,688		8,8	0,074±0,063		4,3	0,589±0,982		16,5	0,39±0,442		25,5	0,334±0,205		30,7	1,864±2,179		7,06	0,159±0,164		21,3	2,622±2,535		22,6	0,093±0,061		27,7	1,076±1,478		14,5	0,142±0,228	
2-Ethyl-3,5-dimethylpyrazine	7,88	0,007±0,016		18,3	0,094±0,095		8,21	0,002±0,003		5,3	0,024±0,046		17,9	0,064±0,077		36,9	0,026±0,027		47	0,366±0,229		11,9	0,027±0,009		29,7	0,185±0,101		22,4	0,019±0,032		22,7	0,148±0,221		8,49	0,007±0,015	
5,6,7,8,9,10-Hexahydrobenzocyclooctene	3,92	0,023±0,037		12,6	0		4,02	0,03±0,045		12,3	0,77±1,59		9,77	0,07±0,114		9,45	0		6,84	0		10,5	0,002±0,003		17,5	0,329±0,356		7,34	0,001±0,001		3,07	0,001±0,001		3,65	0,001	
Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-	4,27	0		12,8	0		5,74	0		13,3	0,295±0,486		6,56	0		5,86	0		2,49	0		12,9	0		4,1	0		5,25	0		6,41	0		4,54	0	
Isoprene	4,51	0,478±0,637		14,3	0,629±0,739		3,08	0,328±0,414		8,09	0,9±0,795		16,9	0,378±0,176		25,7	1,24±0,477		41,1	5,455±8,964		17,7	0,732±0,442		32,1	0,202±0,046		20,2	0,545±0,211		11,2	0,475±0,314		5,48	0,185±0,129	
Cyclohexene,3-butyl-	1,75	0		20,1	0,77±0,741		3,7	0,159±0,295		17,4	0,206±0,281		8,67	0		9,53	0,581±0,923		6,26	0,179±0,245		8,36	0,036±0,038		13,7	0		6,03	0,142±0,236		2,58	0,07±0,106		0,74	0,019±0,034	
Benzene, 1-ethenyl-3-ethyl-	2,11	0		13	0		7,09	0,013±0,029		13,7	0,377±0,475		3,74	0,004±0,009		5,6	0		1,3	0		13,7	0		4,33	0		2,93	0		3,85	0		2,28	0	
5,6,7,8,9,10-Hexahydrobenzocyclooctene	3,27	0,045±0,073		16,8	0,005±0,007		9,14	0,021±0,038		12,8	0,077±0,112		6,79	0,009±0,009		6,5	0,001±0,003		7,52	0,01±0,014		14,2	0,002±0,003		8,03	0		4,89	0,007±0,009		5,87	0		3,68	0,001	
(3aS,8aS)-6,8a-Dimethyl-3-(propan-2-ylidene)-1,2,3,3a,4,5,8,8a-octahydroazulene	7,48	0,00E+00		8,92	0,187±0,186		7,74	0,095±0,187		8,68	0,236±0,329		5,55	0,001		4,37	0,214±0,293		4,02	0,138±0,189		10,5	0,042±0,04		5,49	0		4,27	0,048±0,082		3,92	0,005±0,011		5,47	0,02±0,031	
1-Undecene	2,42	0,00E+00		7,08	0,001±0,001		16	0,112±0,153		2,74	0,19±0,308		15,1	0,016±0,03		6,69	0,095±0,062		9,67	0,061±0,04		1,9	0,046±0,043		10,8	0,001		10,2	0,032±0,059		5,9	0,06±0,063		3,9	0,097±0,158	
Benzene, 1,3,5-tris(1-methylethyl)-	3,92	0		14,5	0		4,43	0,001±0,002		10,9	3,331±7,448		5,76	0,002±0,002		8,33	0		3,09	0,001		10,4	0,001±0,001		7,16	0,001±0,001		5,59	0		5,22	0,002±0,004		3,81	0	
Thiophene, 3-methyl-	4,03	0,001±0,002		14,8	0,001		4,64	0,001±0,001		10,8	0,181±0,405		5,85	0		8,17	0,001±0,001		2,26	0,008±0,011		9,96	0,003±0,003		7,09	0		5,56	0		4,94	0		3,59	0,001	
1-Hexene, 5,5-dimethyl-	4,15	0		12	0,001		11,1	0,045±0,058		6,99	0,334±0,735		5,48	0,002±0,003		8,55	0,004±0,004		4,78	0,003±0,002		5,92	0,003±0,002		9,69	0,002±0,002		4,78	0,006±0,006		3,3	0,007±0,007		3,26	0,015±0,021	
2,4,4,6,6,8,8-Heptamethyl-1-nonene	21	0,001±0,001		12,5	0,09±0,201		12,6	0,308±0,269		8,03	0,275±0,216		14,9	1,021±0,323		19,5	0,355±0,133		13,1	0,361±0,116		7,58	0,386±0,215		15,8	1,625±0,618		14,1	0,467±0,41		25,3	0,917±0,432		20	0,22±0,251	
D-Limonene	24,1	0,193±0,307		9,57	0,476±0,991		9,17	1,464±1,749		10,3	0,801±0,869		37,5	15,722±8,948		11,8	1,417±1,072		25,3	1,377±0,621		10,6	2,372±2,061		13,2	15,859±5,389		18,5	3,718±3,809		25,3	4,228±5,975		25,3	1,826±2,346	
6-Dodecyne	10,7	1,35±1,731		9,68	0,89±1,498		15,7	1,808±0,857		8	2,852±2,398		21,9	0,856±0,314		14,8	0,729±0,241		29,5	1,288±0,838		12,1	2,816±1,326		17,1	4,854±2,481		17,2	8,299±7,011		22,9	8,359±3,298		17,6	2,016±1,26	
Halide																																				
8-Heptadecyne, 1-bromo-	6,82	0,169±0,36		18,5	0,04±0,054		12,3	0,612±0,812		10,9	1,694±2,287		15	0,003±0,007		11,3	0		17,5	0,606±0,619		6,04	0,049±0,074		14	0,001±0,001		12,7	0,01±0,021		9,45	1,363±1,544		8,59	0,797±0,868	
S-Methyl 3-methylbutanethioate	3,12	0		20,8	0		11,1	0,615±0,926		12,1	1,506±2,136		13,2	0		11,2	0		9,6	0,348±0,354		5,98	0,047±0,07		13,3	0		9,71	0,004±0,008		8,02	0,679±0,785		4,14	0,281±0,393	
Hexadecane, 1-iodo-	10,2	0,002±0,003		2,34	0,003±0,007		9,59	0,028±0,058		3,03	0		3,9	0		1,5	0		5,03	0,001±0,002		3,01	0		4,65	0,004±0,008		4,69	0		2,42	0		8,2	0,007±0,008	
Hexadecane, 1,16-dichloro-	5,24	4,279±6,354		15,2	2,252±2,201		9,73	1,008±1,366		14,8	5,196±4,98		10,6	0,103±0,075		4,58	0,13±0,092		3	0,151±0,102		13	0,019±0,015		11,4	1,032±1,18		7,38	0,105±0,131		14,1	0,087±0,085		9,3	0,042±0,016	
Carboxylic acid																																				
9,12-Octadecadienoic acid (Z,Z)-	6,11	1,315±1,864		24,6	0,636±0,164		9,14	0,328±0,419		15,7	1,457±2,002		12,6	0,023±0,05		12,2	0,022±0,024		4,89	0,768±0,757		11,5	0,009±0,016		15	0,796±0,258		7,91	0,008±0,017		16,3	0,05±0,037		9,44	0,014±0,018	
2-Propenoic acid, pentadecyl ester	5,87	0,316±0,619		4,56	0,548±1,024		9,53	4,03±2,93		11,7	6,418±6,787		7,44	14,22±6,774		5,84	2,59±1,299		43,4	2,57±1,441		31,8	23,787±9,354		2,13	12,48±2,16		14,5	4,383±7,042		5,93	5,45±2,918		5,44	2,67±3,511	
Pentadecafluorooctanoic acid, undecyl ester	5	0,198±0,442		3,07	0,003±0,007		8,76	0,461±0,627		8,34	0,468±0,412		3,3	0,955±1,003		4,57	0,747±0,187		30,5	0,749±0,366		25,1	2,468±1,654		2,11	0,019±0,016		8,69	0,053±0,052		4,56	0,386±0,491		6,1	0,267±0,29	
Pentadecafluorooctanoic acid, tridecyl ester	16,5	0,682±0,474		30,5	2,03±3,609		9,16	0,353±0,283		12,4	0,643±0,96		27,2	2,85±2,319		40,9	11,325±6,641		26	4,044±0,704		13,7	1,843±1,049		47,7	5,247±7,747		16,5	5,037±3,643		17,3	3,855±3,436		14,8	0,731±0,652	
Pentadecafluorooctanoic acid, tetradecyl ester	10,3	5,12±11,203		1,67	0,502±0,202		19,9	12,441±8,831		4,41	3,273±3,227		15,8	0,64±0,565		2,1	0,698±0,111		8,4	0,616±0,146		7,11	0,755±0,205		5,22	0,383±0,248		6,75	4,197±3,75		8,87	7,589±7,548		9,98	11,288±7,04	
Butanoic acid, 3-methyl-	24,1	0,543±1,109		2,99	0,306±0,442		12	2,048±3,649		4,56	0,289±0,235		15,7	0,263±0,417		5,46	1,036±0,272		12,3	0,151±0,058		9,09	0,034±0,031		8,43	0,43±0,398		9,03	1,186±0,893		17,7	3,82±2,845		24,6	3,123±3,108	
Butanoic acid, 4-hydroxy-	6,71	2,254±5,001		5,96	0,086±0,16		14	3,646±3,225		4,6	2,929±4,049		10,6	5,345±5,148		9,88	0,767±0,765		9,28	5,246±8,122		4,95	3,076±4,673		13,8	0,015±0,014		4,31	0,077±0,106		8,68	0,968±0,962		6,81	5,588±4,745	
Heterocyclic																																				
Oxazole, trimethyl-	7,62	0,005±0,01		13,3	0,004±0,006		14,1	0,163±0,178		7,36	0,136±0,304		14,1	0		8,56	0		9,56	0,014±0,015		6,41	0,004±0,005		8,56	0,001±0,001		8,96	0,018±0,021		10,5	0,168±0,366		7,71	0,042±0,048	
1,3-Dioxane, 4,5-dimethyl-	11,5	0,006±0,013		3,55	0,003±0,004		12,3	0,596±0,926		1,99	0,252±0,56		6,3	0,001		1,12	0,001±0,002		5,27	0,022±0,019		4,84	0		1,13	0,001		3,08	0,007±0,011		3,64	0,123±0,264		10,1	0,08±0,125	
Alcohol																																				
Cyclohexanol, 1,3-dimethyl-, cis-	4,88	0,026±0,058		23,5	0,033±0,036		11,6	0,063±0,055		13,4	0,322±0,558		21,9	0,17±0,152		18	0,125±0,045		8,3	0,218±0,203		4,68	0,144±0,081		24,4	0,004±0,004		11,6	0,037±0,029		11,8	0,16±0,124		6,35	0,038±0,025	
Phenol, 2-ethyl-	4,75	0		4,27	0,587±1,312		8,02	4,832±10,422		1,9	2,48±5,545		5,49	10,731±13,996		4,83	1,195±1,259		12,8	0,476±1,064		3,81	8,499±11,767		5,28	7,438±10,251		4,65	6,034±17,933		2,66	3,287±4,762		3,62	3,962±8,755	
Methanethiol	20,4	0,09±0,201		6,58	0,128±0,14		20,4	0,367±0,432		8,37	0,788±0,758		18,5	0,085±0,099		8,09	0,25±0,222		21,4	0,855±0,599		11	0,238±0,107		15,8	0,028±0,062		10,6	0,074±0,063		9,09	0,159±0,113		16,8	0,249±0,122	
1-Butanol, 3-methyl-	13	0,289±0,645		3,12	0,015±0,02		14,2	0,916±0,93		3,45	0,276±0,521		3,95	0,412±0,44		1,72	0,015±0,021		2,95	0,173±0,259		4,72	0,06±0,093		3,64	0,001±0,002		2,79	0,001		5,14	0,042±0,055		12	0,258±0,331	
1-Butanol, 2-methyl-	11,2	0,174±0,386		7,05	0,486±0,24		24,8	1,133±1,155		7,11	2,524±1,901		18,4	0,326±0,301		8,4	0,781±0,514		19,2	2,176±0,792		9,65	0,681±0,248		12,3	0,183±0,096		11,1	0,301±0,125		9,41	0,419±0,305		9,61	0,597±0,271	
Heptan-2-ol	7,2	0,124±0,278		4,59	0,032±0,071		13,7	0,128±0,134		2,92	0,033±0,042		9,55	0,005±0,007		5,58	0,006±0,01		8,21	0		5,87	0,004±0,006		8,49	0,007±0,009		5,18	0,017±0,02		4,05	0,16±0,161		4,81	0,126±0,155	
2-Propanol, 1-(2-methoxypropoxy)-	9,05	0,706±0,775		6,6	0,467±0,275		5,23	0,185±0,094		11,3	0,509±0,257		10,3	0,457±0,217		6,01	0,762±0,19		42,4	0,845±0,187		35,5	14,083±13,924		6,02	0,682±0,591		15,6	0,492±0,236		5,35	0,294±0,077		7,93	0,184±0,079	
4-Hexen-1-ol, 2-methylpropionate	12,8	0,041±0,091		3,59	0		14,7	0,111±0,117		4,24	0,019±0,028		5,82	0		4,06	0		4,69	0		5,7	0		7,68	0		4,24	0		5,25	0		11,7	0,03±0,051	
Phenylethyl Alcohol	8,94	0,046±0,104		5,19	0,003±0,006		10,8	0,129±0,128		2,68	0,032±0,037		10,6	0		6,36	0		15,5	0		8,73	0		6,23	0		8,86	0,005±0,018		13	0,32±0,676		10,6	0,145±0,169	
cis-Cyclohexane-1,4-dimethanol, diacetate	18,8	0,015±0,033		10,4	0,016±0,024		18,3	0,13±0,12		6,33	0,015±0,02		12,7	0		11,1	0		5,45	0,002±0,005		6,42	0		13,5	0		7,75	0		10,7	0		16,8	0,011±0,018	
2,3-Butanediol	23,3	5,627±12,573		3,78	0,121±0,107		15	7,797±7,319		4,13	2,833±3,157		19,3	1,032±1,281		6,25	4,965±2,213		11,1	3,358±0,964		8,34	2,528±0,52		6,85	0,172±0,1		9,61	1,414±2,504		16,4	2,313±2,323		21,8	13,347±10,828	
2,3-Butanediol	2,61	0,442±0,548		32,5	0,292±0,105		10,7	0,493±0,448		18,9	2,519±2,989		18	0,025±0,029		16,7	0,022±0,022		6,39	0,017±0,025		10,5	0,273±0,568		21,2	0,29±0,109		9,78	0,069±0,106		12,9	0,62±1,175		5,15	0,15±0,246	
1-Octanol, 2-butyl-	3,47	0,026±0,058		5,58	0,022±0,049		12,7	0,173±0,19		6,93	0,094±0,126		2,36	0,003±0,008		4,74	0,019±0,018		14,1	0,231±0,171		13,6	0,358±0,324		5,15	0,01±0,011		6,85	0,025±0,018		4,08	0,087±0,101		4,9	0,115±0,11	
trans-2-Dodecen-1-ol	7,55	0,107±0,239		25,7	0,004±0,008		2,87	0,088±0,127		15,6	0,15±0,242		12,4	0,759±0,96		29,2	4,484±2,476		9,48	1,703±0,992		27	1,604±0,938		27,7	0,001±0,001		11,5	0,027±0,038		18,1	0,165±0,149		9,77	0,127±0,149	
3-Pentanol, 2,4-dimethyl-	3,24	0,024±0,053		2,97	0		17,3	0,056±0,051		2,45	0,022±0,037		5,39	0		3,75	0		6,72	0		5,28	0,015±0,021		3,86	0		5,73	0,06±0,105		2,61	0,02±0,035		2,37	0,035±0,024	
L-,alpha,-Terpineol	12	0,032±0,064		2,98	0,002±0,003		11,5	0,062±0,056		2,85	0,021±0,032		6,97	0,004±0,006		4,06	0,015±0,018		12,3	0,014±0,01		8,54	0,016±0,005		3,14	0,002±0,002		6,53	0,015±0,015		6,2	0,059±0,098		10,4	0,087±0,121	
2-Propanol, 1-methoxy-	6,44	0		2,66	0,021±0,046		12	1,265±1,959		2,53	0,45±0,656		11,1	2,238±2,142		6,25	0,043±0,085		10,4	0,114±0,183		4,63	1,254±1,082		6,46	2,415±0,696		8,33	0,684±1,997		10,8	1,284±1,565		6,1	0,83±1,38	
2-Pentanol	12,7	0,005±0,011		0,61	0		12,6	0,039±0,055		1,22	0,048±0,066		2,01	0,07±0,157		0,64	0,006±0,008		3,58	0,008±0,014		0,12	0,026±0,011		3,3	0		2,03	0		1,69	0,003±0,004		10,6	0,01±0,01	
Prenol	10,9	0,611±1,268		3,76	0,009±0,012		11,3	1,606±2,048		3,53	0,206±0,186		7,68	0,045±0,045		3,94	0,024±0,02		14,1	0,047±0,044		9,22	0,045±0,033		3,24	0,032±0,017		7,94	0,143±0,114		5,85	1,509±3,126		9,4	2,182±3,035	
1-Dodecanol, 3,7,11-trimethyl-	10,7	0,054±0,099		3,02	0		9,86	1,091±2,283		3,13	0,03±0,03		5,1	0,024±0,045		1,01	0,008±0,018		4,79	0,047±0,044		3,12	0,009±0,009		4,38	0,019±0,026		4,67	0,044±0,149		3,51	0,151±0,326		9,01	0,153±0,185	
3-Heptanol, 5-methyl-	4,29	1,577±3,448		3,45	0,067±0,081		12,1	5,319±5,552		3,42	1,066±0,72		3,54	0,192±0,183		2,03	0,32±0,298		11,2	0,517±0,198		8,16	0,617±0,256		3,06	0,232±0,202		4,33	0,827±0,884		3,27	5,346±10,582		3,72	4,553±5,183	
Phenol, 2-ethyl-	11,7	0,044±0,065		18,7	0,378±0,189		8,26	0,212±0,314		8,9	0,587±0,508		6,84	0,348±0,231		13,3	0,757±0,324		44,7	0,889±0,253		18,8	0,151±0,055		11	0,303±0,124		10,8	0,314±0,367		4,03	0,32±0,264		6,9	0,077±0,014	
Propyl alcohol	14,4	0,111±0,149		17,9	0,958±0,467		9,59	0,537±0,787		8,42	1,496±1,245		9,09	0,829±0,559		13,8	1,867±0,794		45,5	2,154±0,609		17,3	0,348±0,118		12,6	0,744±0,304		12,3	0,771±0,896		4,8	0,746±0,611		9,18	0,171±0,038	
1-Butanol	15,6	0,029±0,064		4,93	0,009±0,02		13,2	0,164±0,21		3,85	0,167±0,281		8,65	0,532±0,754		6,23	0,043±0,028		4,96	0,063±0,038		2,59	0,061±0,045		12,8	0,002±0,005		5,46	0,042±0,036		5,41	0,031±0,039		13,2	0,049±0,039	
2-Pentanol	8,24	0,065±0,145		4,81	0		10,6	0,087±0,135		3,11	0,007±0,015		7,15	0		4,39	0,001±0,003		13,8	0,003±0,004		9,19	0,007±0,005		4,83	0,13±0,273		7,62	0,002±0,003		2,92	0,095±0,19		6,23	0,116±0,151	
Pyrrole	2,11	0,027±0,06		2,81	0		11,7	0,102±0,157		3,46	0,009±0,013		3,61	0		2,89	0,001		7,76	0		5,38	0,007±0,006		3,51	0		5,35	0,007±0,013		3,07	0,017±0,039		2,07	0,062±0,081	
2-Pentanol, 4-methyl-	2,99	0,344±0,769		2,28	0,019±0,023		13,4	0,872±0,854		5,12	0,12±0,123		4,08	0,099±0,09		2,46	0,106±0,152		3,95	0,292±0,094		7,32	0,328±0,213		3,47	0,114±0,058		3,85	0,745±0,583		3,81	0,35±0,31		2,28	0,809±0,661	
2-Pentanol, 3-methyl-	7,11	0,022±0,049		3,1	0		10,3	0,056±0,074		6,88	0,026±0,036		4,57	0,016±0,022		2,04	0,138±0,035		14,6	0,16±0,057		16,2	0,15±0,128		3,41	0		5,84	0,054±0,07		3,42	0,013±0,013		8,24	0,031±0,027	
1-Hexanol	12,9	0,003±0,007		2,3	0		13,3	0,025±0,036		4,29	0,015±0,02		7,62	0,004±0,006		3,8	0,066±0,017		6	0,067±0,028		9,67	0,044±0,022		5,4	0		5,11	0,001±0,003		5,69	0		12,8	0,006±0,005	
1-Octen-3-ol	8,33	0,03±0,033		7,27	0,012±0,027		9,46	0,191±0,298		11,3	0,009±0,012		11,6	0,005±0,007		13,5	0,519±0,515		16,5	0,45±0,164		21,6	0,524±0,086		16,5	0		9,34	0,013±0,018		14,4	0,269±0,572		8,34	0,148±0,281	
Benzenethiol, 3-methyl-	16,3	0,372±0,458		13,3	0,219±0,339		10,4	0,093±0,072		11,1	0,341±0,178		11,4	0,237±0,055		11,1	0,731±0,707		17,7	0,607±0,358		20,2	0,257±0,223		13,5	0,211±0,115		11,8	0,168±0,134		15	0,659±0,535		15,9	0,188±0,1	

1-Nonanol	12,9	0,04±0,089	13,8	0,039±0,062	16,5	0,301±0,265	4,21	0,193±0,383	20	0,04±0,05	20,4	1,031±1,336	18,5	0,134±0,03	5,56	0,317±0,227	22,4	0,122±0,217	14,5	0,6±0,787	13,9	0,149±0,118	12,4	0,132±0,12
5-Decen-1-ol, (E)-	25,4	3,087±2,849	8,84	2,389±2,366	18	0,211±0,378	2,9	0,937±0,894	23,1	4,531±2,836	16,1	4,952±3,263	34,4	6,193±2,769	4,91	0,369±0,355	15,4	5,838±4,533	19,1	0,794±0,681	20,7	1,678±1,169	23,3	0,023±0,029
2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol	20	33,634±22,125	65,3	41,539±35,586	4,06	4,831±6,997	21,5	4,499±4,843	22	0,938±1,551	37,2	1,297±0,929	23,6	0,451±0,284	14,2	0,146±0,11	35,1	0,457±0,462	17,4	0,835±1,313	17,2	0,177±0,168	20,5	0,007±0,015
2,7-Octadien-1-ol, acetate	13,8	0	5,46	0,003±0,005	13,9	0,02±0,036	5,79	0,002±0,004	11,9	0,003±0,004	8,01	0,008±0,012	9,78	0	9,31	0,008±0,012	8,59	0,002±0,003	9,69	0,007±0,014	17,8	0	14,5	0,054±0,054
Hydroxytoluène butylé	24,5	21,035±15,775	10,6	8,989±11,001	11,7	7,213±10,605	8,38	2,646±3,229	19,1	0,001±0,002	17,2	0,017±0,023	24,3	0,013±0,021	14,4	0,008±0,014	18,4	0,008±0,01	15,4	0,002±0,004	23,5	0,006±0,009	25,1	0
Aldehyde																								
2-Butenal, 3-methyl-	7,99	0,06±0,052	10,2	0,39±0,724	17,9	2,063±2,888	7,9	0,606±1,339	25,4	0,053±0,071	15,9	0,025±0,035	14,9	0,051±0,069	6	0	18,4	0,12±0,098	17,5	0,026±0,035	25,9	0,012±0,02	12,4	0
Octanal	4,07	0,076±0,142	1,21	0,268±0,56	8,77	0,825±1,843	3,08	0,921±1,832	1,44	0,003±0,007	7,04	0,01±0,022	15,3	0,298±0,213	8,76	0,325±0,281	3,55	0,058±0,105	7,1	0	8,17	0,161±0,284	3,45	0,039±0,04
Octanal, 7-methoxy-3,7-dimethyl-	25	0,174±0,196	38,8	0,106±0,056	19	0,075±0,115	11,9	0,261±0,352	66,3	4,421±3,691	50,6	0,758±0,21	27,7	0,744±0,474	2,14	0,063±0,031	70,4	0,114±0,072	36,2	0,124±0,088	41,9	0,112±0,118	27,7	0,013±0,007
2-Cyclopentene-1-butanal, .gamma.,.gamma.,2,3-tetramethyl-	5,01	0,079±0,175	12,1	0,001	5,61	0,008±0,007	13,6	1,681±2,569	5,56	0,001±0,002	4,95	0,002±0,004	3,28	0,082±0,085	13,7	0,004±0,008	3,88	0,005±0,005	5,04	0	5,02	0	4,48	0
2,4,6-Octatrienal	3,08	0,004±0,009	13	0,534±0,539	3,17	0,099±0,16	14,9	0,202±0,295	4,21	0,001	5,09	0,328±0,45	7,27	0,175±0,258	8,53	0,022±0,024	6,02	0	3,18	0,204±0,342	6,14	0,031±0,042	3,73	0,02±0,035
Alkane																								
Heptadecane, 1-bromo-	4,09	0	18,8	1,372±1,356	3,83	0,273±0,456	15,4	0,592±0,858	5,04	0,002±0,004	11,2	1,022±1,406	5,41	0,547±0,795	8,21	0,064±0,096	10,8	0	5,45	0,565±0,944	9,46	0	4,33	0,062±0,096
Octane, 3,6-dimethyl-	27,2	0	10,3	0,381±0,366	5,79	0,071±0,124	8,6	0,098±0,136	8	0	7,98	0,301±0,412	11,8	0,128±0,179	3,92	0,018±0,017	7,88	0,001±0,001	8,57	0,179±0,239	12,9	0,092±0,095	24,8	0,09±0,084
Cyclopentane, 1,2-dimethyl-	2,52	0	16,1	0,001±0,002	8,92	0,329±0,586	14,7	2,08±2,563	0,42	0	5,6	0	1,81	0,001±0,001	15,6	0,004±0,004	2,86	0,001	1,57	0,002±0,005	3,2	0	2,17	0
Dodecane, 4,6-dimethyl-	5,06	0,065±0,108	8,89	0,147±0,14	14,4	0,171±0,287	8,21	0,483±0,685	6,33	0,045±0,03	9,12	0,064±0,075	13,5	0,061±0,073	14,4	0,037±0,026	8,15	0,033±0,027	5,37	0,121±0,042	3,98	0,072±0,071	3,53	0,016±0,017
Hexane, 2,4-dimethyl-	15,8	0	6,75	0,121±0,14	22,3	0,487±0,7	7,52	0,788±0,758	16,1	0,087±0,078	4,31	0,319±0,198	13	0,623±0,532	9,4	0,238±0,107	7,98	0,06±0,059	8,14	0,061±0,077	10,9	0,13±0,137	15,3	0,249±0,122
Cyclohexane, 1,3,5-trimethyl-	4,03	0	14,7	0,554±0,526	2,86	0,085±0,151	15,5	0,199±0,276	6,5	0	3,93	0,409±0,561	1,71	0,227±0,319	7,93	0,047±0,046	4,63	0,001	3,47	0,152±0,257	7,24	0,045±0,063	3,82	0,018±0,029
Cyclohexane, 1-ethyl-4-methyl-, trans-	9,63	0,085±0,118	4,98	0,074±0,06	13,1	0,271±0,539	5,93	0,487±0,878	8,96	0,029±0,027	6,54	0,017±0,023	1,41	0,032±0,038	5,55	0,007±0,007	5,62	0,04±0,067	5,51	0,022±0,031	14,5	0,132±0,119	11,5	0,051±0,041
Cyclohexane, 1-ethyl-2,3-dimethyl-	23,9	0,001±0,003	6,93	0,119±0,11	13,5	2,45±5,108	5,73	2,833±3,157	17,9	1,032±1,274	6,98	4,965±2,213	11,9	3,396±0,907	6,2	2,516±0,521	11	0,248±0,135	13,5	1,653±2,333	19,9	2,313±2,323	24,3	7,467±7,376
Cyclohexane, 1-ethyl-2,4-dimethyl-	2,05	0,001±0,002	16,5	0	7,34	0,04±0,086	12,7	0,172±0,272	0,48	0	6,75	0	1,65	0	13,1	0	3,33	0	1,11	0	1,9	0	1,44	0
Cyclohexane, 1-methyl-2-propyl-	2,73	0,021±0,047	14,6	0,165±0,135	12,1	0,149±0,287	10,2	0,222±0,302	9,64	0,02±0,028	10,5	0,023±0,02	15,2	0,006±0,008	14,3	0,001	8,94	0,015±0,016	8,15	0,049±0,073	7,69	0,002±0,005	3,79	0,003±0,004
Tetradecane	16,4	0,05±0,103	16,4	0,945±0,482	12,2	0,552±0,68	8,94	1,496±1,245	11,5	0,819±0,551	16,5	1,867±0,794	41,5	1,97±0,513	16,2	0,362±0,133	18,7	0,777±0,315	12,7	0,771±0,896	6,23	0,736±0,615	9,91	0,173±0,033
Trachylobane	5,62	0	19,4	0,002±0,005	8,03	0,004±0,009	19,3	0,646±0,636	8,85	0,004±0,008	7,25	0	2,84	0	17,8	0	6,33	0	5,12	0,001	6,67	0,005±0,009	5,96	0
Cyclohexane, 1-methyl-2-pentyl-	7,12	0	20,5	0	8,38	0,002±0,004	17,6	0,1±0,103	7,53	0	8,82	0,007±0,017	5,94	0	16,7	0	6,41	0,001±0,002	5,82	0	6,74	0,007±0,015	7,19	0,001±0,002
Dodecane, 4,6-dimethyl-	4,36	0	25,1	0	7	0,01±0,014	18,4	0,685±0,839	8,37	0	10,4	0	2,64	0	15,6	0	9,61	0	4,41	0	5,36	0	4,64	0
Cyclohexane	9,79	0,012±0,021	3,67	0	1,33	0,047±0,051	9,35	0,228±0,359	4,15	0	2,16	0,906±1,289	7,94	1,356±1,577	23,5	0,985±0,94	5,26	0	3	0	2,26	0	6,55	0,205±0,458
Propane, 1-chloro-2-methyl-	3,66	0	20,8	0	3,93	0	12,6	0,11±0,238	6,37	0	9,2	0,001±0,001	2,68	0,001	10,4	0	7,69	0	3,29	0	4,15	0	3,76	0
Hexane, 3,3-dimethyl-	4,58	0,103±0,19	14,3	0,01±0,022	14,2	0,038±0,054	9,69	0,107±0,178	13,2	0,006±0,008	12,9	0,006±0,014	4,71	0,034±0,038	11,4	0,004±0,009	11	0,008±0,019	11,9	0,004±0,009	18	0,007±0,015	7,69	0
Octane, 3-methyl-	32,8	0,139±0,224	9,85	0,322±0,155	12,8	0,107±0,089	5,74	0,403±0,395	30,7	0,966±0,793	27,4	1,24±1,134	33,8	0,481±0,237	9,53	0,116±0,109	32,3	1,096±0,428	27,9	3,52±3,55	29,6	0,692±0,674	29,2	0,099±0,082
Nonane	5,12	0,016±0,032	24,4	0,187±0,085	5,3	0,041±0,055	5,56	0,22±0,314	22,1	0,047±0,041	25,2	0,086±0,074	20,4	0,385±0,178	3,55	0,032±0,023	20,5	0,01±0,01	20,4	0,029±0,06	29,3	0,717±1,539	11,4	0,007±0,016
4,4-Dimethyl octane	21,1	0	8,54	0,029±0,062	7,58	0,168±0,24	8,15	0,12±0,181	15	0	14,1	0,004±0,008	13,6	0,688±0,533	13,8	0,387±0,176	15	2,322±1,304	18,8	2,189±1,114	26,7	2,392±2,187	28,3	0,636±0,272
Octane, 2,5-dimethyl-	24,5	1,987±1,302	3,58	2,178±2,49	18,9	0,083±0,142	3,34	0,151±0,128	24,6	4,866±3,143	12,1	3,858±1,351	25,9	4,971±2,261	6,84	0,519±0,548	10,8	5,663±4,131	17,7	0,934±0,76	17,8	2,153±1,588	20,5	0,05±0,047
Cyclohexane, 1,1,4,4-tetramethyl-	23,1	0,034±0,061	6,14	0,021±0,046	12,4	1,659±3,372	6,24	1,881±2,16	16	1,177±1,176	6,03	3,437±1,509	8,95	2,308±0,592	7,22	2,346±0,434	10,6	0,161±0,119	11,9	1,21±1,632	17,6	2,281±1,912	23,1	4,896±5,425
Cyclohexane, 1,2,3-trimethyl-	13,8	0,029±0,034	6,98	0,071±0,077	14,1	4,827±5,945	6,41	1,062±0,723	12,9	0,269±0,162	12,8	0,492±0,085	10,2	0,517±0,198	8,3	0,617±0,256	15,8	0,275±0,184	11,7	10,666±20,987	19,2	0,502±0,255	15,1	11,025±13,054
Cyclohexane, 1-ethyl-2-propyl-	23,6	0	20,9	0,039±0,087	11,8	0	6,94	0	21,8	0,473±0,435	26,4	0,382±0,372	9,46	0,017±0,014	2,75	0,006±0,005	36,4	0,004±0,005	21	0,227±0,178	35,8	0,207±0,378	22,2	0,005±0,01
Octane, 2,3-dimethyl-	11,8	0,01±0,02	19,1	0,025±0,056	7,66	0	6,17	0,018±0,041	23,5	0,035±0,048	23,8	0,098±0,138	8,21	0,041±0,067	4,78	0,023±0,032	30,1	0,08±0,169	14,3	0,082±0,184	28,4	0,198±0,275	18,1	0,009±0,019
Octane, 3,6-dimethyl-	1,68	0,006±0,014	5,17	0,102±0,155	14	0,13±0,133	2,29	0,071±0,142	5,35	0,001	3,05	0	2,97	0	2,88	0,086±0,083	4,26	0	3,16	0,001	4,42	0,042±0,093	1,7	0,095±0,135
Hexacosane <n->	8,03	0	2,71	0,001±0,002	10,5	0,152±0,254	3,3	0,004±0,005	3	0	2,01	0	5,14	0,001±0,002	3,21	0,004±0,005	4,19	0	4,3	0,008±0,009	2,26	0,002±0,002	6,33	0,071±0,062
Cyclohexane, 1-ethyl-2,3-dimethyl-	10,5	0	1,52	0,001±0,002	15,3	0,07±0,087	0,74	0,065±0,112	4,96	0,001±0,002	1,31	0,018±0,017	1,15	0,047±0,015	0	0,037±0,019	2,78	0	2,75	0,002±0,003	4,39	0,003±0,005	9,62	0,029±0,032
Tridecane	12,9	0	3,94	0	11	0,038±0,06	3,89	0,001±0,002	3,04	0	2,55	0,001±0,001	4,36	0	3,47	0,001	6,37	0	3,17	0	2,42	0	10,3	0,009±0,01
Dotriacontane	3,42	0,009±0,019	4,39	0	5,54	0,043±0,044	6,39	0,009±0,012	2,11	0,003±0,007	2,65	0,287±0,402	3,98	0,186±0,261	12,5	0,12±0,269	3,04	0,009±0,021	3,83	0,013±0,018	2,57	0	4,17	0,031±0,027
Heneicosane	6,99	0	14,8	0	2,1	0,022±0,023	11,9	0,018±0,023	11,6	0,02±0,045	20,9	0,843±0,254	15,9	0,229±0,24	22,3	0,24±0,329	23,8	0	8,04	0,003±0,008	11,5	0,011±0,024	8,74	0,01±0,009
Amine																								
Stearyltrimethylammonium chloride	38,6	0,18±0,186	12,2	0	7,71	0	7,79	0	13,9	0	7,34	0	10,2	0,029±0,034	6,91	0,002±0,003	12,4	0	9,56	0	12,5	0	31,8	0
Pyrazine, 2,5-dimethyl-	37,9	0,185±0,216	9,42	0,001±0,001	7,82	0	6,12	0	15,1	0	7,72	0	10,6	0,044±0,042	3,67	0,006±0,008	14,2	0	10,8	0	13	0	31,6	0
Pyrazine, trimethyl-	39,8	0,386±0,411	14,9	0	6,91	0,001	8,9	0	11,2	0	5,99	0	13,6	0,019±0,03	9,46	0,002±0,003	8,34	0,001±0,002	8,19	0	12	0	32,3	0
Stearyltrimethylammonium chloride	42,1	0,587±0,492	15,4	0,001±0,002	7,1	0	9,27	0	11,7	0,001±0,003	6,18	0	13,6	0,029±0,03	9,67	0,002±0,004	9,14	0	8,23	0	11,5	0	33,5	0,003±0,005
Methylamine, N,N-dimethyl-	41,7	0,712±0,592	15,8	0	7,46	0,001±0,002	9,43	0	12,2	0	6,7	0	14,3	0,044±0,06	9,92	0,002±0,003	9,41	0	8,79	0,001	12,7	0	33,9	0
2,6-Lutidine	3,82	0	9,62	0	7,59	0,011±0,019	15,3	0,341±0,485	9,53	0,003±0,007	2,22	0	3,35	0	14,6	0	2,02	0,002±0,004	4,82	0	6,65	0,012±0,027	5,32	0
1-Naphthalenamine, N-phenyl-	9,76	0	35,4	0	4,61	0	10,9	0,144±0,322	25	0,089±0,198	57,2	0,977±1,064	28,8	0	10,7	0,072±0,16	65,1	0	24	0	28,9	0	10,4	0
Pyrazine, tetramethyl-	3,85	0,001±0,002	14,9	0,001	4,56	0,001±0,001	10,8	0,181±0,405	5,42	0,003±0,007	8,1	0	2,74	0,008±0,011	10,2	0,002±0,002	6,62	0	5,55	0	4,97	0	3,5	0,001
Indole	27,8	0,007±0,007	5,83	0,356±0,44	6,4	0,111±0,138	3,63	0,111±0,103	13,1	0,346±0,35	8,62	0,762±0,455	11,2	0,332±0,153	8,56	0,377±0,188	9,46	0,427±0,919	8,85	1,059±1,334	19,7	1,389±0,923	27,6	4,082±7,07
Ether																								
Propyl tetradecyl ether	9,94	0,004±0,009	10	0,012±0,023	13,4	0,115±0,18	3,01	0,131±0,178	17,5	0,02±0,013	12,3	0,003±0,006	23,7	0,011±0,008	5,91	0,07±0,069	14,4	0,032±0,03	11,5	0,322±0,76	5,4	0,059±0,073	7,71	0,013±0,004
Anisole	7,12	0,318±0,406	42,9	0	2,1	0,021±0,028	18,2	0,113±0,162	17,2	0,039±0,056	33,7	0	15,1	0	9,02	0,001±0,002	36,9	0,343±0,498	11,4	0,003±0,013	18,6	0,003±0,007	8,2	0,015±0,024
Eicosyl octyl ether	9,09	0,013±0,016	7,86	0,001±0,002	7,77	0	5,19	0,002±0,005	25,5	0	21,6	0,001	31,3	0,001±0,002	7,24	0	28,1	0	24,7	2,447±5,472	17,7	0	10,4	0
Nonadecyl pentafluoropropionate	14	0,137±0,183	8,91	0,037±0,039	8,11	0,015±0,019	4,33	0,026±0,023	17,5	0,04±0,008	9,78	0,024±0,025	24,5	0,022±0,02	8,74	0,005±0,006	13,5	1,365±0,816	19,6	1,88±4,18	15	0,019±0,017	13,2	0,006±0,005
Ester																								
Pentafluoropropionic acid, nonyl ester	12,8	0,137±0,183	7,16	0,032±0,041	7,98	0,005±0,004	4,18	0,019±0,025	18,9	0,041±0,007	11,6	0,017±0,026	25,5	0,035±0,031	8,36	0,002±0,004	15,9	1,131±1,027	20,2	1,88±4,179	15,2	0,019±0,007	12,4	0,011±0,011
Ethyl Acetate	16,3	0,479±0,366	4,93	2,597±3,674	14,4	1,613±1,045	5,73	2,852±2,398	24,6	0,851±0,294	11,4	0,729±0,241	31,3	1,288±0,838	11,7	2,795±1,32	14,9	5,313±2,488	17,1	13,603±8,454	20,5	8,369±3,294	22,4	2,001±1,251
Carbonic acid, hexyl prop-1-en-2-yl ester	22	0,001	11,9	0,518±1,083	5,82	0,033±0,055	7,71	0,112±0,186	17,1	0	17,9	0,001±0,001	10,1	0,688±0,533	11,9	0,363±0,215	14,9	2,301±1,299	16,3	2,127±1,204	31,7	2,498±1,937	31,2	0,568±0,291
Acetic acid, undec-2-enyl ester	1,9	0,045±0,051	7,76	0,029±0,026	15,8	0,098±0,067	4,74	0,031±0,034	12,4	0,05±0,035	7,07	0,034±0,029	7,47	0,009±0,013	4,63	0,005±0,007	8,9	0,043±0,043	7,32	0,03±0,035	6,18	0,088±0,162	2,23	0,077±0,168
2-Propenoic acid, tridecyl ester	37,5	0,313±0,635	8,41	0,045±0,061	13,2	0,096±0,129	7,02	0,167±0,096	19,3	0,047±0,03	7,97	0,031±0,014	11,2	0,11±0,083	8,67	0,069±0,024	14,2	0,039±0,032	11,1	0,111±0,085	14,9	0,284±0,426	34,3	0,179±0,14
Ketone																								
2-Heptanone, 5-methyl-	10,8	0	32,1	0,036±0,078	10,2	0	15,9	0	34,9	0,001±0,002	51,5	0,062±0,038	77,3	1,93±1,305	32,7	0,198±0,182	61,9	0	37,8	0,002±0,003	23,4	0,096±0,116	12,8	0,033±0,024
Methyl octyl ketone	30,4	0,039±0,087	21,2	0,253±0,504	16,3	0,033±0,042	10,4	0,057±0,023	35,5	1,719±1,007	25,6	0,965±0,453	13,3	0,808±0,314	6,59	0,273±0,218	32,4	2,635±1,755	20,4	0,507±0,243	22,3	0,733±0,862	25,3	0,077±0,068
2-Butanone, 3-phenyl-	5,22	0,729±0,742	7,39	0,435±0,496	16,4	1,974±2,724	5,06	1,031±1,779	21,2	0,123±0,132	13,9	0,071±0,104	15,7	0,305±0,215	3,3	0,324±0,278	15,4	0,168±0,207	15,9	0,142±0,277	24,2	0,201±0,269	10,3	0,067±0,052
4,6-Nonanedione, 2,8-dimethyl-	4,38	0	14,2	0,735±1,644	6,05	0,071±0,143	8,51	0,189±0,424	16,4	0,081±0,137	16	0,561±0,857	11,7	0	6,9	0,072±0,16	24,1	0	14,6	0	11,5	0,006±0,008	5,85	0,002±0,003
4,6-Nonanedione, 2,8-dimethyl-	6,4	0,231±0,214	70,5	1,37±1,81	3,76	0,111±0,153	21,6	0,031±0,03	20,6	0	37,5	0	16,3	0	8,84	0	34,4	0,001	15	0	16,7	0	9,94	0
2H-Pyran-2-one, tetrahydro-6-propyl-	2,88	0,021±0,042	11,4	0,475±0,652	4,46	0,145±0,262	12,7	0,24±0,337	1,18	0,006±0,007	3,51	0,281±0,395	8,68	0,131±0,208	9,42	0,017±0,02	2,96	0,008±0,006	3,01	0,169±0,27	1,47	0,07±0,105	2,89	0,017±0,03
2,2-Dimethylheptane-3,5-dione	21	0,119±0,23	54,5	0,107±0,144	11,2	0,106±0,223	18,9	0,117±0,117	53,5	0,035±0,045	76,7	0,054±0,085	34,7	0,041±0,042	9,62	0	100	1,083±0,607	38	0,033±0,04	46,3	0,036±0,024	21,9	0
2(3H)-Furanone, dihydro-5-methyl-5-(2-methylpropyl)-	4,73	0,014±0,028	13,2	0,6±0,823	12,2	0,263±0,309	12,3	0,309±0,437	8,53	0,001±0,002	6,85	0,344±0,488	8,9	0,201±0,275	7,53	0,034±0,036	5,61	0	7,01	0,208±0,352	7,11	0,082±0,113	4,18	0,02±0,038
2-Pentanone	4,75	0,001	8,38	0,019±0,04	7,29	0,017±0,016	14,1	0,65±0,891	5,58	0,001±0,001	2,11	0,011±0,015	2,14	0,012±0,017	15,2	0,001±0,001	1,82	0	4,09	0,013±0,012	4,88	0,009±0,008	4,89	0,002±0,002
Pentan-2-one <3-methyl->	4,59	0,015±0,027	13	0,602±0,821	12,7	0,272±0,318	12,3	0,317±0,439	8,68	0,001±0,002	6,8	0,344±0,488	8,78	0,201±0,276	7,63	0,034±0,036	5,63	0	7,02	0,209±0,351	7,2	0,083±0,114	4,13	0,022±0,037
2-Heptanone	16,9	0	8,38	0,042±0,067	16,9	0,109±0,142	5,34	0,048±0,051	8,56	0	8,59	0	4,29	0	6,77	0	11,3	0	6,81	0,004±0,008	6,11	0,003±0,006	14,2	0,007±0,007
2-Heptanone, 6-methyl-	16,9	0	8,39	0,042±0,067	16,9	0,109±0,142	5,34	0,048±0,051	8,58	0	8,65	0	4,35	0	6,77	0	11,4	0	6,84	0	6,16	0,003±0,006	14,2	0,007±0,007
2-Cyclohexen-1-one, 3-methyl-	23	0	30,8	0,536±1,198	9,05	0,035±0,033	9,24	0,014±0,01	17	0,001±0,001	21,5	0,002±0,002	14,9	0,001±0,001	5,88	0,003±0,001	19,8	0	13,7	0,003±0,003	21,6	0,077±0,09	21,9	0,236±0,335
Acetophenone	10	0,022±0,01	13,9	0,326±0,431	4,16	0,104±0,191	10,8	0,192±0,249	16,6	0,049±0,041	10,1	0,239±0,23	8,19	0,123±0,114	6,49	0,021±0,021	14,6	0,035±0,02	7,29	0,237±0,065	16,1	0,087±0,153	12,4	0
2-Nonanone	1,8	0,537±0,41	43,6	0,304±0,159	1,93	0,071±0,064	8,32	0,238±0,161	11,6	0,222±0,084	38,7	3,378±7,162	21,7	0,283±0,097	3,35	0,072±0,017	34,4	0,263±0,11	12,2	0,119±0,033	13,9	0,208±0,129	3,1	0,072±0,037
Benzyl methyl ketone	4,14	1,689±0,998	13,7	3,098±4,835	14,1	2,208±2,149	3,45	1,981±2,613	8,14	2,467±3,374	11,5	0,643±0,608	7,75	3,283±5,245	3,34	1,959±3,069	12,7	0,125±0,089	4,39	0,034±0,05	9,32	0,633±0,677	5,35	3,305±3,023
2-Decanone	4,32	0,019±0,043	20	0,247±0,305	4,57	0,068±0,125	15	0,046±0,063	4,9	0,008±0,016	6,37	0,098±0,152	5,84	0,067±0,094	6,22	0,009±0,011	3,51	0	3,87	0,048±0,084	4,69	0,018±0,028	2,94	0,008±0,012
2,4,6-Cycloheptatrien-1-one	3,03	0,005±0,009	9,96	0,766±1,049	5,05	0,2±0,357	12,9	0,294±0,387	3,19	0,003±0,005	2,64	0,432±0,592	4,21	0,208±0,274	8,43	0,04±0,047	1,51	0,003±0,003	3,36	0,195±0,328	4,01	0,12±0,17	2,86	0,024±0,044
2-Decanone	8,85	0,00E+00	18	0,251±0,342	10,7	0,095±0,101	9,89	0,095±0,109	7,78	0,002±0,003	8,77	0,256±0,349	3,03	0,108±0,146	2,87	0,02±0,019	7,21	0,003±0,002	6,02	0,121±0,208	5,73	0,026±0,022	7,31	0,014±0,014
2-Undecanone	3,42	0,006±0,01	11	0,588±0,806	4,2	0,159±0,295	13	0,206±0,281	1,61	0	2,87	0,33±0,451	3,89	0,18±0,244	7,59	0,036±0,038	1,85	0,001±0,001	2,29	0,141±0,237	2,87	0,07±0,106	2,2	0,019±0,034
(Z)-Undec-6-en-2-one	13,3	0,009±0,021	14,4	0,171±0,193	6,12	0,033±0,053	10,6	0,027±0,051	13	0,044±0,035	8,88	0,171±0,238	15,5	0,074±0,095	3,98	0,013±0,007	11,6	0,004±0,003	11,8	0,05±0,083	10,4	0,046±0,048	13,6	0,026±0,019
2-Undecanone	12,8	1,00E-03	13,6	0,387±0,52	9,42	0,114±0,111	10,4	0,094±0,139	40,6	1,973±2,411	26,8	0,39±0,491	49,6	0,257±0,18	25,7	1,026±0,854	38,1	0,007±0,005	26,3	0,169±0,264	21	0,076±0,073	15,3	0,027±0,033

Decyl methyl ketone	11,9	0,004±0,007	13,4	0,397±0,544	9,95	0,106±0,139	10,4	0,119±0,155	40,4	1,992±2,411	26,4	0,411±0,51	49,6	0,253±0,175	26,1	1,046±0,882	37,4	0,009±0,006	26,1	0,159±0,274	21	0,067±0,092	14,7	0,025±0,037
2-Tridecanone	1,59	0,001±0,002	29,4	0,583±0,8	4,16	0,134±0,207	15,3	0,235±0,299	6,06	0	14,4	0,664±0,907	2,89	0,313±0,421	6,67	0,057±0,057	8,82	0,001±0,001	5,45	0,259±0,436	5,64	0,029±0,065	1,23	0,024±0,04
2-Tetradecanone	3,73	0,078±0,048	21,7	0,719±0,983	6,83	0,157±0,269	14,3	0,183±0,249	10,3	0,002±0,004	13,4	0,288±0,414	6,14	0,175±0,172	6,81	0,029±0,026	11	0,003±0,005	9,47	0,082±0,138	7,2	0,159±0,27	2,42	0,016±0,027
2-Pentadecanone	7,39	0,013±0,015	10,9	0,124±0,171	8,76	0,063±0,048	8,93	0,045±0,063	4,12	0	9,32	0,088±0,119	15,9	0,101±0,117	12,5	0,089±0,144	6,26	0,001±0,002	8,35	0,038±0,052	8,07	0,022±0,035	7,85	0,006±0,006
(Z)-Pentadec-6-en-2-one	26,6	0,29±0,141	8,81	0,145±0,174	12,4	0,01±0,009	4,73	0,099±0,084	21,7	0,058±0,032	18,2	0,148±0,074	26,6	0,197±0,071	7,22	0,02±0,005	18,1	0,096±0,048	21,1	0,029±0,017	31,7	0,125±0,11	26,4	0,011±0,01
2-Heptadecanone	13,1	0,003±0,006	12,9	0,537±0,688	7,24	0,074±0,063	5,49	0,589±0,982	8,66	0,39±0,442	25,7	0,221±0,12	35,4	2,459±2,295	9,19	0,159±0,164	17,3	2,622±2,535	21,3	0,093±0,061	29,2	0,921±1,476	12,1	0,07±0,072
2,3-Butanedione	12,5	0,018±0,023	11,3	0,106±0,127	15,5	0,177±0,291	12	0,377±0,515	21,7	0,198±0,125	15,3	0,053±0,048	14	0,042±0,028	14,8	0,008±0,006	24	0,004±0,006	11,9	0,005±0,01	17,7	0,02±0,014	13,8	0
Acetoin	1,59	0	29,4	0,276±0,378	2,12	0,061±0,107	15,1	0,093±0,135	4,82	0	13,4	0,34±0,441	5,29	0,186±0,247	5,57	0,034±0,042	7,25	0	5,18	0,115±0,193	4,02	0,017±0,039	0,55	0,011±0,017
3-Hexanone, 4-methyl-	14,6	0	15,3	0,237±0,323	13,3	0,119±0,084	8,37	0,147±0,154	5,67	0	7,13	0,297±0,407	2,92	0,124±0,175	3,73	0,026±0,027	5,45	0,001±0,002	3,26	0,097±0,163	5,21	0,01±0,012	12,6	0,011±0,014
Furan-3-one <2,3-dihydro-, 2,5-dimethyl->	3,89	0	22,8	0	8,41	0,015±0,029	13,4	0,037±0,082	14,5	0	12,3	0	6,73	0,002±0,003	6,69	0,001±0,001	13,6	0	10,8	0,002±0,005	9,5	0,027±0,036	5,16	0,003±0,003
Hexalactone <gamma->	3,23	0,046±0,072	14,1	0,021±0,014	7,74	0,021±0,038	12,3	0,084±0,107	3,66	0,014±0,004	6,55	0,01±0,007	9,62	0,015±0,012	16	0,005±0,003	7,27	0,008±0,005	3,73	0,016±0,003	2,3	0,005±0,004	2,14	0,002±0,001
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	4,64	0,186±0,255	12,5	0,224±0,34	12,6	0,124±0,14	4,57	0,073±0,141	13,7	0	8,16	0,001±0,002	8,43	0,006±0,013	4,26	0,06±0,086	6,78	0,005±0,012	8,49	0	11	0,057±0,123	8,84	0,033±0,07
2(3H)-Furanone, dihydro-5-(2-octenyl)-, (Z)-	12,8	0,006±0,014	24,9	0,261±0,315	16,2	0,116±0,121	7,96	0,091±0,136	18,9	0,003±0,006	15,5	0,009±0,019	21,2	0,006±0,013	4,99	0,133±0,072	13,1	0	13,2	0,046±0,043	15,3	0,056±0,09	14,7	0,007±0,012
Undecan-2-one	13,7	0	17,6	0,281±0,625	9,36	0	10,2	0,002±0,004	29,7	0	17,2	0,246±0,549	22,4	0,008±0,015	5,11	0,015±0,021	23,2	0	15,4	0,449±0,305	27,1	0,285±0,402	21,9	0,022±0,034
4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one	25,6	0,012±0,017	24,3	0,062±0,116	10,1	0,017±0,022	11,5	0,056±0,126	37,6	0,051±0,07	39,1	0,01±0,015	49,8	0,031±0,07	16,8	0,001±0,002	50,4	0,053±0,041	34,9	0,304±0,148	24,7	0,049±0,089	23,4	0,005±0,007
2-Dodecanone	3,96	0,061±0,092	7,76	0	9,2	0,021±0,043	3,24	0,002±0,003	15	0	7,56	0	5,5	0	5,35	0	7,54	0	8,46	0	19,5	0,046±0,102	8,6	0,001
5-Ethyl-4-tridecanone	14,4	0	16,1	0	13,6	0	11	0	21,9	0	15,6	0,268±0,12	78	0,392±0,254	26	0,005±0,012	10,5	0	28,3	0	13,1	0	14,8	0
(Z)-Pentadec-6-en-2-one	5,45	0	3,73	0	10,5	1,055±2,043	2,2	0,552±1,199	10,3	2,107±2,278	6,51	0,039±0,086	6,46	1,544±3,181	4,26	1,254±1,082	5,67	2,305±0,842	6,89	0,684±1,997	8,82	1,285±1,563	4,97	0,829±1,381
Tetradec-(7Z)-en-2-one	18,9	0	5,77	0	5,42	0,019±0,03	2,87	0,016±0,022	9,26	0,016±0,036	5,62	0	17,8	0	8,59	0,002±0,004	6,08	0	9,23	0	6,29	0,147±0,329	17	0,107±0,206
3,3-Dimethyl-2,4-pentane dione	2,34	0,07±0,112	5,48	0	7,71	0,031±0,047	3,75	0,005±0,011	17,6	0,002±0,004	8,92	0,002±0,005	3,59	0	5,62	0,065±0,13	9,16	0,002±0,005	10,8	0,025±0,019	23,9	0,211±0,472	8,86	0,028±0,036
4-Heptanone	21,6	1,251±2,326	16,7	0,074±0,057	2,12	0,02±0,034	7,63	0,218±0,18	10,3	0,059±0,033	8,35	0,177±0,097	11,2	0,114±0,042	6,18	0,097±0,166	5,7	0,124±0,063	7,8	0,105±0,052	4,08	0,174±0,246	13,2	0,05±0,098
2-Octanone	8,74	0,001±0,002	10,7	0,003±0,006	13,2	0,254±0,536	4,82	0,018±0,018	17	0,02±0,045	10,1	0,065±0,134	6,37	0,015±0,025	2,67	0,006±0,005	12,6	0,015±0,006	8,09	0,011±0,013	10,8	0,001±0,002	9,5	0,036±0,07
3-Hexanone, 2,5-dimethyl-	6,99	0	4,84	0,129±0,289	11,4	0,085±0,137	4,02	0,008±0,014	6,07	0,003±0,006	3,94	0,01±0,013	14,5	0,007±0,006	8,49	0,007±0,004	2,45	0,002±0,005	7,03	0,002±0,003	3,94	0,096±0,189	5,83	0,116±0,151
3,5-Heptanedione, 2,6-dimethyl-	13	0,927±1,252	3,25	1,381±1,81	5,83	0,667±0,616	4,65	1,521±0,44	8,45	2,726±2,526	9,78	3,425±2,851	14,3	1,641±1,501	6,79	0,943±1,031	10	2,379±2,166	9,39	4,243±3,422	13,8	2,674±3,836	14,6	0,899±0,609
(Z)-Tetradec-6-en-2-one	4,22	0,104±0,055	3,12	0,006±0,01	10,6	0,042±0,061	3,28	0,002±0,004	4,56	0,005±0,005	2,6	0	9,8	0,003±0,007	7,76	0,001±0,002	4,07	0,017±0,02	4,92	0,01±0,015	2,22	0,033±0,065	2,27	0,036±0,052
5-Octen-2-one, 3,6-dimethyl-	34,4	0,348±0,2	15,6	0,005±0,009	4,42	0,014±0,023	8,12	0,02±0,037	10,1	0,005±0,006	8,44	0,005±0,008	2,8	0,028±0,026	7,8	0,001±0,001	12,6	0,001±0,001	6,37	0,002±0,003	10,3	0,011±0,025	26,2	0,012±0,022
Ethanone, 1-cyclododecyl-	4,62	0,005±0,006	5,99	0,001±0,001	3,88	0,001±0,003	7,72	0,002±0,002	8,16	0	7,37	0,003±0,008	32,7	0	23,5	0,027±0,039	7,55	0,001	10,7	0,003±0,004	4,33	0	5,29	0
2-Pentadecanone	16,4	0,088±0,081	5,63	0,013±0,029	1,84	0,007±0,009	8,14	0,008±0,011	6,98	0,036±0,061	8,05	1,023±0,653	6,16	0,924±0,536	19,8	0,794±0,151	12	0,048±0,051	5,12	0,002±0,008	6,33	0,594±1,274	13,5	0,325±0,716
Methyl Isobutyl Ketone	7,41	0,041±0,043	3,32	0,035±0,038	1,4	0,038±0,085	12,5	0,295±0,52	18,5	1,883±1,57	22,9	2,471±0,64	35	4,551±2,355	35,1	1,512±0,8	34,6	0,023±0,035	13,5	0,058±0,055	8,47	0,574±0,601	7,74	0,477±0,627
Hexatriacontane	16,6	0,037±0,04	5,3	0,023±0,036	2,29	0,009±0,007	8,15	0,009±0,011	8,47	0,036±0,061	8,52	1,024±0,651	9,76	0,665±0,774	20,2	0,793±0,151	13,8	0,021±0,047	6,31	0	6,25	0,565±1,224	13,8	0,323±0,717
Sulfur compound																								
Sulfur dioxide	14,2	0,041±0,038	20,9	0,039±0,05	6,59	0,016±0,012	7,23	0,029±0,036	28,3	0,06±0,05	26,1	0,128±0,121	8,99	0,047±0,064	6,49	0,027±0,028	33,5	0,09±0,163	17,4	0,091±0,179	34,4	0,222±0,266	20,9	0,014±0,017
Borane-methyl sulfide complex	26,6	0,29±0,141	8,81	0,145±0,174	12,4	0,01±0,009	4,73	0,099±0,084	21,7	0,058±0,032	18,2	0,148±0,074	26,6	0,197±0,071	7,22	0,02±0,005	18,1	0,096±0,048	21,1	0,029±0,017	31,7	0,125±0,11	26,4	0,011±0,01
Methanesulfonic anhydride	16,7	0,183±0,352	15,8	0,003±0,005	2,68	0,001±0,003	4,81	0,012±0,021	8,53	0,022±0,029	16,1	0,003±0,004	10,7	0,026±0,016	6,28	0,003±0,003	14,5	0,052±0,049	12,1	0	20,5	1,103±2,437	18,3	0,275±0,612
Carbon disulfide	5,08	0	11,9	0,001±0,002	6,59	0	15,1	2,38±2,807	6,96	0	4,39	0	2,2	0	14,9	0	2,82	0	4,92	0	6,82	0	5,32	0
Dimethyl disulfide	2,38	0	20,2	2,228±2,151	0,84	0,388±0,696	15,4	0,572±0,815	5,47	0,055±0,068	8,22	2,306±3,157	2,68	1,011±1,384	4,75	0,204±0,182	4,15	0	3,26	0,918±1,585	6,14	0,402±0,603	2,86	0,075±0,118
Dimethyl trisulfide	3,48	0,078±0,108	20,8	0,931±0,894	5,73	0,174±0,292	17,1	0,245±0,293	10,7	0,015±0,01	8,03	0,541±0,727	8,29	0,254±0,299	8,29	0,067±0,049	10,2	0,045±0,034	6,9	0,216±0,364	6,9	0,127±0,176	3,57	0,036±0,031
Tetrasulfide <methyl->	13,1	0,01±0,013	8,9	0,097±0,12	16	0,179±0,289	11,4	0,376±0,516	20,9	0,202±0,135	11,7	0,053±0,049	13,7	0,023±0,025	14,8	0,008±0,003	20,2	0,007±0,007	11,3	0,003±0,01	17	0,009±0,014	14,4	0,002±0,004

To identify differences among the complete volatile profile released by larvae infested by EPNs and by larvae killed by liquid nitrogen, a PermMANOVA was performed. The analysis reveals that the odour profile of larvae differs according to the killing method ($F_{2.57}$=6.3643; *p*-value=0.001). These results are illustrated by the PCA (Figure 3).

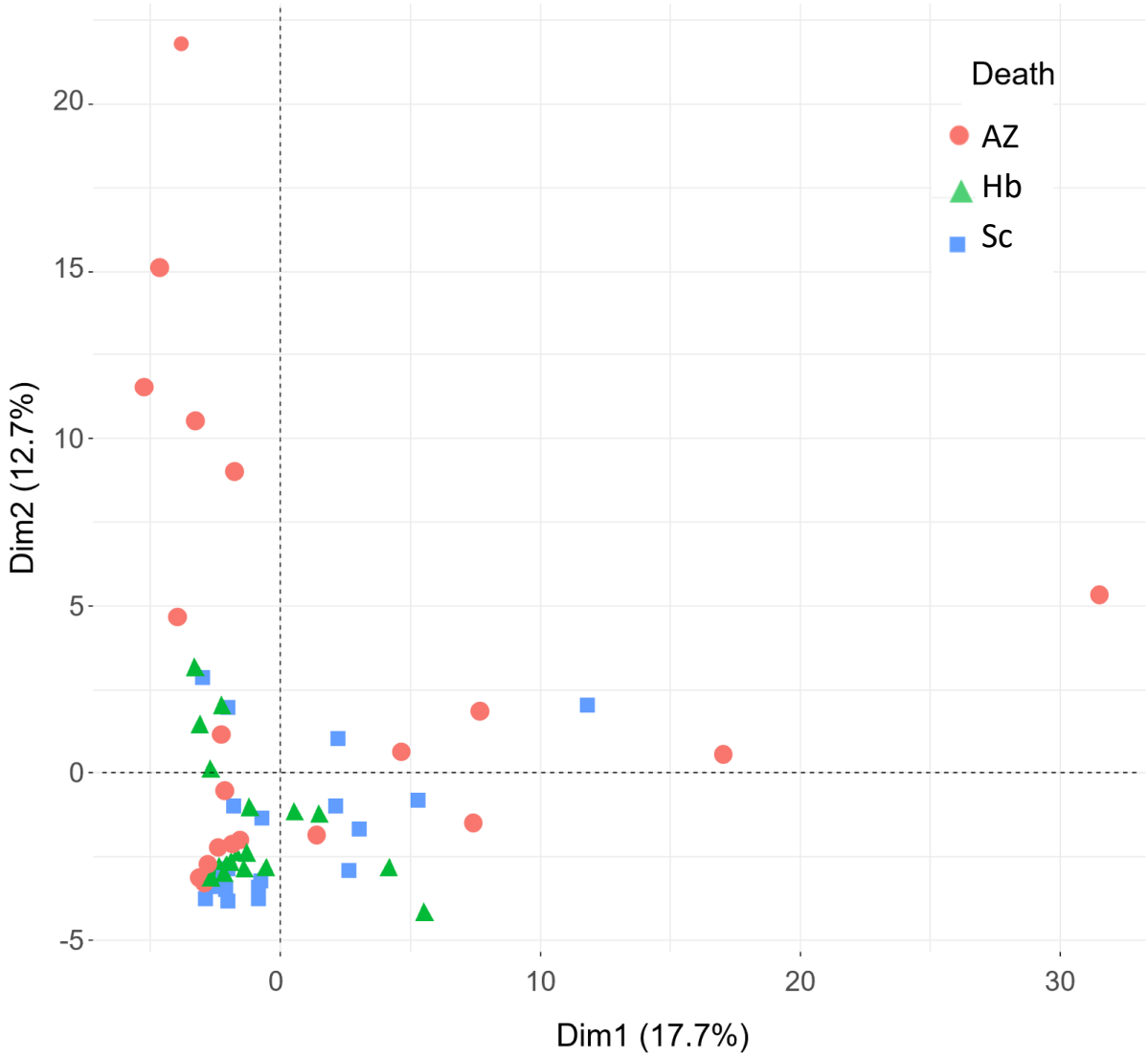

Figure 3 Principal Component Analysis (PCA) performed on the VOC profiles of liquid nitrogen (AZ), *Heterorhabditis bacteriophora* (Hb), and *Steinernema carpocapsae* (Sc) conditions (n = 20).

To identify differences among the odour profile originating from the three killing methods and various PMI, a predictive analyse has been performed using a PLS-DA test. The analysis of volatile fingerprints highlights the important variables of the different conditions. This model includes 12 factors and achieves an overall accuracy of 100% (Kappa = 1). The high sensitivities (all factors = 1.00) and high specificities (all factors = 1.00) demonstrate strong reliability of the model. The high accuracy of this model indicates that each modality is characterised by a VOC profile in both qualitative and quantitative terms.

Table 2 Classification performance metrics for the PLS-DA models based on the odour profile

Reference	AZJ1	AZJ3	AZJ7	AZJ11	HbJ1	HbJ3	HbJ7	HbJ11	ScJ1	ScJ3	ScJ7	ScJ11
AZJ1	5	0	0	0	0	0	0	0	0	0	0	0
AZJ3	0	5	0	0	0	0	0	0	0	0	0	0
AZJ7	0	0	5	0	0	0	0	0	0	0	0	0
AZJ11	0	0	0	5	0	0	0	0	0	0	0	0
HbJ1	0	0	0	0	5	0	0	0	0	0	0	0
HbJ3	0	0	0	0	0	5	0	0	0	0	0	0
HbJ7	0	0	0	0	0	0	5	0	0	0	0	0
HbJ11	0	0	0	0	0	0	0	5	0	0	0	0
ScJ1	0	0	0	0	0	0	0	0	5	0	0	0
ScJ3	0	0	0	0	0	0	0	0	0	5	0	0
ScJ7	0	0	0	0	0	0	0	0	0	0	5	0
ScJ11	0	0	0	0	0	0	0	0	0	0	0	5
Sensitivity	1	1	1	1	1	1	1	1	1	1	1	1
Specificity	1	1	1	1	1	1	1	1	1	1	1	1

Each identified VOC was associated with a confidence coefficient, indicating its degree of influence in the prediction profile for each class/factor (AZJ1, AZJ3, AZJ7, AZJ11, HbJ1, HbJ3, HbJ7, HbJ11, ScJ1, ScJ3, ScJ7, and ScJ11). A higher coefficient suggests a greater contribution of the compound to the discrimination of the factor compared to others. The representation of these coefficients on a spider chart (Figure 4) revealed that the odour profiles of larvae infested by the two entomopathogenic nematode strains (*S. carpocapsae* and *H. bacteriophora*) at PMI1, PMI3, and PMI7 are influenced by a reduced number of significant variables, unlike the odour profile of larvae killed by liquid nitrogen. Moreover, each of these variables has a higher coefficient, which makes it stand out from the other variables. Furthermore, starting from PMI11, a similarity emerges between the odour profiles of infected larvae and those of larvae killed by liquid nitrogen.

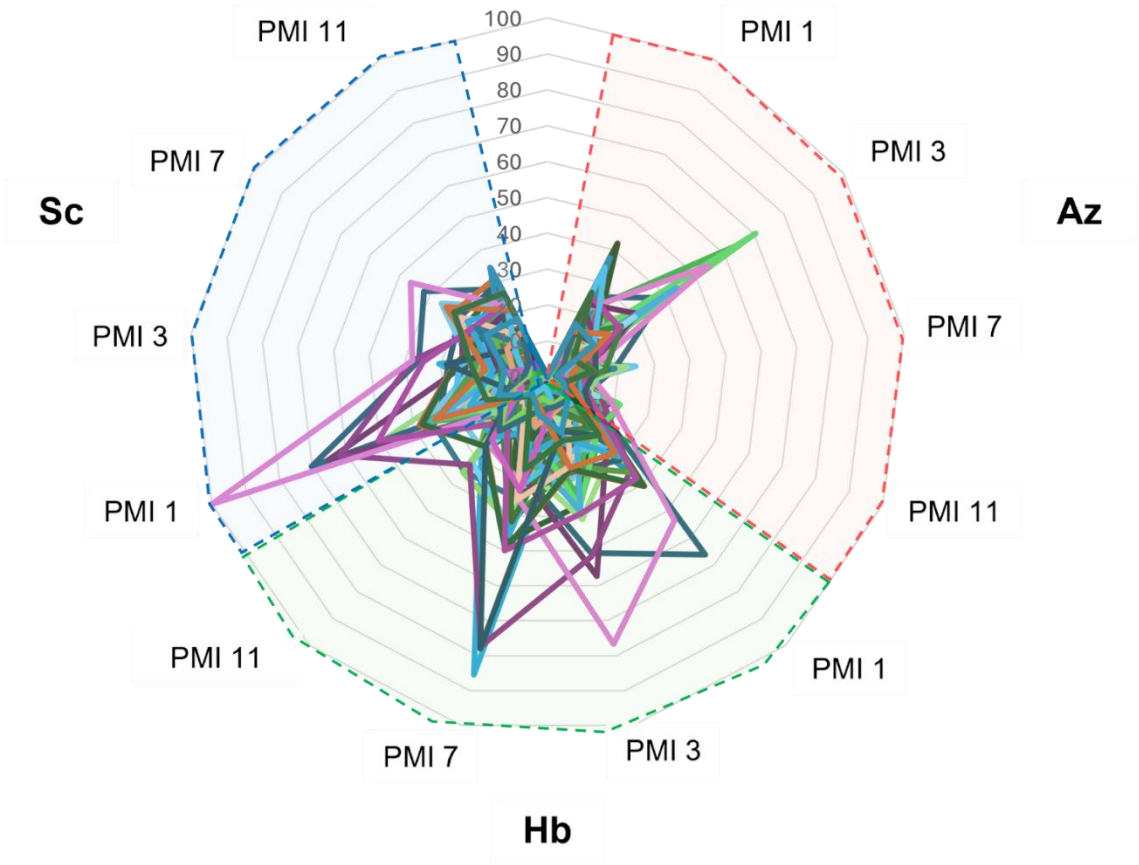

Figure 4 The spider chart displays all detected compounds represented by each line and their respective contribution in the predicted models generated from the PLS-DA analysis. Compounds that do not contribute to the predicted model are centralised on the chart, whereas those extending towards the periphery significantly influence the model predictions. Colours allow to individualise each compound on the chart.

The PLS-DA indicated that the chemical compounds present in each olfactory profile allow for significant differentiation of modalities (*p*-value = 2.2e-16).

AZ-PMI1 profiles are characterised by six compounds with a confidence coefficient of ≈ 40%: pentadecafluorooctanoic acid tridecyl ester, pentadecafluorooctanoic acid tetradecyl ester, dodecane 4,6-dimethyl-, 1-dodecanol 3,7,11-trimethyl-, pentadecafluorooctanoic acid undecyl ester, and pyrrole. Regarding their respective relative abundances (Table 2), we can observe that their high contribution to the profile characterization is associated with high abundances. In comparison, the major compounds acetoin and 2,3-butanedione also contribute highly to the profile specificity but with a lower confidence coefficient of 24% and 20%, respectively.

AZ-PMI3 profiles are characterised by Trimethyl oxazole, 2,3-butanedione, and 1-Hexanol are the compounds with the highest confidence coefficients, at 70%, 65%, and 54%, respectively. This aligns with the ranking as major compounds and confirms the importance of 2,3-butanedione in identifying the AZJ3 odour profile.

AZ-PMI7 profiles are characterised by 2-Propanol, 1-(2-methoxypropoxy)-, 3-Pentanol, 2,4-dimethyl-, Octanal, and 2-Heptanone are key variables with confidence coefficients of at least 20%. There is little difference in confidence among these compounds, which corresponds to the increased number of major compounds described earlier for AZ-PMI7.

AZ-PMI11 profiles are characterised by Trimethyl oxazole and 2,3-butanedione are the key variables with confidence coefficients exceeding 20%. As with AZ-PMI7, the difference in confidence values across all compounds in the AZ-PMI11 odour profile is minimal, which aligns with the increase in the number of major compounds.

Hb-PMI1 profiles are characterised by Hexane, 2,4-dimethyl- and 1-Hexanol have confidence coefficients of 66% and 53%. These trace compounds have very low relative abundance, but still contributes to the characterization of the profile of this modality.

Hb-PMI3 profiles are characterised by 1-Hexanol, 3-Heptanol 5-methyl-, 2-Pentanol 4-methyl-, and Hexane 2,4-dimethyl- have confidence coefficients of 76%, 57%, 51%, and 50%, respectively. Given their high percentages, these traces are significant for the odour profile.

Hb-PMI7 profiles are characterised by Pentafluoropropionic acid nonyl ester, Hexadecane, 1,16-dichloro-, and 2-Pentanol 4-methyl- have confidence coefficients of 85%, 77%, and 77%, respectively. Although these trace compounds have low relative abundance, they carry significant confidence coefficients.

Hb-PMI11 profiles are characterised by Acetophenone, Methyl Isobutyl Ketone, 2-Pentanol 4-methyl-, and Dimethyl disulfide are the four compounds with confidence coefficients reaching 30%. Notably, the presence of Dimethyl disulfide as a major compound highlights its importance in the odour profile.

Sc-PMI1 profiles are characterised by This profile has compounds with relatively high confidence coefficients compared to the previous ones. The five compounds with percentages exceeding 50% are 1-Hexanol (100%), Hexane, 2,4-dimethyl- (70%), 3-Heptanol, 5-methyl- (65%), 2-Pentanol 4-methyl- (61%), and 1-Nonanol (50%). The significance of 1-Hexanol as a trace compound in the SCJ1 odour profile should be noted.

Sc-PMI3 profiles are characterised by 1-Hexanol, 2-Pentanol 4-methyl-, Hexane, 2,4-dimethyl-, 1-Nonanol, and Pentafluoropropionic acid nonyl ester are the key variables with confidence coefficients exceeding 30%. These trace compounds have low relative abundance.

Sc-PMI7 profiles are characterised by 1-Hexanol and Hexane, 2,4-dimethyl- have confidence coefficients of 46% and 41%, respectively. Given their recurrence across the different odour profiles previously described, these trace compounds should be considered.

Sc-PMI11 profiles are characterised by Pyrrole, Pentadecafluorooctanoic acid tetradecyl ester, Pentadecafluorooctanoic acid tridecyl ester, Dodecane, 4,6-dimethyl-, 1-Dodecanol, 3,7,11-trimethyl-, Pentadecafluorooctanoic acid undecyl ester, and 2-Pentanol are the compounds that reach 30%. It is worth noting, once again, that the gap in confidence coefficients across all compounds narrows as the number of major compounds increases (Figure 2).

Behavioural test

*Test 1 - Preference Test_* This test assesses whether a *G. mellonella* larva prefers to stay near a larva infected by entomopathogenic nematodes (*H. bacteriophora*) rather than in an empty zone. The results for PMI1, PMI3, and PMI11 showed an initial preference for dead zones, with 67%, 53%, and 60 %, respectively. For the final choice, only PMI3 reached a percentage greater than 50% (63%).

Table 3 Preference zone test results. The *p*-values are obtained through the Chi-square independence test.

		Initial Choice				Final Choice				Average time (seconde±Sd)			
		Empty	Dead	p-value	X-squared	Empty	Dead	p-value	X-squared	Empty	Dead	p-value	X-squared
Infected	PMI1	33	67	0,020	5,454	53	47	0,549	0,360	145,5±11,3	154,5±11,6	0,603	0,270
	PMI3	47	53	0,549	0,360	37	63	0,009	6,760	133,3±10,8	166,7±12,5	0,1	3,700
	PMI7	53	47	0,549	0,360	50	50	1,000	1,000	143,3±11,5	156,5±11,7	0,455	0,455
	PMI11	40	60	0,046	4,000	63	37	0,020	5,454	155,3±12,1	144,7±11,6	0,616	0,252
AZ	PMI1	50	50	1,000	0,000	62	38	0,016	5,760	177,9±14,7	122,1±14,7	0,001	10,390
	PMI3	71	29	<0,001	17,640	57	43	0,162	1,960	229,4±11,8	70,6±11,8	<0,001	84,058
	PMI7	57	43	0,162	1,960	43	57	0,162	1,960	87±13	213±13	<0,001	52,920
	PMI11	38	62	0,016	5,760	50	50	1	0	136±13,4	164±13,4	0,106	2,613

The larvae were significantly attracted to the infested larvae in the initial choice for the PMI1 and PMI11 modalities (Table 3). The larvae were significantly attracted to the infested larvae (death zone) in the final choice for the PMI3 modality and PMI11.
For AZ, the larvae were significantly attracted to the empty zone in the initial choice for the PMI3 and PMI11. The larvae were significantly attracted to the empty zone in the final choice for the PMI1. Regarding the average time, the larvae showed a significant preference for the empty zone in PMI3 and a significant preference for the dead zone for the death zone in PMI7.

*Test 2 - Pitfall Test_* This test assesses if a larva was attracted to a larva infested by Hb EPNs at PMI1, PMI3, PMI7, and PMI11, and to larva killed by liquid nitrogen (AZ). For the PMI1, PMI7, PMI11, and AZ conditions, only 4% of the 30 larvae made a choice (n=12). Over 63% of the tested larvae had made a choice during the PMI3 assays.
Larvae that made a choice were attracted to liquid nitrogen-killed larva (X-squared = 0.667; *p*-value = 0.321). Larvae were also attracted to infested larvae at PMI3 (X-squared = 0.889; *p*-value = 0.346) and PMI11 (X-squared = 0667; *p*-value = 0.321). At PMI7, a similar number of larvae were attracted to the infested side as to the empty side. The chi-square test showed no significant difference (Figure 5).

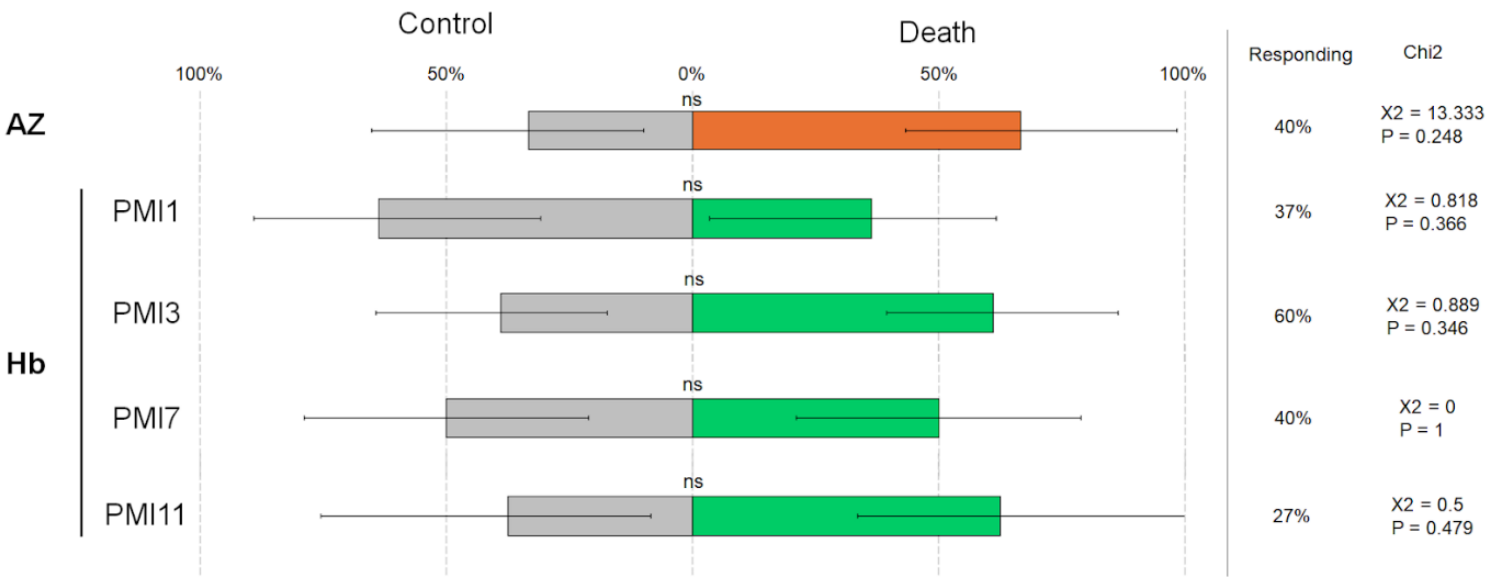

Figure 5 Percentage of larvae reaching one side of the glass cylinder tube during the pitfall test. Statistical values were determined by Chi-square independence test (ns = not significant differences).

*Test 3 - Escape Time Test_* This test was performed to assess the escape behaviour of larvae in an arena containing either a larva infested with *H. bacteriophora* EPNs or a larva killed by nitrogen (AZ). On average, larvae exit the area after 19.0±14.1 in presence of AZ killed larva, while they escaped after 50.4±8.5, 32.9±6.3, 23.4±1.9, and 40.1±7.5, for infested larva at PMI1, PMI3, PMI7, and PMI11, respectively (Figure 6).

The results from the Kruskal-Wallis test showed that the larvae escape times measured in PMI, PMI3 and PMI7 were significantly different (PMI1: chi-squared = 9.6, *p*-value = 0.008; PMI3: chi-squared = 8.4, *p*-value = 0.015: PMI7: chi-squared = 8.3, *p*-value = 0.016). The times measured in PMI11 were not significantly different (chi-squared = 4.0, *p*-value = 0.138). Post-hoc tests showed that larvae took longer to escape in the presence of Hb-infested larvae compared to larvae killed by liquid nitrogen in PMI1 (Z = -2.7; *p*-value = 0.022) and PMI3 (Z = -2.8; *p*-value = 0.014). The comparison between the PMI of the AZ modality and the comparison between the PMI of the Hb modality are not significant (all p-values > 0.05).

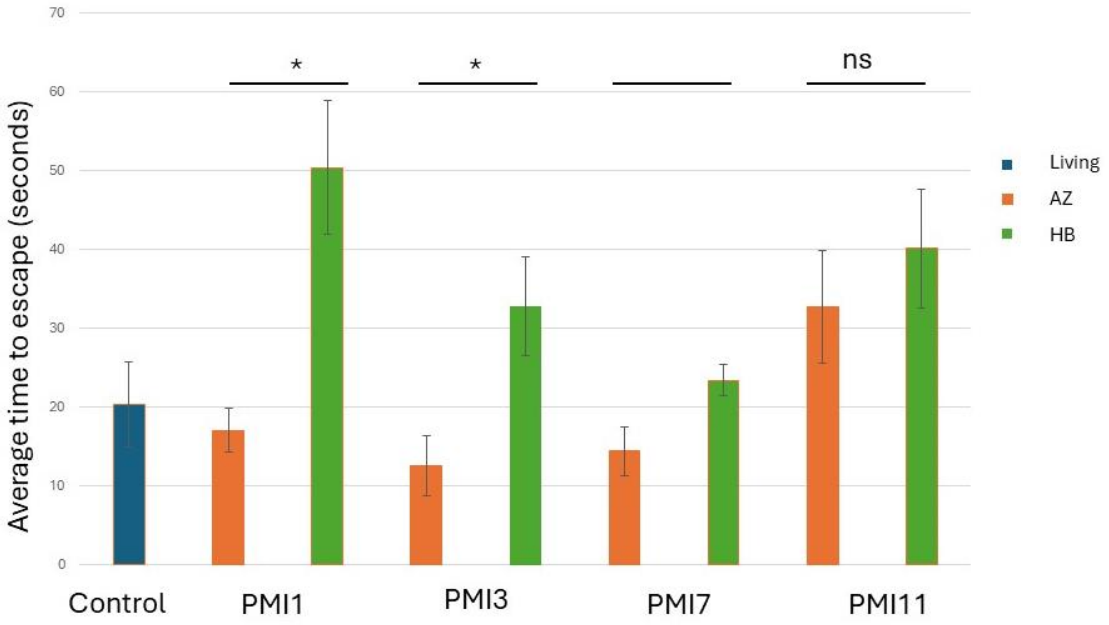

Figure 6 Larvae time to escape (mean ± SD) in presence of alive, or dead larvae. Significance was determined using the Kruskal-Wallis test. The figure 6 shows the p-values of the Dunn test between liquid nitrogen (AZ), and *Heterorhabditis bacteriophora* (Hb). ns: not significant; *: <0,05 ; **:<0,01 ; ***: <0,001.

Discussion

The first objective of this study was to identify the odour profiles of larvae infected by entomopathogenic nematodes. We observed differences in odour profiles between infected and non-infected insects, as well as between different stages of infection. The second objective was to determine whether these volatile organic compounds (VOC) attract non-infected insects. We observed an attractive effect on non-infected insects, at least at the beginning of the entomopathogenic nematodes' reproductive cycle.

The volatile profile of dead insects infected by entomopathogenic nematodes differs between the two species of entomopathogenic nematodes, as well as from the odour of non-infected dead insects, and each profile was statistically distinct. However, we noted similarities in the composition of volatile profiles, particularly in terms of major compounds, some of which are markers of decomposition (Verheggen et al., 2017). Among these, the sulphide class and 2-heptanone are characteristic and particularly abundant ((Benbow & Pechal, 2019; LeBlanc et al., 2020)). The sulphide class is predominant at the beginning and end of the entomopathogenic nematode cycle (PMI1 and PMI11). However, the physical appearance of the larvae during the experiments was quite different, with marked decomposition observed only at the end of the cycle. Indeed, a dark coloration of the larvae and a loss of rigidity, which was present at the beginning of the cycle, were observed. Previous studies (Tarasco et al., 2023) have shown that the integrity of the larvae supports the functioning of the nematode reproductive cycle. This integrity is maintained due to the role of symbiotic bacteria, which kill the host and produce secondary metabolites that slow the

decomposition process (Vallet-Gely et al., 2008). The exponential growth of bacteria occurs throughout the entomopathogenic nematode cycle. Once the cycle is complete, the bacteria are reabsorbed by the nematodes and enter an inactive state. Thus, this leads to the cessation of secondary metabolite production that preserves the host's integrity (Tarasco et al., 2023b). This could also explain the increasing number of compounds released during decomposition, up until the final moment when this number decreased. Most major compounds (e.g., 2,3-butanedione; 2-nonanone; 2-heptanone) exhibit high coefficients, reinforcing the idea that these compounds play a central role in distinguishing odour profiles between different entomopathogenic nematode species and the AZ odour profile corresponding to larvae killed with liquid nitrogen. Interestingly, some compounds, although present in very low quantities, also have high predictive coefficients. This observation highlights that beyond the major compounds, certain low-concentration or absent compounds can significantly influence the total odour profiles. These minor compounds could act as attractants or repellents to other larvae (Gajger & Dar, 2021).

Regarding the behavioural tests, our results did not show a significant attraction of live larvae towards infected larvae, except during the initial choices in test1 (PMI1 and PMI11) . However, studies have shown that pathogens can attract insects by producing VOC. For example, this is the case with an entomopathogenic fungus (Muslim & Al-Zurfi, 2019).

We propose several explanations for the lack of general attraction. The first is the choice of the insect model, *Galleria mellonella*. These larvae naturally exhibit an instinct to seek food and avoid danger. This suggests that they might not be naturally attracted to others of the same species, and therefore, a cooperative system may not have developed in this species, unlike what is observed in some other insects. Indeed, this species is not known to be gregarious (Kwadha et al., 2017). During the tests, it was observed that the larvae often circled the glass dish, seemingly trying to find an exit. Additionally, in the "pitfall" test, the larvae's response rate was low (<50%), which may reflect their natural behaviour, more focused on escaping the setup rather than making a definitive choice. To improve the response rate, we suggest extending the duration of both the "pitfall" and preference tests. Moreover, increasing the number of replicates could help confirm or refute the observed attraction trend. Finally, the experimental setup for the behavioural tests used a single infected larva compared to seven larvae for odour profile determination. If there is any attraction or repulsion by *G. mellonella*, it is possible that the amount of volatile organic compounds influencing the larvae's behaviour was insufficient in the test setup. We found that uninfected larvae display significant escape behaviour when exposed to infected conspecifics. Other examples of insect-pathogen relationships have already been demonstrated by (Mann et al., 2012). A pathogen found in the plant's phloem produced VOC that attracted the insect. This reaction is lower at the beginning of the entomopathogenic nematode reproductive cycle, while the reaction is quicker in the presence of larvae killed with liquid nitrogen. This suggests that uninfected larvae detect a scent, and their reaction varies depending on the odour

profile identified earlier. It is noteworthy that there is a correlation between the behavioural test results and the obtained odour profiles. Specifically, the VOC emitted throughout the entomopathogenic nematode reproductive cycle influence the reactions of *G. mellonella* (Chantab et al., 2024).

Samples were collected after the death of *G. mellonella* larvae. We suggest collecting odour samples from larvae infected before their death (Baiocchi et al., 2017). Indeed, during this very short phase, the nematodes enter their host and release symbiotic bacteria. These bacteria then become pathogenic and produce various chemical compounds (toxins, secondary metabolites). It is interesting to analyse this phase because the internal chemical reactions could be reflected externally. Moreover, the significance of these compounds could vary, with some being predominant at the beginning but only present in trace amounts compared to the odour profile of still-living infected larvae.
The odour profiles identified for the two entomopathogenic nematode species may differ in terms of major compounds. Each of the two nematode species is associated with a specific bacterial species. It would be interesting to identify the odours produced by *G. mellonella* larvae in the presence of only these bacteria, for example, by directly injecting the bacteria into the host.
It would also be interesting to conduct behavioural tests with the second species of entomopathogenic nematode (*Sc*) and compare the results. Given that their profile is different, it may be possible to identify chemical compounds - whether trace or major - that affect the behaviour of *G. mellonella*. Finally, it would be interesting to identify characteristic compounds emitted by *G. mellonella* larvae after infection by entomopathogenic nematodes. These could be tested separately or in combination on insect behaviour.

Bibliographie

Baiome, B. A., Ye, X., Yuan, Z., Gaafar, Y. Z. A., Melak, S., & Cao, H. (2022). Identification of Volatile Organic Compounds Produced by Xenorhabdus indica Strain AB and Investigation of Their Antifungal Activities. *Applied and Environmental Microbiology*, *88*(13). https://doi.org/10.1128/AEM.00155-22/SUPPL_FILE/AEM.00155-22-S0001.PDF

Barsics, F., Haubruge, E., & Verheggen, F. J. (2013). *Le taupin : un ravageur souterrain préoccupant*. http://www.gerbeaud.com/jardin/fiches/taupin.phphttp://www.syngenta-agro.frorganicagcentre.caLarve

Benbow, M. E., & Pechal, J. L. (2019). Forensic Entomology and the Microbiome. *Forensic Entomology*, 499–517. https://doi.org/10.4324/9781351163767-25

Boemare, N. E., Akhurst, R. J., & Mourant, R. G. (1993). DNA relatedness between Xenorhabdus spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer Xenorhabdus luminescens to a new genus, Photorhabdus gen. nov. *International Journal of Systematic Bacteriology*, *43*(2), 249–255. https://doi.org/10.1099/00207713-43-2-249/CITE/REFWORKS

Boemaret A N, N. E. (1988). Biochemical and Physiological Characterization of Colony Form Variants in Xenorhabdus spp. (Enterobacteriaceae). In *Journal of General Microbiology* (Vol. 134).

Campbell, J. (2010). High-Throughput Assessment of Bacterial Growth Inhibition by Optical Density Measurements. *Current Protocols in Chemical Biology*, *2*(4), 195–208. https://doi.org/10.1002/9780470559277.CH100115

Chacón-Orozco, J. G., Bueno, C. J., Shapiro-Ilan, D. I., Hazir, S., Leite, L. G., & Harakava, R. (2020). Antifungal activity of Xenorhabdus spp. and Photorhabdus spp. against the soybean pathogenic Sclerotinia sclerotiorum. *Scientific Reports 2020 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-77472-6

Chantab, K., Rao, Z., Zheng, X., Han, R., & Cao, L. (2024). Ascarosides and Symbiotic Bacteria of Entomopathogenic Nematodes Regulate Host Immune Response in Galleria mellonella Larvae. *Insects*, *15*(7), 514. https://doi.org/10.3390/INSECTS15070514/S1

ChatGPT. (n.d.). Retrieved March 27, 2024, from https://chat.openai.com/
Use for translation assistance and spelling check

Eidt, D. C., & Thurston, G. S. (1995). PHYSICAL DETERRENTS TO INFECTION BY ENTOMOPATHOGENIC NEMATODES IN WIREWORMS (COLEOPTERA: ELATERIDAE) AND OTHER SOIL INSECTS. In *The Canadian Entomologist* (Vol. 127).

Erdem, E., Yüksel, E., & Canhilal, R. (2024). Evaluation of cell-free supernatants from the symbiotic bacteria of entomopathogenic nematodes for controlling the colorado potato beetle [Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae)]. *Journal of Plant Diseases and Protection*, *131*(3), 731–742. https://doi.org/10.1007/S41348-024-00894-1/TABLES/9

Forst, S., & Nealson, K. (1996). Molecular Biology of the Symbiotic-Pathogenic Bacteria Xenorhabdus spp. and Photorhabdus spp. In *MICROBIOLOGICAL REVIEWS* (Vol. 60, Issue 1). https://journals.asm.org/journal/mr

Gabriela Ioan, B., Manea, C., Hanganu, B., Statescu, L., Solovastru, L. G., & Manoilescu, I. (n.d.). *The Chemistry Decomposition in Human Corpses*. http://www.revistadechimie.ro

Gajger, I. T., & Dar, S. A. (2021). Plant Allelochemicals as Sources of Insecticides. *Insects 2021, Vol. 12, Page 189*, *12*(3), 189. https://doi.org/10.3390/INSECTS12030189

Gangwar, P., Trivedi, M., & Tiwari, R. K. (2022). Entomopathogenic Bacteria. *Microbial Approaches for Insect Pest Management*, 59–79. https://doi.org/10.1007/978-981-16-3595-3_2/FIGURES/6

Hiltpold, I., Hibbard, B. E., French, B. W., & Turlings, T. C. J. (2012). Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. *Plant and Soil*, *358*(1–2), 11–25. https://doi.org/10.1007/s11104-012-1253-0

Hurtado, A. C., Ruhland, F., Boullis, A., Verheggen, F. J., & Verheggen, F. (2023). Potato varietal susceptibility to wireworms: feeding behaviour, fitness and semiochemical-based host selection. *Article Published in Entomologia Generalis*, *43*, 1193–1201.

Jari Oksanen [aut, cre], Gavin L. Simpson [aut], F. Guillaume Blanchet [aut], Roeland Kindt [aut], Pierre Legendre [aut], Peter R. Minchin [aut], R.B. O'Hara [aut], Peter Solymos [aut], M. Henry H. Stevens [aut], Eduard Szoecs [aut], Helene Wagner [aut], Matt Barbour [aut], Michael Bedward [aut], Ben Bolker [aut], Daniel Borcard [aut], Gustavo Carvalho [aut], Michael Chirico [aut], Miquel De Caceres [aut], Sebastien Durand [aut], & Heloisa Beatriz Antoniazi Evangelista [aut]. (n.d.). *Vegan community ecology package*. Retrieved August 16, 2024, from https://www.researchgate.net/publication/360782912_vegan_community_ecology_package_version_26-2_April_2022#fullTextFileContent

Jorjão, A. L., Oliveira, L. D., Scorzoni, L., Figueiredo-Godoi, L. M. A., Prata, M. C. A., Jorge, A. O. C., & Junqueira, J. C. (2018). From moths to caterpillars: Ideal conditions for galleria mellonella rearing for in vivo microbiological studies. *Virulence*, *9*(1), 383–389. https://doi.org/10.1080/21505594.2017.1397871

Kim, J., Hiltpold, I., Jaffuel, G., Sbaiti, I., Hibbard, B. E., & Turlings, T. C. J. (2021). Calcium-alginate beads as a formulation for the application of entomopathogenic nematodes to control rootworms. *Journal of Pest Science*, *94*(4), 1197–1208. https://doi.org/10.1007/s10340-021-01349-4

Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K., Fombong, A. T., Stout, M. J., Davis, J., Diaz, R., & Beuzelin, J. M. (2017). The Biology and Control of the Greater Wax Moth, Galleria mellonella. *Insects 2017, Vol. 8, Page 61*, *8*(2), 61. https://doi.org/10.3390/INSECTS8020061

LA FORGIA, D., Bruno, P., Campos-Herrera, R., Turlings, T., & Verheggen, F. (2021). The lure of hidden death: Development of an attract-and-kill strategy againsagriotes obscurus (coleoptera: Elateridae) combining semiochemicals and entomopathogenic nematodes. *Turkish Journal of Zoology*, *45*(Special issue 1), 347–355. https://doi.org/10.3906/ZOO-2106-38

Larsen, N. A., Nuessly, G. S., & Cherry, R. H. (2016). Efficacy of pyrethroid and neonicotinoid insecticides for Melanotus communis (Gyll.) (Coleoptera: Elateridae) control in Florida

sugarcane (Saccharum spp.). *Journal of Applied Entomology*, *140*(7), 517–526. https://doi.org/10.1111/JEN.12285

LeBlanc, H. N., Perrault, K. A., & Ly, J. (2020). The Role of Decomposition Volatile Organic Compounds in Chemical Ecology. *Forensic Entomology*, 485–498. https://doi.org/10.4324/9781351163767-24/ROLE-DECOMPOSITION-VOLATILE-ORGANIC-COMPOUNDS-CHEMICAL-ECOLOGY-H

Leroy, P. D., Sabri, A., Heuskin, S., Thonart, P., Lognay, G., Verheggen, F. J., Francis, F., Brostaux, Y., Felton, G. W., & Haubruge, E. (2011). Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. *Nature Communications 2011 2:1*, *2*(1), 1–7. https://doi.org/10.1038/ncomms1347

Mann, R. S., Ali, J. G., Hermann, S. L., Tiwari, S., Pelz-Stelinski, K. S., Alborn, H. T., & Stelinski, L. L. (2012). Induced Release of a Plant-Defense Volatile 'Deceptively' Attracts Insect Vectors to Plants Infected with a Bacterial Pathogen. *PLOS Pathogens*, *8*(3), e1002610. https://doi.org/10.1371/JOURNAL.PPAT.1002610

Mollah, M. M. I., & Kim, Y. (2020). Virulent secondary metabolites of entomopathogenic bacteria genera, Xenorhabdus and Photorhabdus, inhibit phospholipase A2 to suppress host insect immunity. *BMC Microbiology*, *20*(1), 1–13. https://doi.org/10.1186/S12866-020-02042-9/TABLES/3

Muslim, S., & Al-Zurfi, A. (2019). *Biological control of the red flour beetle, Tribolium castaneum using entomopathogenic fungi*. http://theses.ncl.ac.uk/jspui/handle/10443/4768

Ogier, J.-C., Pages, S., Thaler, O., Frayssinet, M., Jumas-Bilak, E., Esteves, K., Givaudan, A., Tailliez, P., & Gaudriault, S. (n.d.). *Caractérisation moléculaire du microbiome bactérien associé aux nématodes entomopathogènes*. https://www.genoscope.cns.fr/agc/mage/

Poggi, S., Le Cointe, R., Lehmhus, J., Plantegenest, M., & Furlan, L. (2021). Alternative strategies for controlling wireworms in field crops: A review. *Agriculture (Switzerland)*, *11*(5). https://doi.org/10.3390/agriculture11050436

P.S.Grewal, R. U. EhlersD. I. S. (2005). *Nematodes as biocontrol agents* (P. S. Grewal, R. U. Ehlers, & D. I. Shapiro-Ilan, Eds.). CABI Publishing. https://doi.org/10.1079/9780851990170.0000

Schelkens, R. (2020). *ÉTUDE DES INTERACTIONS MAÏS-TAUPINS À FINALITÉ DE DÉVELOPPEMENT DE MÉTHODES DE LUTTE BIOLOGIQUE*.

Selmani, N. (2021). *Développement d'un protocole de microvinification*. https://sonar.ch/documents/320288/files/Selmani_N_2021.pdf

Shapiro-Ilan, D., & Gaugler, R. (2002). Production technology for entomopathogenic nematodes and their bacterial symbionts. *Journal of Industrial Microbiology & Biotechnology*, *28*(3), 137–146. https://doi.org/10.1038/sj/jim/7000230

Smeets, T. (2022). *Développement d'une méthode de type "attract-and-kill" dans le cadre d'une stratégie de lutte biologique contre les taupins du genre Agriotes spp*. https://lib.uliege.behttps://matheo.uliege.be

Stock, S. P. (2019). Partners in crime: symbiont-assisted resource acquisition in Steinernema entomopathogenic nematodes. In *Current Opinion in Insect Science* (Vol. 32, pp. 22–27). Elsevier Inc. https://doi.org/10.1016/j.cois.2018.10.006

Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., & De Luca, F. (2023a). Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses. *Frontiers in Insect Science*, *3*, 1195254. https://doi.org/10.3389/FINSC.2023.1195254/BIBTEX

Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., & De Luca, F. (2023b). Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses. *Frontiers in Insect Science*, *3*, 1195254. https://doi.org/10.3389/FINSC.2023.1195254/BIBTEX

Ulug, D. (2023). *Response of Rattus norvegicus (Berkenhout, 1769) (Rodentia: Muridae) to entomopathogenic bacteria infected insect cadavers1*.

Vallet-Gely, I., Lemaitre, B., & Boccard, F. (2008). Bacterial strategies to overcome insect defences. In *Nature Reviews Microbiology* (Vol. 6, Issue 4, pp. 302–313). https://doi.org/10.1038/nrmicro1870

Verheggen, F., Perrault, K. A., Megido, R. C., Dubois, L. M., Francis, F., Haubruge, E., Forbes, S. L., Focant, J.-F., & Stefanuto, P.-H. (2017). *The Odor of Death: An Overview of Current Knowledge on Characterization and Applications*. *67*(7). https://doi.org/10.1093/biosci/bix046

Vitta, A., Thimpoo, P., Meesil, W., Yimthin, T., Fukruksa, C., Polseela, R., Mangkit, B., Tandhavanant, S., & Thanwisai, A. (2018). Larvicidal activity of Xenorhabdus and Photorhabdus bacteria against Aedes aegypti and Aedes albopictus. *Asian Pacific Journal of Tropical Biomedicine*, *7*(1), 31–36. https://doi.org/10.4103/2221-1691.221134

Wollenberg, A. C., Jagdish, T., Slough, G., Hoinville, M. E., & Wollenberg, M. S. (2016). Death Becomes Them: Bacterial Community Dynamics and Stilbene Antibiotic Production in Cadavers of Galleria mellonella Killed by Heterorhabditis and Photorhabdus spp. *Applied and Environmental Microbiology*, *82*(19), 5824. https://doi.org/10.1128/AEM.01211-16

Annexes

Annex 1. Additional manipulations conducted during this study related to entomopathogenic nematodes (not presented).

Materials and Methods

Insect rearing _ Wireworms (*Agriotes spp.*) were collected from a field located in Gembloux (Belgium) and then reared under laboratory conditions (21°C ± 1; 40% RH). The larvae were raised in pots (11x11x6 cm) containing a mix of potting soil (Terreau universel “La Plaine Chassart”) and vermiculite (v:v, 1:1) at 65% Water Holding Capacity (WHC). They were fed *ad libitum* with germinating organic barley (Walagri, Belgium). Fifth-stage larvae, which are the most voracious and thus the most likely to consume many beads, were selected for the experiments (Poggi et al., 2021).

Nematodes _ Entomopathogenic nematodes (*Steinernema carpocapsae, Heterorhabditis bacteriophora*) were bred on *Galleria mellonella* larvae in Petri dishes (Ø = 5.5 cm) on filter paper. In each Petri dish, 300 µl of a solution containing entomopathogenic nematodes (834 EPN/ml) was applied per group of five *G. mellonella* larvae. Once the larvae are dead, a White trap system (White, 1927) was used to collect the emerging infective entomopathogenic nematode juveniles (IJs). The IJs were stored in 100 mL of tap water in 250 ml cell culture flasks (75 cm^2 Falcon Brand Products, New-York, United-States) at 13°C to ensure their viability until the experiments.

Bacterial culture _ Bacteria, *Photorhabdus laumondii*, were removed from cryopreservation (-80°C) using a loop and streaked onto Lysogeny Broth Agar (LBA) medium in a Petri dish (Ø=9 cm) under a laminar hood. The dishes were then placed in an incubator at 28°C for 48 hours to allow the bacteria to reproduce sufficiently for the experiments. Then, to conduct the experiment with wireworms and *G. mellonella*, an isolated bacterial colony was transferred from LBA medium to an Erlenmeyer flask containing Lysogeny Broth (LB) medium. The Erlenmeyer flask was placed in a shaking incubator at 35°C for 24 hours to ensure a homogeneous distribution of nutrients, uniform thermal exchange, and to prevent cell sedimentation (Vitta et al., 2018).

Optimisation of the formulation

To identify the compounds most attractive to wireworms and address the first objective (1) (cf. introduction), different solutions encapsulated in beads were prepared (Table 4). The factors that varied to find the optimal formulation included the matrix (alginate or agar), the sucrose concentration (1% and 5%), and the infecting agent (entomopathogenic nematodes, bacteria with their secondary metabolites, secondary metabolites alone, or bacteria alone).

Testing different sucrose concentrations aimed to enhance the beads' attractiveness to wireworms (Forst & Nealson, 1996). Examining various infecting agents is important because entomopathogenic nematodes can face difficulties penetrating the wireworm, while bacteria, being smaller, might offer a more effective solution (Gangwar et al., 2022). Additionally, some beads were prepared without infecting agents (Alg-3, Agr-2) (Table 4) to assess their impact on wireworm attraction.

Alginate formulation _ The beads were produced by mixing sodium alginate (2%, w/v) with water at 50°C. The solution was homogenised and heated using a heated magnetic stirrer and a magnetic stirring bar (Heidolph ; 750 rpm). Sucrose (1% or 5%, w/v; acting as a phagostimulant) (Table 4) and glycerol (18%, w/v; allowing for entomopathogenic nematode dormancy) were then added (Hiltpold et al., 2012; Kim et al., 2021). Once the solution returned to room temperature, alpha-tocopherol (1.5%, w/v; antioxidant) and potato juice (50%, w/v; variety Monalisa; attractant) were incorporated. To obtain the beads, the final solution was conveyed through a tube using a peristaltic pump (Shenchen), producing droplets that fell into a solution of calcium chloride (1.48% w/v; 0.1M) under magnetic stirring (750 rpm). The polymerization process lasted 30 minutes. Finally, the different infecting agents were added into the beads (Table 4) following the procedures described below.

- *EPNs in alginate formulation (Alg-1 and Alg-2)_* 300 entomopathogenic nematodes were added into each bead (Hiltpold et al., 2012). The beads were then stored in an incubator at 14°C until used for the tests described below (cf. Testing of formulation).

- *Bacteria alone in alginate formulation (Alg-4)_* The bacteria, *Photorhabdus laumondii*, were added at 35°C during the final stage of the alginate bead preparation. Prior to incorporation into the beads, the bacterial culture was centrifuged to isolate the bacteria from their secondary metabolites. Then the bacterial concentration was determined using optical density and the "Thoma chamber" method (Campbell, 2010; Selmani, 2021). The quantities were adjusted to reach a concentration of 39k bacteria per bead.

*Agar formulation_* To produce agar baits, agar (1% w/v, Sigma-Aldricht) was dissolved in boiling water for 10 minutes. The solution was homogenised and heated using a heated magnetic stirrer and a magnetic stirring bar (Heidolph, 750rpm). Then, glycerol (18% w/v) and sucrose (5% w/v) were added. The temperature was reduced to 50°C before adding potato juice (50% w/v). Finally, alpha tocopherol was added at 35°C. The solution was then poured into a Petri dish (Ø = 9 cm), and cubes of 0.02 g were cut to match the mass of alginate beads. After

preparing the solution, the infecting agents were added (Table 4) following the procedures described below.

- *EPNs in agar formulation (Agr-1)_* Entomopathogenic nematodes were added at 35°C (Smeets, 2022). The concentration of IJs in the solution was evaluated under a binocular microscope and adjusted to 3000 entomopathogenic nematodes per agar cube by filtration (Hiltpold et al., 2012). It is estimated that one agar cube is equivalent to 10 alginate beads, resulting in 3000 entomopathogenic nematodes (10*300) per agar cube to maintain the same EPNs concentration in both the alginate and agar matrices.

- *Entomopathogenic bacteria in agar formulation (Agr-3, Agr-4 and Agr-5)_* The bacteria and the cell-free supernatant were added separately or together (Table 4) at 35°C during the final stage of agar preparation. The cell-free supernatant is separated from the bacteria through centrifugation of the culture. Thus, an agar formulation with bacteria was obtained, an agar formulation with cell-free supernatant, an agar formulation with both bacteria and cell-free supernatant (Table 4). Each agar cube contained approximately 195000 *Photorhabdus laumondii bacteria.*

Testing of the formulation

All beads' formulations were then presented to wireworms and *Galleria mellonella*, which served as a positive control. Either an appetence test or a dead/ alive test was conducted to determine which biodegradable formulation was most palatable to the wireworms (Table 4).

Appetence test _ To assess bead attractiveness, 10 beads of each formulation (Alg-1, Alg-3, Alg-4, Agr-2, Agr-3, Agr-4, Agr-5) (Table 4) were placed in a plastic pot (5.8x4x4.7 cm) with a pierced lid containing 10 g of sterile sand (Hubo river sand 0-2 mm) at 90% Water Holding Capacity (WHC). Then, either a wireworm or a *Galleria mellonella* (reference model) was placed into the pot. For each solution formulation and each insect model, 10 replicates were made (n=10) (Figure 7). The insects were exposed for one week in a climate-controlled chamber (21 ± 1°C) in darkness. After 7 days, all 10 beads in each plastic pot were examined under a binocular microscope to detect bite marks from either wireworms or *G. mellonella,* estimating feeding activity and the attractiveness of the solution formulation.

*Dead/ alive test_* This test was conducted on two solution formulations (Alg-2, Agr-3) (Table 4). Like the appetence test, 10 beads of each formulation were placed in a plastic pot (5.8x4x4.7 cm) with a pierced lid containing 10 g of sterile sand (Hubo river sand 0-2 mm) at 90% WHC, and either a wireworm or a *Galleria mellonella*. Each formulation and insect model had 10 replicates (n=10). The exposure lasted for one week in a climate-controlled chamber (21 ± 1°C) in darkness. After 7 days, all beads were removed from the plastic pots, and the number of dead or alive wireworms and *G. mellonella* were counted in each replicate. Dead individuals were placed in White traps to confirm that the death was caused by entomopathogenic nematode infection.

To ensure that no infections were missed, which might have occurred towards the end of the seven-day period and had not yet led to the death of the insects, the plastic pots containing only the insect's model (wireworms or *G. mellonella*) were kept in the same dark chamber for an

additional seven-day post-exposure to the beads. On the 14th day, the insects' dead or alive status was reassessed, and the number of dead insects per formulation was recorded.

Matrix		Alginate matrix				Agar matrix				
Solution's name		Alg -1	Alg-2	Alg-3	Alg-4	Agr-1	Agr-2	Agr-3	Agr-4	Agr-5
Sucrose concentration	1%	X								
	5%		X	X	X	X	X	X	X	X
Infecting agent	Nematodes with their symbiont bacteria	X	X			X				
	Bacteria (*P.laumondi*) with their secondary metabolites									X
	Secondary metabolites alone, called supernatant								X	
	Bacteria (*P.laumondi*) alone				X			X		
Test realised	Dead / alive test		X			X				
	Appetence test	X		X	X		X	X	X	X

Table 4 Bead formulations based on the matrix (alginate or agar), the sucrose concentration (1% and 5%), and the infecting agent (entomopathogenic nematodes with their symbiont bacteria, bacteria with their secondary metabolites, secondary metabolites alone, or bacteria alone), along with the tests conducted using these beads.

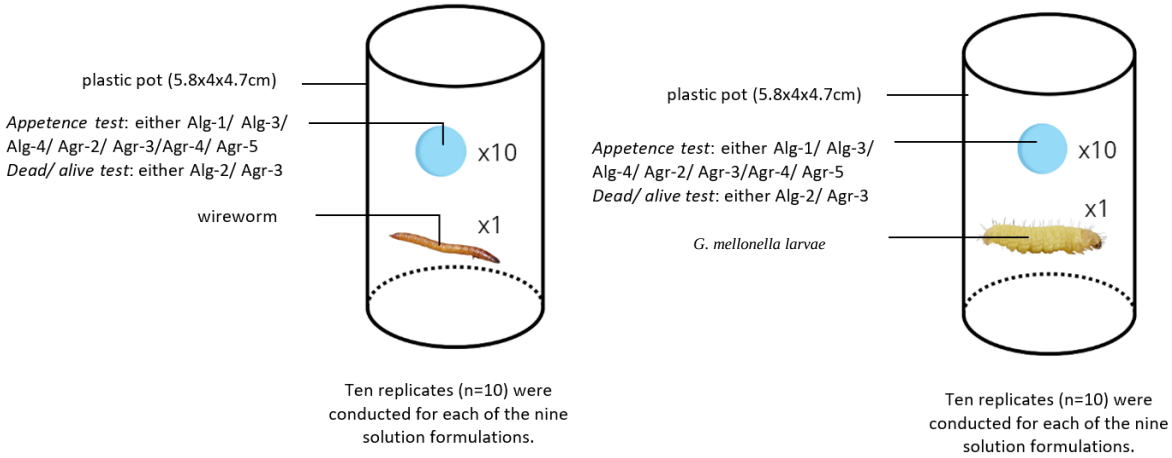

Figure 7 Experimental unit for the appetence and dead/ alive tests.

Result

Testing of the formulation

Comparison of sucrose percentage in alginate beads fabrication with EPN (Alg-1; Alg-2):
To optimise the palatability of alginate beads containing entomopathogenic nematodes, we first tested the impact of sucrose concentration by comparing beads containing 1% and 5% sucrose. The number of bitten beads was recorded. The results were 56% for the 1% sucrose concentration and 69% for the 5% sucrose concentration. The result reveals that the sucrose concentration does not impact the appetence of the beads (t-student = 0.141; *p*-value = 0.161).

Assessment of entomopathogenic nematode strategies in the appetence of alginate beads:
When comparing the palatability of alginate beads containing EPNs with beads without EPNs, the number of bitten beads was recorded. There were 69% bitten beads containing EPNs compared to 0% for beads without EPNs. The results reveal that the presence of entomopathogenic nematodes in the granules affects the wireworm's appetite. (t-student = 8.835, *p*-value = 5.8e-08). Subsequently, the underlying reasons for this observation were explored by testing with entomopathogenic bacteria.

Assessment of entomopathogenic bacteria (*P. laumondi*) strategies in the appetence of alginate of beads:
To determine the wireworms' interest in alginate beads containing entomopathogenic nematodes, we encapsulated the entomopathogenic bacteria that live symbiotically within these EPNs. The number of bitten beads was measured. The biting rate for the beads containing bacteria was 40% compared to 0% for the control treatment. The results reveal that the presence of bacteria in the beads affects the wireworm's appetite (W = 0.80176, *p*-value = 0.000918).

Gellant impact on wireworms' feeding behaviour (Alg-3; Agr-2):
To assess whether wireworms exhibit a preference in feeding behaviour depending on the gellant used, the percentage of bitten beads or cubes was recorded. 50% of agar cubes were bitten, while no bites were recorded for alginate beads. The results reveal that the agar matrix affects the wireworm's appetite compared to the alginate matrix. (X-squared = 5, df = 1, *p*-value = 0.02535), the pathogenicity of entomopathogenic nematodes was tested on this specific gellant.

Pathogenicity of entomopathogenic nematodes in agar cube on *Galleria mellonella* (Agr-1):
It was studied whether the pathogenicity of entomopathogenic nematodes on G. mellonella remained identical when replacing an alginate matrix with an agar matrix. The mortality rate was measured and found to be 100%. The results reveal that the presence of entomopathogenic nematodes in an agar matrix influences the mortality of wireworms (X-squared = 16.2, df = 1, *p*-value = 5.699e-05).

Gellant impact on wireworm feeding behaviour with *P. laumondii Bacteria*: Given the palatability of alginate beads containing bacteria and the wireworms' preference for the agar matrix (test no. 2), the percentage of bitten beads or cubes was recorded. We compared the two matrices containing entomopathogenic bacteria. The obtained percentages were 40% for the agar matrix compared to 30% for the alginate beads. The results show that the presence of bacteria in the agar does not influence the wireworm's appetite ($F = 0.449$, p-value = 0.654). Although the results were not significant, the agar matrix was chosen because the appetence was 10% higher.

Role of secondary metabolites produced by *P. laumondii* in agar for wireworms' appetence:
The previous result revealed a significant appetence for the beads containing bacteria. It is of interest to know if the secondary metabolites produced by these bacteria have an impact on wireworms' appetence. Thus, four treatments (Agr-1; Agr-2; Agr-3; Agr-4) based *on P. laumondii* bacteria were applied in agar, and appetence was measured. 80% of agar pieces containing the bacteria and the cell-free supernatant (Agr-2) were bitten. 50% of agar pieces containing the bacteria were bitten, and 10% of agar pieces containing secondary metabolites were bitten. However, the results reveal that the presence of secondary metabolites does not affect the wireworm's appetite (zvalue = -1.788 ; p-value = 0.0739).

Annex 2. Personal Contribution

My final year project (TFE) began with extensive bibliographic research to better understand and assimilate the subject I would be working on. After familiarising myself with the state of the art regarding the click beetle (*Agriotes sp*) and entomopathogenic nematodes, I proposed several ideas to my supervisors to address the optimization of an "Attract and Kill" method. Following discussions, we developed protocols to initiate the first tests. In parallel, I had the opportunity to carry out some microbiology applications, such as performing multiple PCRs and bacterial cultures.
The optimization tests for the "Attract and Kill" method were conducted over a period of approximately two months. During this time, I learned to use the appropriate laboratory equipment. These tests required continuous exploration of new ideas to develop an effective application.
Once most of the optimization tests were completed, I began odour sampling in May. The method applied for these samples had been optimised by other members of the lab. Through these collections, I became familiar with volatolomic equipment (Hisorb) as well as gas chromatography coupled with mass spectrometry. This work required careful planning and daily monitoring.
Finally, I conducted behavioural tests to correlate them with the expected results from the odour samples. The protocols for these tests were designed in collaboration with my supervisors.
Once all the experiments were completed, I focused on data analysis. I primarily used Excel for data entry, and the data processing was done with RStudio.

Throughout my TFE period, I managed the rearing of entomopathogenic nematodes, click beetles, and *G. mellonella*, which took considerable time alongside the tests. The rearing ensured a constant supply of specimens for the experiments. Similarly, the writing of this document was done in parallel with the tests, until the end of July, when I could fully dedicate myself to it as the submission deadline approached.