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Article

Impact of necrophagous insects on the emission of volatile organic compounds released during the decaying process

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With 4 figures and 2 tables

Abstract: After death, corpses undergo a complex decomposition process, during which volatile organic compounds (VOCs) are released. Several groups of organisms, including insects, use these VOCs to select their mating and feeding sites. While the presence of insects on a corpse influences the decaying process, we do not know whether insects impact on the VOC profile released by the cadaver. Using decomposing rats exposed to dipterans (*Lucilia sericata*) and/or coleopterans (*Dermestes frischii*), we assessed how the presence of insects impacted the cadaver volatilome by using dynamic sampling. As expected, the decomposition of rats in presence of insects was faster than in absence of insects. All rats went through the five decomposition stages with the exception of rats decomposing without insects. The composition of their volatile profiles differed among decomposition stages. We also found that insects do not affect the volatilome of decomposing rats, and no indicator compound could be associated to the presence of specific insect groups.

Keywords: thanatochemistry, decaying process, forensic sciences

1 Introduction

Thanatochemistry is the branch of forensic science that investigates the chemical reactions that occur during the decomposition of a cadaver (Dermengiu et al. 2010, Salam et al. 2012, Tumram, Ambade & Dongre 2014). After death, a corpse undergoes a complex process called decomposition, which includes the mechanisms of autolysis and putrefaction (Pinheiro 2006, Dekeirsschieter et al. 2009). Autolysis impairs cells, tissues and organs through aseptic chemical processes. In comparison, putrefaction is the consequence of the activity of endogenous and exogenous, anaerobic and aerobic bacteria. Moreover, fungal and insect activity also contributes to the decomposition process (Campobasso & Introna 2001, Dent, Forbes & Stuart 2004, Saukko & Knight 2004, Pinheiro 2006). The joint action of autolysis and putrefaction leads to physical and chemical changes on the body during the decomposition, allowing to draw five decomposition stages: fresh, bloated, active decay, advanced decay and dry remains (Vass 2001, Grassberger & Frank 2004, Dekeirsschieter et al. 2009, Amendt et al. 2010, Statheropoulos et al. 2011, Rivers & Dahlem 2014b). Several abiotic and biotic factors affect the process of decomposition, including temperature (Campobasso & Di Vella 2001, Adlam & Simmons 2007, Amendt et al. 2010, Rivers & Dahlem 2014b), humidity (Campobasso & Di Vella 2001), and availability of oxygen (Dent, Forbes & Stuart 2004), as well as the diversity of microorganisms, insects and scavengers exploiting the corpse (Campobasso & Di Vella 2001) [14, 16].

During decomposition, volatile organic compounds (VOCs) originating from the chemical degradation of macromolecules (proteins, lipids and carbohydrates) are released (Vass et al. 2004, Rivers & Dahlem 2014a). These volatile compounds belong to a wide range of chemical families (alkanes, alkenes, aromatic compounds, alcohols, sulphur compounds, nitrogen compounds, and carboxylic acids) and form the cadaveric volatilome (Dekeirsschieter et al. 2009, Paczkowski & Schutz 2011, Rosier et al. 2016, Pirrone & Albertini 2017, Verheggen et al. 2017, Martin & F. Verheggen 2018a). The volatilome of a decaying corpse differs among species. For example, pig remains are distinguishable from human remains based on five esters (Rosier et al. 2015). Several previous studies have collected and identified the odours released by cadavers during decomposition (Vass et al. 2008, Dekeirsschieter et al. 2009, Vass 2012, Agapiou

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et al. 2015, Rosier et al. 2015, Stefanuto et al. 2015, 2016, Verheggen et al. 2017). These odours differ with the stage of decomposition, and have been evaluated in relation to various biotic and abiotic factors. Previous studies also showed that the environment in which the corpse is decaying impacts the VOCs released by cadavers (Dekeirsschieter et al. 2009).

Like vertebrate scavengers, necrophagous insects are able to perceive these cadaveric VOCs and use them to select suitable mating and feeding sites (Visser 1986, Archer & Elgar 2003, Dekeirsschieter et al. 2013, Verheggen et al. 2017, Martin & Verheggen, 2018b). Because insects are important actors in decomposition processes, the present study aimed to assess how insects (i.e. flies and beetles) influence the VOC profiles released by a vertebrate cadaver. We hypothesized that the presence of flies and beetles impacts the VOCs profiles of decaying bodies.

2 Material & Methods

2.1 Insect rearing

To assess the impact of insects on the VOC profiles released by decaying rats, we used a dipteran and a coleopteran species as models of this study. *Lucilia seriacta* (Meigen) (Diptera: Calliphoridae) was chosen as dipteran model because Calliphoridae are commonly found on decaying carcasses, whatever the stage of decomposition (Pohjoismäki et al. 2010, Pérez-Marcos et al. 2016, Abdullah et al. 2017). *Dermestes frischii (Kugelan)* (Coleoptera: Dermestidae) was selected as coleopteran model because *D. frischii* is among the most abundant Dermestidae species found on carcasses (Mayer & Vasconcelos 2013, Charabidzé et al. 2014).

Blowflies pupae were placed in net cages (45 × 45 × 80 cm) inside an incubator (Snijders Scientific®), and were maintained at 23.0 ± 0.1 °C and 73.7 ± 0.4% relative humidity (RH) under a 12:12 h light:dark photoperiod (Charabidzé et al. 2015). After the adults emerged, water and sugar were provided in a Petri dish, along with beef liver as a protein source. When eggs were observed, the Petri dish was removed and placed on sand. Liver was supplied during the entire development of the larvae. At the end of their life-cycle, the larvae migrated to the sand to pupate. Pupae were then stored in a fridge before being transferred to the same incubator to complete the metamorphosis (Clark, Evans & Wall 2006, Tarone & Foran 2006, Shiravi et al. 2011, Martin & Verheggen 2018b).

D. frischii were reared on wood chips in a plastic box (50 × 30 × 40 cm) and were kept in darkness under room conditions (22.1 ± 0.1 °C et 36.4 ± 0.1% RH). Beef liver was introduced daily to ensure mating and feeding. Pieces of polystyrene were provided for larval pupation (Rąkowski & Cymborowski 1981, Richardson & Goff 2001, Menezes, Rossi & Godoy 2006).

2.2 Decomposing rats

Twelve female laboratory rats (3–5 months old, 291.6 ± 12.1 g, kept in identical laboratory conditions) were stifled with carbon dioxide under the supervision of a veterinarian, before being frozen for several days. After thawing, the rats were placed in separate glass vivariums (40 × 30 × 30 cm) on a mixture of 200 g of dry sand (required for the pupation of *L. sericata* larvae), 25 g of wood chips, 10 g of polystyrene (required for the pupation of *D. frischii*) and sugar (required to feed *L. sericata*). Each vivarium was placed in a net cage (45 × 45 × 80 cm). The 12 rats were left to decompose under four modalities: the absence of insects (n = 3), the presence of 10 newly emerged adults of *L. sericata* (sex ratio 1:1, n = 3), the presence of 10 adults of *D. frischii* of less than two months (sex ratio 1:1, n = 3), and the presence of 10 newly emerged adults of *L. sericata* and 10 adults of *D. frischii* of less than two months (sex ratio 1:1, n = 3). All rats were left in the same greenhouse composed of four compartments separated by plexiglass walls with each 'rat-insect' association being allocated in the same greenhouse compartment to avoid the cross-contamination of VOCs. Temperature and humidity were monitored during the decomposition to ensure that the same conditions were met among compartments.

2.3 Volatile collection and analysis

The headspace of each decaying rat was sampled by using a dynamic "push-pull" pump system (Volatile Assay System®, PVAS11). The pushed (charcoal filtered) airflow was set at 1.2 L/min and the pulled air flow was set at 0.7 L/min. This overpressure avoided that VOCs from the greenhouse entered the vivarium. The VOCs from the headspace were trapped on a 60 mg Tenax TA® cartridge (Gerstel®, Germany) made of a microporous polymer of 2,6-diphenylen oxide, which was placed at the exit of the vivarium. VOCs were sampled twice a week during the first month and once a week until the dry remains stage to ensure that each stage was sampled at least once. After sampling, the cartridges were kept in a fridge at 4 °C with silica gel crystals to avoid water adsorption, until analysis (Statheropoulos et al. 2011, Rosier et al. 2015). Within a week, the VOCs were thermally desorbed in a gas chromatograph (Agilent Technology® 7890A) coupled with an automatic thermal desorber (ATD, Agilent Technology®). VOCs were detected with a mass spectrometer (Agilent Technology® 5975C, inert XL EI/CIMS with triple axis detection). See Table 1 for all analytical parameters. After desorption, the VOCs were cryo-focused at −80 °C in a glass liner (CIS4, Agilent technology®). The liner was then heated at 260 °C with a temperature ramp of 12 °C/sec. All compounds were identified by interpreting their mass spectra and by injecting standards when available (Rosier et al. 2014, 2015, 2016). Blanks were also performed for each modality by collecting the VOCs from the headspace of vivarium containing everything but the rat (including wood substrate, insect diet and insects).

Table 1. Analytical parameters of the TDU-GC-MS analysis (adapted from Rosier et al. 2014).

TDU	GC-MS
Desorption temperature: 350 °C / 4 min **Trap temperature:** −80 °C – 260 °C **Transfer line temperature:** 40 °C **Desorption mode:** splitless	GC 7890 A **Carrier gas:** Helium **Column:** VF-624 ms 60 m × 0.25 um × 1,4 mm **Initial temperature:** 40 °C / 1 min **First ramp:** 1 °C / min until 80 °C **Second ramp:** 3 °C / min until 120 °C **Third ramp:** 5 °C / min until 250 °C hold during 10 min **Detector:** MS MS 5975C **Mass scan:** m/z 35–350

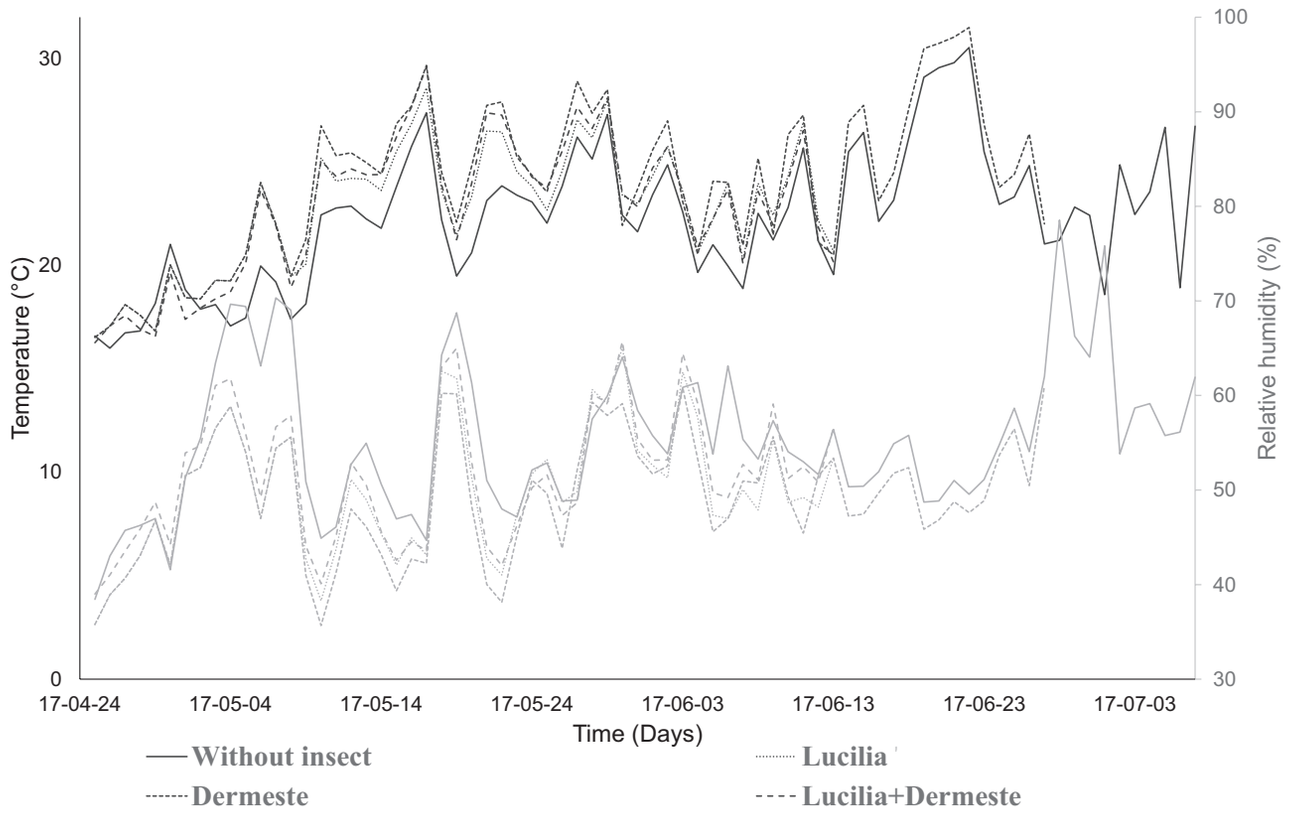

Fig. 1. Temperatures (black) and relative humidity (grey) monitoring in the different compartments of the greenhouse.

2.4 Statistical analyses

After aligning the different peaks of the chromatogram by using the GCAligner 1.0 program (Dellicour & Lecocq 2013) and removing VOCs identified the blank, we calculated the relative abundance of each compound based on the peak areas. Correlation plot was performed on data matrix for each modality to observe the variation of correlation due to the presence of insects ("corrplot" command, R-package cran). Data were analysed via multivariate analysis in R 3.0.2 program (R Core Team 2013) after arcsine transformation allowing to transform finite data (percentages) into infinite data. To detect differences in the fragrance profiles among the different insect modalities, a permutational multivariate analysis of variance (i.e. perMANOVA) was performed using an Euclidian distance matrix and 999 permutations ("adonis" command, R-package vegan, (Oksanen et al. 2017)). As this analysis is tough to the violation of data normality, only homoscedasticity was checked using the "betadisper" function. When a *p*-value was significant, pairwise comparisons were performed. The *P*-values of theses comparisons underwent Bonferroni's adjustment to avoid type I errors due to multiple analyses. Indicator compound analysis was also performed using the "indval" function from the labdsv package (Roberts 2016) to identify the VOCs that were indicative of a single decomposition stage or insect modality. The analysis produced a *p*-value and an indicative value based on

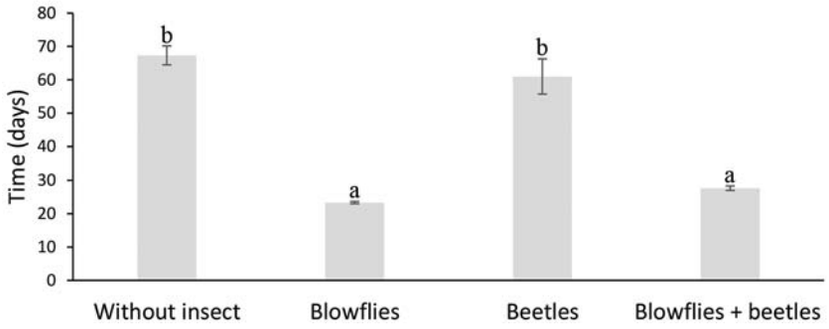

Fig. 2. Duration (number of days) of the decomposition of rats exposed or not to insects (without insect, with blowflies, with hide beetles and with both blowflies and hide beetles) during the entire decomposition process. Modalities sharing the same letter are not different from each other (threshold: $p < 0.05$).

the abundance and relative frequency of the VOC. *P*-values were adjusted with a Holm correction to avoid type I errors due to multiple analyses.

3 Results

The evolution of temperature and relative humidity in the four greenhouse compartments over the entire period of decomposition is provided in Fig. 1. No difference is detected between greenhouse compartments (Temperature: $F_{742,7} = 0.027$, $p = 1$, Humidity: $F_{742,7} = 1.170$, $p = 0.456$). All rats from all four modalities passed through each of the five decomposition stages, except for those decaying without insect, which were flattened and dry. The final stage differed with respect to modality. In the absence of insects, rats became mummified (dried undecomposed cadaver). In the presence of just blowflies, most rat tissues were consumed by larvae, with the bones being stacked under the skin, which became leathery on the upper side of the cadaver. In the presence of beetles (with and without blowflies), the cadaver ended up as a heap of bones and hairs. Besides the expected difference in the stages of decomposition (GLMM, decomposition stage effect, $F_{3,32} = 5.98$, $p = 0.002$), we found that insects significantly impacted the rate of decomposition (GLMM, modality effect, $F_{3,32} = 6.80$, $p = 0.001$). The decomposition rate was significantly faster in presence of blowflies (23.3 ± 0.3 days in the presence of *L. sericata*, 27.6 ± 0.7 days in the presence of both *D. frischii* and *L. sericata*) compared to the modalities without blowflies (67.3 ± 2.8 days without insects, 61.0 ± 5.3 days in the presence of just *D. frischii*) (Fig. 2).

3.1 Volatile organic compounds

67 different VOCs belonging to nine chemical families were collected and identified (Table 2). Based on the relative abundance of the VOCs, the main compounds present in samples, regardless of insects' modalities and decomposition stages, is the disulphide, dimethyl- with a mean relative abundance of 27.66 ± 0.36%. It reached 46.60 ± 4.28% during the active decay stage and down to 10.69 ± 3.88% during the bloated stage. However, it is almost constant among insect modalities (without insect: 28.59 ± 6.12%, with *L. sericata*: 26.15 ± 6.76%, with *D. frischii:* 33.97 ± 5.35%, with *L. sericata* and *D. frischii:* 18.85 ± 5.52%). Even if we identified a wide diversity of molecules, three compounds only accounted for 50% of the total quantity VOCs: disulphide, dimethyl-, butanal, 3-methyl (14.80 ± 1.92%), butanal 2- methyl (8.06 ± 1.32%). We found trisulfide, dimethyl- in higher concentration in rats decaying in presence of *D. frischii* (3.44 ± 0.76 %) than for rats decaying under the others insect's modalities (without insect: 1.87 ± 0.82%, with *L. sericata*: 0.78 ± 0.45%, with *L. sericata* and *D. frischii:* 0.86 ± 0.34%) ($F_{3,57} = 3.219$, $p = 0.0293$).

We build up a correlation plot to illustrate the impact of insects on the families of compounds released during the decomposition (Fig. 3). While aldehydes and sulphured compounds are released simultaneously in absence of insects, their emission is differed in presence of *L. sericata* and *D. frischii.*

As attended, statistical analysis revealed that VOC profiles differed among the decomposition stages ($F_{3,57} = 7.896$, $p < 0.001$), all stages being significantly different from each other (pairwise comparisons, $p < 0.05$). However, no clear discrimination may be visually assessed on the PCA ordination (Fig. 4) and no indicative compound has been highlighted. Concerning the impact of insects on the volatilome released during all the decomposition process, regardless of the decomposition stage, no effect was statistically detected ($F_{3,57} = 0.760$, $p = 0.681$). Impact of insect presence was then analysed considering each decomposition stage (combination decomposition stage*modality leading to 16 different qualitative levels) and a significant difference was detected ($F_{13,47} = 3.020$, $p < 0.001$). Presence of *L. sericata* and *D. frischii* impacted the VOC profile released during the fresh stage compared to rats decaying without any insect ($p = 0.013$) while no significant impact was detected in presence of either *L. sericata* or *D. frischii* alone. The same observa-

Table 2. List of the 67 volatile organic compounds released by rat remains in absence (-) or presence of insects (Δ).

Volatile organic compounds	Current experimentation				Literature
	Without insect	With *L. sericata*	With *D. fichii*	With *L. sericata* and *D. frischii*	
Alkanes					
Hexane	Δ	Δ	Δ	Δ	(Caldwell et al. 2011, Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel 2012)
Nonane	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011, Dekeirsschieter et al. 2012)
Undecane	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011, Dekeirsschieter et al. 2012)
Octane, 3-methyl-	Δ	Δ	Δ	Δ	
Dodecane	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Tetradecane	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011, Dekeirsschieter et al. 2012)
Sulphur compounds					
Sulfoxyde, dimethyl-	Δ	Δ	Δ	Δ	
Methanethiol	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2009, Statheropoulos et al. 2011)
Disulfide, dimethyl-	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2009, 2012, Hoffman et al. 2009, Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel, 2012)
Disulfide, Methylethyl-	-	Δ	Δ	Δ	(Statheropoulos et al. 2011)
Dimethyltrisulfide	Δ	Δ	Δ	-	(Vass et al. 2008, Statheropoulos *et al.*, 2011, Dekeirsschieter *et al.*, 2012)
2-Thiaheptane	Δ	Δ	Δ	Δ	
Methional	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011)
Tetrasulfide, diméthyl-	-	Δ	Δ	-	
Aldehydes					
Propanal, methyl-	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2009, 2012, Statheropoulos et al. 2011)
Butanal, 3-methyl-	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2007, 2011)
Butanal, 2-methyl-	Δ	Δ	Δ	Δ	
Pentanal	Δ	Δ	Δ	Δ	(Statheropoulos, Spiliopoulou & Agapiou 2005)
Hexanal	Δ	Δ	Δ	Δ	(Lorenzo et al. 2003, Tolliver, 2005, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Heptanal	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2012)
Benzaldehyde	Δ	Δ	Δ	Δ	(Statheropoulos, Spiliopoulou & Agapiou, 2005, Dekeirsschieter et al. 2009, 2012, Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel 2012)
Octanal	Δ	Δ	Δ	Δ	(Hoffman et al. 2009, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Octen-2-al	Δ	Δ	Δ	Δ	(Hoffman et al. 2009, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Nonanal	Δ	Δ	Δ	Δ	(Tolliver 2005, Hoffman et al. 2009, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Decanal	Δ	Δ	Δ	Δ	(Vass et al. 2008, Caldwell et al. 2011, Degreeff & Furton, 2011, Cablk, Szelagowski & Sagebiel 2012)
Alcohol					
Propan-1-ol	Δ	Δ	Δ	Δ	
Propan-1-ol, 2-methyl-	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011)
Butanol	Δ	Δ	-	-	(Dekeirsschieter et al. 2012)
Butanol, 3-methyl	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2012)

Volatile organic compounds	Current experimentation				Literature
	Without insect	With *L. sericata*	With *D. fichii*	With *L. sericata* and *D. frischii*	
Butanol, 2-methyl	Δ	Δ	Δ	Δ	
Butan-1-ol, 3-methyl-, acetate	Δ	Δ	Δ	Δ	
Butan-1-ol, 2-methyl-, acetate	Δ	Δ	Δ	Δ	
Hexenol	Δ	Δ	Δ	Δ	
2-butoxy ethanol	Δ	Δ	Δ	Δ	(Statheropoulos et al. 2011)
Heptan-2-ol	Δ	Δ	Δ	Δ	
1-Octen-3-ol	Δ	Δ	Δ	Δ	
Ketones					
Butan-3-one	Δ	Δ	Δ	Δ	
Butan-2-one	Δ	Δ	Δ	Δ	(Statheropoulos, Spiliopoulou & Agapiou 2005, Statheropoulos et al. 2007)
Butan-2-one, 3-hydroxy-	-	-	Δ	-	
Butan-3-one, 3-methyl-	Δ	Δ	Δ	Δ	
Pentan-2-one, 3-methyl-	-	Δ	-	-	
Pentan-2-one, 4-methyl-	Δ	-	Δ	-	
Hexen-2-one	Δ	Δ	Δ	Δ	
Octan-3-one	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2012)
Octan-2-one	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2012)
3-Octen-2-ol	Δ	Δ	Δ	Δ	(Caldwell et al. 2011)
Acetophenone	Δ	Δ	Δ	Δ	
Organic acids and esters					
Borate, trimethyl	Δ	Δ	Δ	Δ	
Acetic acid	Δ	Δ	Δ	Δ	(Caldwell et al. 2011, Degreeff & Furton 2011, Statheropoulos et al. 2011)
Ethyl propionate	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2012)
Methyl-3-methylbutanoate	Δ	Δ	Δ	-	
Ethyle-3-methylbutanoate	Δ	Δ	Δ	Δ	
Butylpropanoate	-	-	Δ	-	
Methyl acetate	Δ	Δ	Δ	Δ	(Dekeirsschieter et al. 2009)
Butanoïc acid	Δ	Δ	Δ	Δ	
Aromatics					
Toluene	Δ	Δ	Δ	Δ	(Statheropoulos, Spiliopoulou & Agapiou 2005, Hoffman et al. 2009, Degreeff & Furton 2011, Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel 2012, Dekeirsschieter et al. 2012)
Isobutylbenzene	-	-	Δ	Δ	
Propylbenzene	Δ	Δ	Δ	Δ	(Statheropoulos, Spiliopoulou & Agapiou 2005, Statheropoulos et al. 2011)
Limonene	Δ	Δ	Δ	Δ	
Ethanol-Benzene	Δ	Δ	Δ	Δ	
Nitrogen compounds					
Trimethylamine	-	-	-	Δ	
Alkenes					
Oct-2-ene	Δ	Δ	Δ	Δ	
Non-1-ene	Δ	Δ	Δ	Δ	

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Volatile organic compounds	Current experimentation				Literature
	Without insect	With *L. sericata*	With *D. fichii*	With *L. sericata* and *D. frischii*	
Unknown					
Unknown 1	Δ	Δ	Δ	Δ	
Unknown 2	Δ	Δ	Δ	Δ	
Unknown 3	-	-	Δ	Δ	
Unknown 4	-	Δ	-	-	

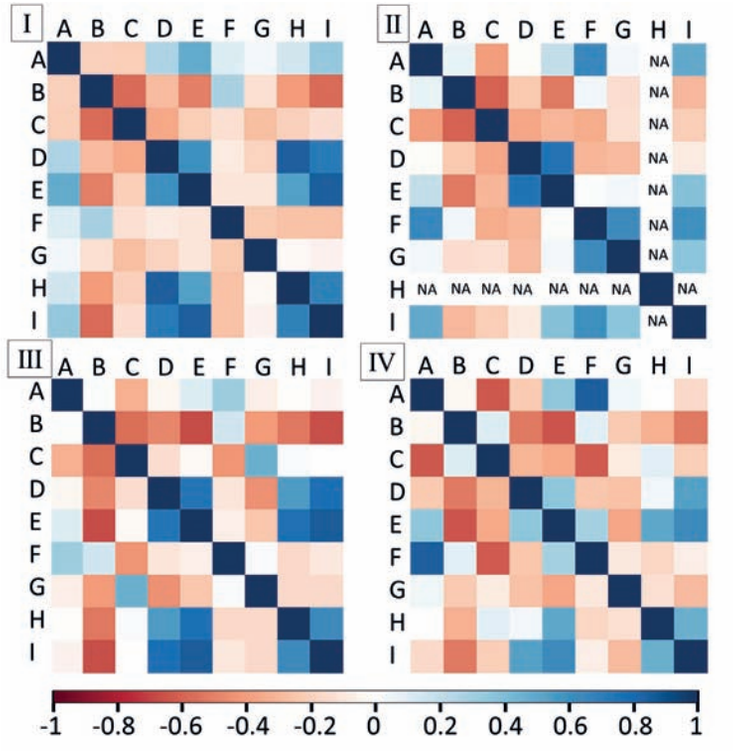

Fig. 3. Correlation plot representing chemical families of compounds released during decomposition of rats without insect (I), with *L. sericata* (II), with *D. frischii* (III) and with *L. sericata* and *D. frischii* (IV). Colours illustrate the correlation between families of compounds (blue: perfected correlated and red: non-correlated), (A: Alkanes, B: Sulphur compounds, C: Aldehydes, D: Alcohols, E: Ketones, F: Organic acids, G: Aromatic compounds, H: Amines, I: Alkenes).

tion was made during the bloated stage, with a significant impact of the simultaneous presence of blowflies and beetles ($p < 0.001$). However, no indicator compound was associated to a specific insect order present on the decaying corpse for a given decomposition stage.

Based on the relative abundances of the nine chemical families, the decomposition stage had a significant impact ($F_{3,57} = 8.766$, $p < 0.001$) while insect modalities did not have any effect, regardless of the decomposition stage ($F_{3,57} = 0.874$, $p = 0.554$). Considering each decomposition stage (16 level-combination variable), a significant difference was detected ($F_{13,47} = 3.113$, $p < 0.001$). Pairwise comparisons on chemical VOC families lead to the same results obtained with the individual VOC analysis. Actually, the simultaneous presence of both insects affected the VOC profiles released by decaying rats during fresh ($p = 0.045$) and bloated stages ($p = 0.001$) compared to those released by rats decaying without insects. However, no indicator compound was associated to a specific insect order present on the decaying corpse for a given decomposition stage.

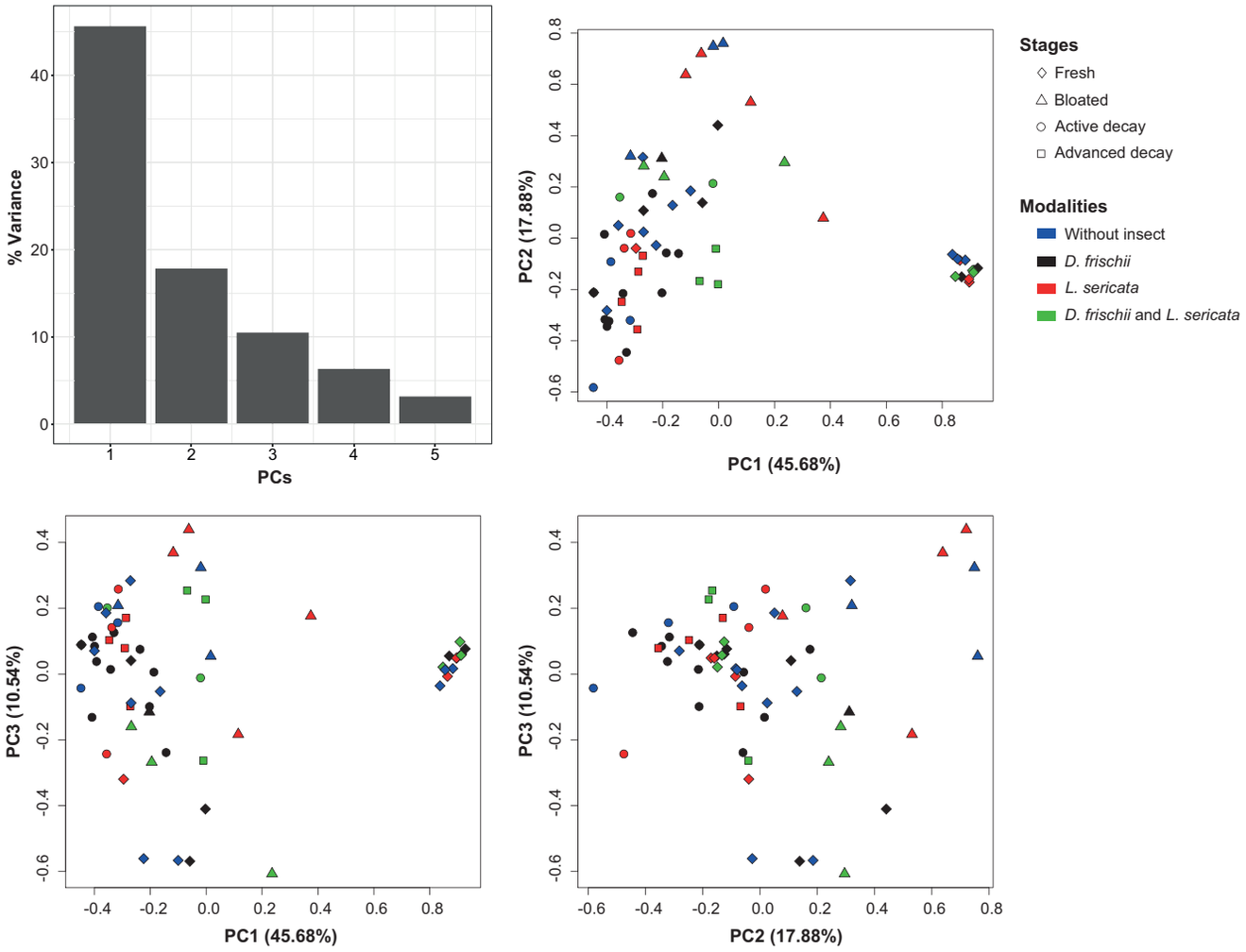

Fig. 4. PCA ordination plots. Visualization of the VOC profiles during the decaying process, depending on the decomposition stage (figures) and the modality (colours). Each sampling is represented in a two dimensional plan constituted by two axes of the principal compounds analysis. Axes are combination of studied factors (insect presence and stage of decomposition) characterized by a percentage of reliability.

4 Discussion

The decomposition process of a corpse is affected by various biotic and abiotic factors, with environmental conditions and the causes of death being the most commonly studied factors (Tomberlin & Adler 1998, Dent, Forbes & Stuart 2004, Dekeirsschieter et al. 2009, Notter et al. 2009, De Donno et al. 2014, Lynch-Aird, Moffatt & Simmons 2015, Mcintosh, Dadour & Voss 2016). This study confirms that the presence of dipterans and coleopterans directly impacts the decomposition rate. Rats decomposing with insects passed through the five stages of decomposition described in the published literature (i.e. fresh, bloated, active decay, advanced decay and dry remains). In contrast, rats decomposing in the absence of insect dried. In this last modality, the only biological actors of decomposition are microorganisms (Amendt, Krettek & Zehner 2004, Pinheiro 2006). Thus, in the absence of insects, no macro organisms were feeding on soft tissues, resulting in no bones being observed. Because the exposition of bones characterises the advanced stage of decay, this stage was not observed (Goff 2010). Consequently, corpses were mummified (i.e. soft tissues were preserved). Low humidity and heat promote mummification, leading to the formation of a dry unskeletonised cadaver (Campobasso & Di Vella 2001, Schotsmans, Forbes & Marquez-Grant 2017). Decomposition in the presence of flies does not lead to the skeleton being revealed, because the bones stick to the dried skin. Indeed, when corpses reach the advanced stage of decay, they become less attractive to flies (Charabidzé et al. 2015). Because hide beetles feed on the dry remains (including skin and cartilage), their presence allows the corpse to reach the skeleton stage (Hefti et al. 1980, Huchet 2008, Charabidzé et al. 2014).

The degradation of proteins, lipids and carbohydrates leads to the emission of VOCs (Dent, Forbes & Stuart 2004, Statheropoulos et al. 2007, Brasseur et al. 2012). More than 60 compounds were identified from the headspace of decomposing rats. The fact that rats were placed in an unnatural environment (i.e. inside a vivarium, placed inside a greenhouse), with a limited diversity of microorganisms and insects, might explain the limited diversity of VOCs collected in our study compared to other studies (Statheropoulos, Spiliopoulou & Agapiou 2005, Statheropoulos et al. 2007, Boumba, Ziavrou & Vougiouklakis 2008, Dekeirsschieter et al. 2009, Hoffman et al. 2009, Agapiou et al. 2015, Rosier et al. 2015). However, most of the VOCs commonly reported in the published literature were found in our study, including sulphur compounds, such as methanthiole, disulphide, dimethyl-, sulphide, methylethyl-, sulphide, dimethyl- (Vass et al. 2004, Dekeirsschieter et al. 2009, 2012, Hoffman et al. 2009, Statheropoulos et al. 2011, Cablk, Szelagowski & Sagebiel 2012, Rosier et al. 2015). Disulfide, dimethyl- and methanthiole were collected under all modalities. Disulphide, methylethyl- was only detected from rats placed in the presence insects. All of these compounds originate from the degradation of sulphured amino acids (methionine and cysteine) (Dent, Forbes & Stuart 2004).

VOCs collected are the products released due to the degradation of tissues of the corpse. Previous studies revealed that each tissues released specific families of compounds. Alkanes derive from the decomposition of muscles, bones and fat (Cablk, Szelagowski & Sagebiel 2012). Aldehydes are typical decomposition by-products and include benzaldehyde, nonanal and decanal (Boumba, Ziavrou & Vougiouklakis 2008, Dekeirsschieter et al. 2009, Caldwell et al. 2011, Statheropoulos et al. 2011), which were all identified under all modalities in our study. Aldehydes are associated with the degradation of carbohydrates, more specifically from the degradation of pyruvate by pyruvate decarboxylase (Boumba, Ziavrou & Vougiouklakis 2008, Dekeirsschieter et al. 2009). Alcohols were released at the bloated stage in our study. Alcohols are products of carbohydrate fermentation, amino acids degradation and lipid oxidation. Ketones were produced in different quantities across all modalities. They are released during the degradation of lipids and carbohydrates (Agapiou et al. 2015). Finally, carboxylic acids and aromatic compounds were also collected, and exhibited high diversity (Dekeirsschieter et al. 2009, Hoffman et al. 2009, Agapiou et al. 2015). Even if all these chemical families were present, the presence of necrophagous insects was found to impact the sequence of the emission of the important chemical families.

By monitoring the VOCs produced from decaying rats in the presence and absence of insects (dipteran and coleopteran) over a two-month period showed that insects do not have an important impact on the odours released during decomposition. Different odours were produced at some stages of decomposition, however, indicator compounds could not be identified. Two dimensional chromatography could provide additional information by revealing the presence of additional compounds (Dekeirsschieter et al. 2012, Stefanuto et al. 2016, Verheggen et al. 2017). The background VOC signal present in the greenhouse could have also hidden interesting compounds (Dekeirsschieter et al. 2009), thus, the use of sterile conditions might improve the quality of the data.

There is extensive documentation that the presence of insects on a corpse influences the process of decay, however, this study was the first to evaluate how insects impact the VOC profile released by a cadaver. Most of the differences in volatile emissions highlighted in this study were made between rats decaying without insects and rats decaying in presence of both necrophagous flies and Dermestes. While no specific compound is released as a results of necrophagous insect feeding, we found that insects impact on the dynamic of emission of the most important chemical families. Two dimensional chromatography could reveal some VOCs not detected in this study, making it possible to deeper understand the impact of insects on the emission of VOCs.

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Supplementary Material

Table S1. Relative abundance (%) of the VOCs released by the decaying rat depending on modalities and decomposition stages (mean ± sd). See page 29.

	Modalities				Decomposition stages			
	Without insect	With L. sericata	With D. frischii	with D. frischii and L. sericata	Fresh	Bloated	Active decay	Advanced decay
Hexane	1.30±0.32	1.72±0.65	1.27±0.63	0.88±0.28	0.79±0.26	1.52±0.44	1.39±0.60	2.67±1.04
Nonane	0.76±0.30	0.79±0.29	0.63±0.23	0.83±0.26	1.45±0.26	0.19±0.09	0.21±0.09	0.62±0.20
Undecane	0.07±0.04	0.24±0.18	0.02±0.04	0.09±0.05	0.17±0.04	0.00±0.00	0.15±0.14	0.00±0.00
Octane, 3-methyl-	0.64±0.24	1.73±0.64	1.25±0.68	2.14±1.53	0.38±0.16	1.23±0.70	1.46±0.66	4.97±2.22
Dodecane	0.01±0.00	0.01±0.01	0.02±0.01	0.02±0.01	0.03±0.01	0.01±0.00	0.00±0.00	0.00±0.00
Tetradecane	0.09±0.04	0.08±0.04	0.05±0.03	0.19±0.1	0.22±0.05	0.03±0.01	0.00±0.00	0.02±0.02
Sulfoxyde, dimethyl-	0.64±0.24	1.73±0.64	1.25±0.68	2.14±1.53	0.38±0.16	1.23±0.70	1.46±0.66	4.97±2.22
Methanethiol	0.46±0.13	0.91±0.37	0.48±0.07	0.60±0.20	0.30±0.08	0.73±0.46	0.66±0.08	1.35±0.32
Disulfide, dimethyl-	28.59±6.12	26.15±6.76	33.97±5.35	18.85±5.52	18.86±4.57	10.69±3.88	46.60±4.28	32.30±5.44
Disulfide, Methylethyl-	0.00±0.00	0.17±0.14	0.01±0.01	0.05±0.03	0.00±0.00	0.20±0.19	0.02±0.01	0.12±0.05
Dimethyltrisulfide	1.87±0.82	0.78±0.45	3.44±0.76	0.86±0.34	0.90±0.35	0.75±0.39	3.79±0.86	1.95±0.92
2-Thiaheptane	0.09±0.05	0.08±0.03	0.10±0.03	0.06±0.03	0.14±0.04	0.01±0.01	0.05±0.02	0.09±0.05
Methional	0.16±0.06	0.32±0.12	0.17±0.04	0.20±0.10	0.23±0.06	0.02±0.01	0.16±0.04	0.62±0.22
Tetrasulfide, diméthyl-	0.00±0.00	0.08±0.06	0.04±0.02	0.00±0.00	0.01±0.00	0.09±0.08	0.03±0.02	0.02±0.02
Propanal, methyl-	0.08±0.04	0.78±0.56	0.33±0.19	0.22±0.08	0.28±0.15	1.03±0.73	0.11±0.03	0.27±0.13
Butanal, 3-methyl-	19.78±4.99	14.20±4.13	12.45±2.44	12.42±3.74	10.29±0.34	34.98±5.58	11.54±2.02	6.30±1.00
Butanal, 2-methyl-	9.66±2.55	10.81±4018	6.15±1.42	5.36±1.63	3.92±1.34	19.92±4.75	7.69±1.47	4.12±0.89
Pentanal	1.46±0.74	1.44±0.45	0.78±0.29	1.11±036	2.31±0.49	0.99±0.48	0.25±0.11	0.24±0.13
Hexanal	2.03±0.88	1.47±0.71	1.39±0.44	2.44±082	2.14±0.66	2.77±0.98	1.02±0.41	1.17±0.52
Heptanal	1.74±0.85	1.04±2.14	1.83±0.89	3.76±1.44	5.15±1.03	0.38±0.15	0.12±0.05	1.17±0.71
Benzaldehyde	1.02±0.46	1.40±0.45	1.04±0.34	1.71±0.58	2.27±0.46	0.27±0.07	0.52±0.12	1.43±0.46
Octanal	0.31±0.18	0.45±0.23	0.34±0.18	0.60±0.34	1.01±0.24	0.04±0.02	0.00±0.00	0.01±0.01
Octen-2-al	0.08±0.04	0.16±0.09	0.15±0.08	0.31±0.16	0.41±0.10	0.03±0.01	0.00±0.00	0.00±0.00
Nonanal	0.53±0.22	0.71±0.29	0.41±0.18	0.81±0.36	1.33±0.25	0.18±0.09	0.08±0.04	0.11±0.05
Decanal	0.18±0.06	0.24±0.09	0.16±0.06	0.21±0.09	0.41±0.06	0.14±0.09	0.02±0.01	0.03±0.03
Propan-1-ol	0.09±0.03	0.24±0.13	0.17±0.05	0.09±0.07	0.07±0.03	0.05±0.02	0.18±0.05	0.43±0.25
Propan-1-ol, 2-methyl-	1.15±0.71	1.54±0.75	1.42±0.65	0.85±0.55	2.95±0.74	0.03±0.02	0.30±0.08	0.12±0.08
Butanol	0.07±0.03	0.62±0.52	0.00±0.00	0.24±0.24	0.03±0.02	0.00±0.00	0.18±0.16	1.34±1.02
Butanol, 3-methyl	2.16±0.90	2.64±0.88	4.69±1.16	2.61±1.36	0.78±0.30	0.45±0.23	6.56±1.07	6.87±1.62
Butanol, 2-methyl	0.95±0.23	1.34±0.33	1.63±0.31	1.23±0.40	0.82±0.08	0.38±0.10	2.15±0.29	2.43±0.65
Butan-1-ol, 3-methyl-, acetate	0.09±0.04	0.13±0.04	0.50±0.19	0.60±0.33	0.11±0.03	0.04±0.01	0.55±0.18	0.96±0.50
Butan-1-ol, 2-methyl-, acetate	0.13±0.06	0.17±0.22	0.22±0.07	0.24±0.08	0.33±0.07	0.08±0.03	0.11±0.04	0.09±0.06
Hexenol	0.06±0.03	0.07±0.04	0.08±0.03	0.17±0.15	0.03±0.02	0.01±0.01	0.22±0.10	0.10±0.07
2-butoxy ethanol	2.82±1.75	2.73±1.38	2.73±1.43	5.71±3.78	7.55±2.32	1.27±0.60	0.30±0.09	0.44±0.16
Heptan-2-ol	0.27±0.17	0.44±0.22	0.40±0.19	0.77±0.39	1.10±0.25	0.04±0.01	0.01±0.01	0.07±0.03
1-Octen-3-ol	0.16±0.10	0.49±0.24	0.44±0.21	0.71±0.32	0.98±0.24	0.01±0.01	0.05±0.01	0.24±0.11
Butan-3-one	0.27±0.16	0.16±0.08	0.16±0.08	0.10±0.06	0.42±0.12	0.03±0.02	0.00±0.00	0.03±0.03
Butan-2-one	3.20±0.62	5.22±1.25	2.64±0.50	5.96±2.30	2.04±0.31	3.43±1.12	4.70±1.18	10.92±2.06
Butan-2-one,3-hydroxy-	0.03±0.03	0.00±0.00	0.25±0.16	0.00±0.00	0.03±0.02	0.00±0.00	0.13±0.12	0.00±0.00
Butan-3-one, 3-methyl-	0.41±0.20	0.41±0.16	0.28±0.10	1.07±0.72	0.34±0.14	0.11±0.05	1.04±0.47	0.34±0.13
Pentan-2-one, 3-methyl-	0.00±0.00	0.23±0.15	0.04±0.03	0.20±0.20	0.00±0.00	0.00±0.00	0.06±0.04	0.78±0.38

	Modalities				Decomposition stages			
	Without insect	**With L. sericata**	**With D. frischii**	**with D. frischii and L. sericata**	**Fresh**	**Bloated**	**Active decay**	**Advanced decay**
Pentan-2-one, 4-methyl-	0.09±0.04	0.07±0.05	0.14±0.07	0.51±0.51	0.06±0.03	0.02±0.01	0.18±0.07	0.88±0.82
Hexen-2-one	4.67±2.62	6.61±3.33	4.82±2.55	7.18±3.85	14.13±3.06	0.46±0.42	0.11±0.04	0.09±0.05
Octan-3-one	0.06±0.04	0.15±0.06	0.13±0.04	0.19±0.05	0.22±0.05	0.02±0.01	0.07±0.01	0.16±0.08
Octan-2-one	0.1±0.06	0.15±0.07	0.08±0.03	0.91±0.57	0.19±0.05	0.02±0.01	0.03±0.01	1.54±0.88
3-Octen-2-ol	0.00±0.00	0.06±0.04	0.05±0.02	0.05±0.03	0.08±0.03	0.00±0.00	0.00±0.00	0.06±0.03
Acetophenone	0.18±0.08	0.17±0.08	0.25±0.11	0.21±0.11	0.44±0.09	0.02±0.01	0.09±0.08	0.03±0.03
Borate, trimethyl	0.19±0.10	0.47±.34	0.15±0.09	0.53±0.24	0.22±0.09	0.91±0.43	0.19±0.15	0.00±0.00
Acetic acid	0.48±0.21	0.64±0.28	0.77±0.30	0.26±0.15	0.74±0.17	0.02±0.02	0.74±0.31	0.44±0.41
Ethyl propionate	0.64±0.15	0.12±0.05	0.46±0.15	0.66±0.30	0.29±0.10	0.31±0.12	0.69±0.19	0.69±0.34
Methyl-3-methylbutanoate	0.04±0.02	0.02±0.01	0.06±0.04	0.03±0.03	0.00±0.00	0.06±0.03	0.08±0.04	0.05±0.04
Ethyle-3-methylbutanoate	0.19±0.07	0.09±0.04	0.45±0.21	1.10±1.01	0.14±0.04	0.04±0.01	0.50±0.21	1.84±1.62
Butylpropanoate	0.01±0.01	0.01±0.01	0.02±0.01	0.00±0.00	0.01±0.01	0.00±0.00	0.03±0.01	0.00±0.00
Methyl acetate	1.26±0.32	1.81±0.44	0.87±0.21	3.51±2.31	1.25±0.24	1.30±0.57	1.12±0.24	5.75±3.60
Butanoïc acid	0.71±0.04	0.07±0.04	0.22±0.07	0.56±0.23	0.30±0.16	0.34±0.10	0.50±0.15	0.42±0.35
Toluene	7.14±3.49	3.47±1.34	8.38±2.84	8.62±47	9.22±2.96	11.81±4.61	2.64±0.98	2.27±1.39
Isobutylbenzene	0.00±0.00	0.01±0.01	0.01±0.01	0.11±0.06	0.05±0.03	0.00±0.00	0.00±0.00	0.07±0.03
Propylbenzene	0.10±0.06	0.68±0.57	0.10±0.04	0.13±0.07	0.25±0.06	0.79±0.75	0.02±0.01	0.01±0.01
Limonene	0.26±0.12	0.29±0.13	0.09±0.04	0.07±0.03	0.31±0.01	0.17±0.13	0.06±0.02	0.06±0.03
Ethanol-Benzene	0.04±0.02	0.21±0.13	0.11±0.04	0.16±0.11	0.01±0.01	0.20±0.16	0.13±0.04	0.42±0.17
Trimethylamine	0.10±0.07	0.00±0.00	0.02±0.01	0.02±0.02	0.09±0.05	0.00±0.00	0.00±0.00	0.00±0.00
Oct-2-ene	0.02±0.01	0.31±0.25	0.03±0.02	0.08±0.05	0.01±0.01	0.37±0.32	0.03±0.02	0.21±.10
Non-1-ene	0.34±0.18	0.20±0.09	0.20±0.09	0.20±0.08	0.53±0.13	0.08±0.02	0.03±0.02	0.05±0.03