Characterization of volatile organic compounds emitted by insect vectors of *Xylella fastidiosa*

Bianca Adelina Bispo

Final thesis report presented to

Escola de Tecnologia e Gestão

Instituto Politécnico de Bragança

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Within the context of the double diploma with

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Dedico todo e qualquer sucesso do mundo aos meus pais, que, mesmo sob muito sol, me fizeram chegar até aqui, na sombra. Dedico também à minha Tia Andreza (in memoriam), que estaria muito orgulhosa de tudo isso.

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"*Consagre ao Senhor tudo o que você faz, e os seus planos serão bem-sucedidos.*"

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RESUMO

O presente estudo teve como objetivo caracterizar os compostos orgânicos voláteis (COVs) emitidos por *Philaenus spumarius*, principal vetor da bactéria *Xylella fastidiosa*, causadora de doenças graves em oliveiras, videiras e amendoeiras. Considerando a ausência de tratamentos eficazes, a identificação desses compostos pode auxiliar no desenvolvimento de armadilhas e estratégias de manejo sustentável. Os insetos foram capturados em ambientes naturais, mantidos em laboratório e submetidos à análise de COVs por cromatografia gasosa acoplada à espectrometria de massas (GC-MS A metodologia experimental foi otimizada, definindo-se as condições ideais como fibra exposta por 3 horas, utilização de 2 insetos por ensaio, o que garantiu maior confiabilidade e reprodutibilidade dos resultados. Machos isolados emitiram dois compostos, fêmeas nove, e casais apresentaram 21 compostos, evidenciando maior diversidade química na interação entre os sexos. Destacam-se o 2-etil-1-hexanol e o nonano, 2,2,4,4,6,8,8-heptametil-, presentes em todas as condições, sugerindo papel basal na comunicação química. A emissão variou sazonalmente, com maior diversidade no outono, período reprodutivo, e menor na primavera, refletindo o ciclo de vida. O estudo fornece um protocolo otimizado e identifica compostos-chave, oferecendo subsídios para futuras investigações sobre feromônios e manejo integrado de *P. spumarius*.

Palavras-chave: *Philaenus spumarius*; compostos orgânicos voláteis; *Xylella fastidiosa*; feromônios;

ABSTRACT

This study aimed to characterize the volatile organic compounds (VOCs) emitted by *Philaenus spumarius,* the main vector of the bacterium *Xylella fastidiosa*, which causes serious diseases in olive, grapevine, and almond trees. Given the lack of effective treatments, identifying these compounds could aid in the development of traps and sustainable management strategies. The insects were captured in natural environments, kept in the laboratory, and subjected to VOC analysis by gas chromatography-mass spectrometry (GC-MS). The experimental methodology was optimized, defining ideal conditions as fiber exposure for 3 hours, using two insects per experiment, which ensured greater reliability and reproducibility of the results. Single males emitted two compounds, females nine, and pairs emitted 21 compounds, demonstrating greater chemical diversity in the interaction between the sexes. 2-ethyl-1-hexanol and nonane, 2,2,4,4,6,8,8-heptamethyl-, stand out, present in all conditions, suggesting a basal role in chemical communication. Emission varied seasonally, with greater diversity in autumn, the reproductive period, and lower in spring, reflecting the life cycle. The study provides an optimized protocol and identifies key compounds, offering support for future research on pheromones and integrated management of *P. spumarius.*

Keywords: *Philaenus spumarius*; volatile organic compounds; *Xylella fastidiosa*; chemical communication; pheromones;

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1. INTRODUCTION AND OBJECTIVES

As the global population continues to grow, the demand for food is increasing accordingly, placing greater pressure on agricultural systems. Among the challenges faced by modern agriculture, plant diseases stand out as a major factor compromising both crop productivity and quality (Zhang et al., 2024). Bacteria play a particularly significant role in this context, as they are ubiquitous microorganisms that perform essential ecological functions but can also act as pathogens, causing substantial losses across a wide range of crops (Machini,Oliveira-Brett, 2021).

One of the most concerning bacterial pathogens is *Xylella fastidiosa* (Gammaproteobacteria), a gram-negative bacterium described by Wells et al. (1987). Belonging to the class Gammaproteobacteria, this xylem-limited species is considered one of the most dangerous for agriculture due to its remarkably broad host range. This bacterium affects more than 30 families of *monocots* and *dicotyledons*, generating substantial environmental, social and economic impacts (Camino et al., 2021; Bajocco et al., 2023). Its transmission occurs naturally by insect vectors that feed on xylem sap, belonging to the *Aphrophoridae* and *Cicadellidae* families (Zicca et al., 2020; Raparelli et al., 2024; Imam et al., 2024; Anastasaki et al., 2021). Some of the main species being *Homalodisca vitripennis* (Germar, 1821) *Graphocephala atropunctata* (Signoret, 1854), *Dilobopterus costalimai* (Lin Young, 1977), *Philaenus spumarius (P. spumarius)* (Linnaeus, 1758), *Neophilaenus campestris* (Fallén, 1805) and *Cicadella viridis* (Linnaeus, 1758).

The identification of volatile organic compounds (VOCs) that can affect the behavior of these insects is an important factor in developing for more effective control strategies. Animals' sensory systems, shaped by selective pressures, influence their behavioral responses to natural stimuli, making the study of perception mechanisms fundamental for pest management (Santer, Allem, 2025). Among the main chemical signals involved in this process, semiochemicals stand out, small molecules used in intra and interspecific communication. In the case of insects, these compounds can perform defense functions or act as pheromones, regulating interactions between individuals of the same species (Rebholz et al., 2023).

Currently, control of *X. fastidiosa* is based on the removal of infected plants, drastic pruning, the use of healthy seedlings for new plantings and the management of insect vectors (Sabri et al., 2025; Gilioli et al., 2023). However, the use of chemical pesticides to control pests has raised environmental and health concerns, leading to the search for alternative methods, such as the use of VOCs to manipulate the behavior of vectors and reduce their spread (El

Arroud et al., 2024).

In this context, the identification and characterization of these volatiles allow the development of attractive traps that mimic natural signals, inducing insects to approach controlled baits. According to Wang (2024), the use of synthetic sex pheromones is one of the most promising approaches within integrated pest management, as it attracts adult males to specific traps, enabling their monitoring or capture. Furthermore, as highlighted by Nahar (2024), the large-scale application of such traps can significantly reduce the reproductive population of vectors, which in the long term contributes to a decrease in egg laying and, consequently, in the incidence of associated diseases, such as those caused by *X. fastidiosa*. Thus, understanding volatile compounds represents a strategic step toward the development of more precise and environmentally conscious pest control strategies.

Given this scenario, the present study aims to investigate the VOCs emitted by *X. fastidiosa* vectors, contributing to the development of sustainable strategies to control this bacterium and minimizing the impact of pesticides in agriculture (Abenaim et al., 2025).

General Objective

To develop and apply an analytical methodology for the identification and characterization of VOCs emitted by *P. spumarius*, with the aim of elucidating their role in chemical communication and their potential relationship with reproductive behavior. In order to achieve the main objective, several specific objectives were established:

- **i.** To develop and optimize an analytical protocol suitable for the collection, identification, and quantification of VOCs emitted by *P. spumarius* under controlled laboratory conditions;
- **ii.** To identify and chemically characterize the VOCs emitted by *P. spumarius*, establishing the qualitative composition and relative abundance of the detected compounds;
- **iii.** To compare the volatile emission profiles of unmated males, unmated females, and couple (female and male together), in order to identify compounds potentially involved in chemical communication and mating-related behavior;

2. BIBLIOGRAPHIC REVIEW

2.1 *Xylella fastidiosa*

The increase in global trade and the growth of travel, combined with climate change, have driven the spread of several plant pests, generating significant impacts on the agricultural, environmental and socioeconomic sectors (Camino et al., 2022). Among these threats, *X. fastidiosa* stands out, an aerobic and phytopathogenic Gram-negative bacterium that affects several plant species and can significantly compromise agricultural productivity (Boutigny et al., 2023; Fagerquist et al.,2023; Tortorici et al., 2024; Raparelli et al., 2024). According to the European Food Safety Authority (EFSA), the total number of host plants for this bacterium in category E, which includes all reported positive plant species, regardless of the detection methods applied, now reaches 696 species, distributed across 307 genera and 88 botanical families.

This bacterium includes several subspecies with different hosts and impacts. *X. fastidiosa subspecies fastidiosa* (Xff) affects crops such as grapes (*Vitis vinifera*), alfalfa (*Medicago sativa*) and some almond varieties (*Prunus dulcis*), being the main cause of Pierce's disease (PD) in grapevines (Donegan et al., 2025). The *multiplex subspecies* (Xfm) infects species such as peach (*Prunus persica*), plum (*Prunus domestica*) and blueberry (*Vaccinium* spp.), in addition to several hardwood trees native to North America (Burbank, Ortega, 2018).

In turn, the *pauca subspecies* (Xfp) has strains that affect crops such as sweet orange (*Citrus sinensis*) and coffee (*Coffea arabica*), putting olive production in Europe at risk (Schneider et al., 2021; Yuan et al., 2015). Additionally, the *subspecies sandyi* (Xfs) infects oleander (*Nerium oleander*), causing damage such as leaf burn and death of ornamental plants (Burbank, Ortega, 2018).

According to the International List of Quarantine Organisms EPPO, the bacteria spread mainly through xylem sap-boring insect vectors, which feed on infected plants.

Once in the host, the bacteria colonize the xylem, obstructing the absorption of water and nutrients, which impairs sap flow and generates the characteristic symptoms of the disease. Furthermore, xylem-related signals are often associated with the formation of bacterial biofilms and other plant defensive structures. As the infection progresses, these colonies accumulate on the walls of the xylem vessels, making sap transport even more difficult and worsening the damage to the affected plants. Its diffusion rate varies depending on the species of insects and plants affected, making its control a major challenge (Hall-Stoodley et al.,2004; Walker et al., 2024).

Furthermore, the first records of diseases associated with this bacterium date back to the end of the 19th century, when it was identified in vines in California, causing the so-called Pierce's disease, which significantly compromised grape production in the region (Scortichini et al., 2024).

Xylella fastidiosa was first reported in Europe in 2013, associated with olive groves in the Apulia region, Italy (Saponari et al., 2013) resulting in Rapid Olive Decline Syndrome, which affected around 8,000 hectares of olive groves. In just one year, the infected area almost tripled (Boutigny et al., 2023; Raparelli et al., 2024), and the estimated costs of the epidemic in Apulia could reach up to €5.2 billion over the next 50 years if infected trees are not replaced (Raparelli et al., 2024).

Faced with these challenges, the bacteria cause significant losses in several crops both in the Americas and in other areas such as Europe and Israel. Its direct damage affects essential crops, such as almonds, citrus, vines, olives and stone fruits, in addition to impacting forest, landscape and ornamental trees, generating serious economic consequences (Camino et al., 2022; Beretta et al., 2022).

Among the main diseases associated with this bacterium, variegated chlorosis of citrus (Li et al., 2013), the rapid decline of olive trees (Bruno et al.,2021) and Pierce's disease, which affects grapevines (Aldrich et al., 2015), stand out.

Unfortunately, there are no treatments available to deal with the *X. fastidiosa* bacteria (Telesca et al., 2023). However, control strategies focused on reducing the population of insect vectors can help limit the access of these insects to infectious plants, preventing subsequent inoculation of the pathogen (Tortorici et al., 2024).

2.2 Insect vectors of *Xylella fastidiosa*

Xylella fastidiosa is transmitted exclusively by xylem-sap-feeding insects (Frazier, 1965). These insects feed on the xylem vessels of plants, where the bacterium resides, making them efficient vectors. When an infected insect feeds on a healthy plant, it introduces the bacterium into the xylem, where it multiplies and spreads, causing diseases (Redak et al., 2004; Almeida et al., 2005). The insects' vectors of *X. fastidiosa* belong to the order *Hemiptera*, suborder *Cicadomorpha*, including sharpshooters (*Cicadellidae: Cicadellinae*), spittlebugs (*Aphrophoridae)*, and froghoppers (*Cercopidae*) (Novotny,Wilson, 1997; Krugner et al., 2019). Their efficiency in transmitting *X. fastidiosa* depends on their feeding behavior, biology, ecology, and ability to acquire and inoculate the bacterium.

In the Americas, sharpshooters such as *Homalodisca vitripennis* (Figure 1a), the most epidemiologically relevant vector of Pierce's Disease in North America, *Graphocephala atropunctata (*Figure 1b*)* formerly key in California, and *Draeculacephala minerva (*Figure 1c*)* in central California are the main vectors, while in South America, species including *Oncometopia facialis*, *Dilobopterus costalimai*, *Acrogonia citrina (*Figure 1d*)*, and *Bucephalogonia xanthophis* play significant roles in spreading Citrus Variegated Chlorosis (Hernandez-Martinez et al., 2007, 2009; Daane et al., 2011; Paiva et al., 1996).

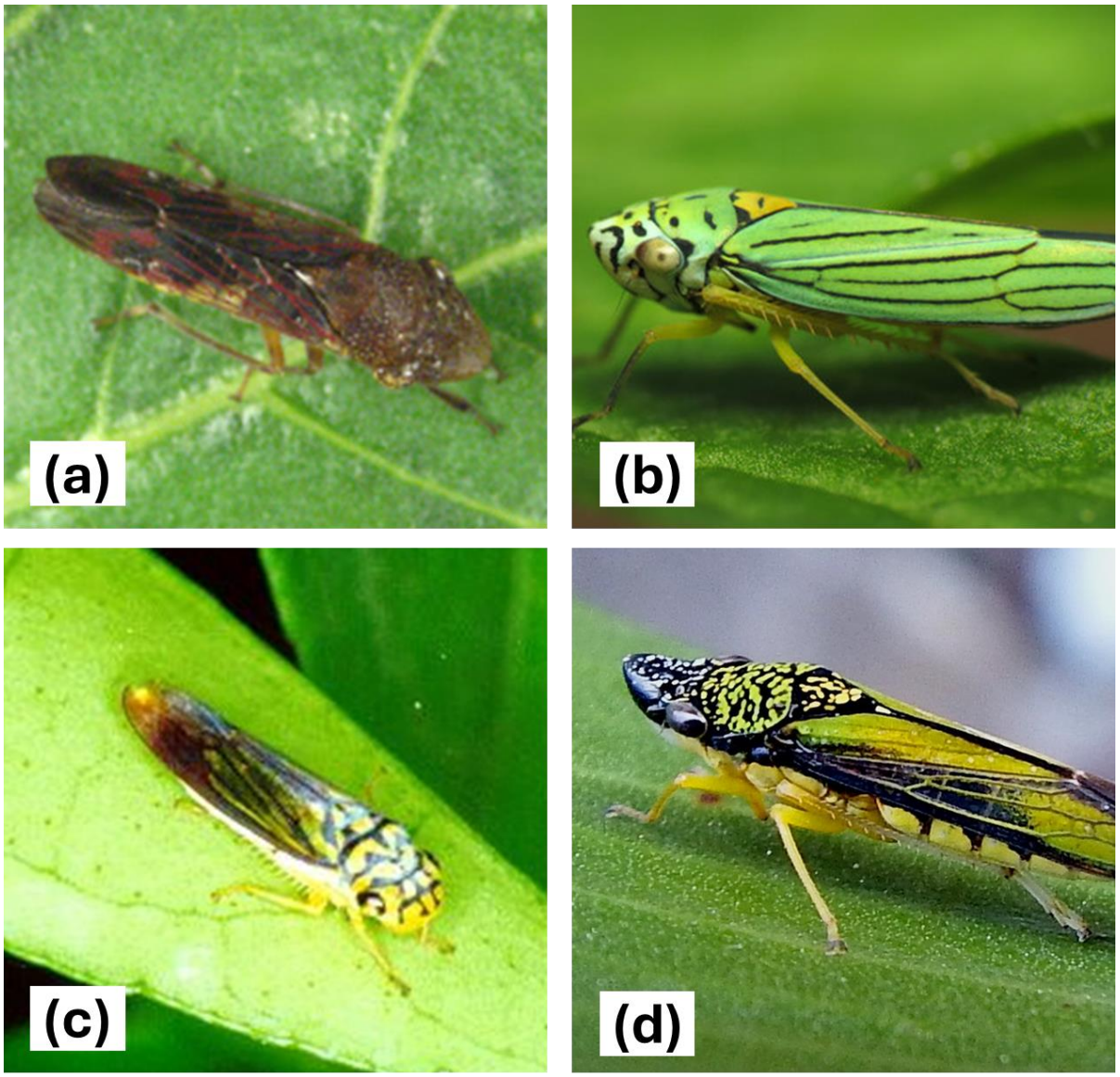

Figure 1 : American insect vectors of *Xylella fastidiosa*: (a) *Homalodisca vitripennis* (Cabanillas, Jones, 2013); (b) *Graphocephala atropunctata* (Biodiversity4all, 2019); (c) *Dilobopterus costalimai* (Agrolink, 2024); (d) Acrogonia citrina (Flickr,2014).

In Asia, *Kolla paulula* has been identified as a key vector during Phony Peach Disease outbreaks (Bragard et al., 2019). In contrast, Europe exhibits a different vector dynamic.

Sharpshooters are relatively scarce, and spittlebugs, particularly the meadow spittlebug *P. spumarius* (Figure 2a), are considered the primary vectors of *X. fastidiosa*, especially in olive groves and other crops (Cornara et al., 2019; Jacques et al., 2019; Avosani et al., 2022). Other European vectors include *N. campestris* (Figure 2b), which has been shown to transmit *X. fastidiosa* under experimental conditions, although with lower efficiency compared to *P. spumarius* (EFSA, 2015; Cornara et al., 2019), and *P. italosignus*, another species with potential vector status (Cavalieri et al., 2019). Additionally, *C. viridis* (Figure 2c), an abundant European member of the *Cicadellidae*, has been demonstrated as a competent vector under laboratory conditions, although its acquisition and inoculation rates are lower than those of *P. spumarius* (Bodino et al., 2022). Recently, the sharpshooter *Draeculacephala robinsoni* (Figure 2d) has also been detected in Europe, further contributing to concerns about vector diversity (Rösch et al., 2022).

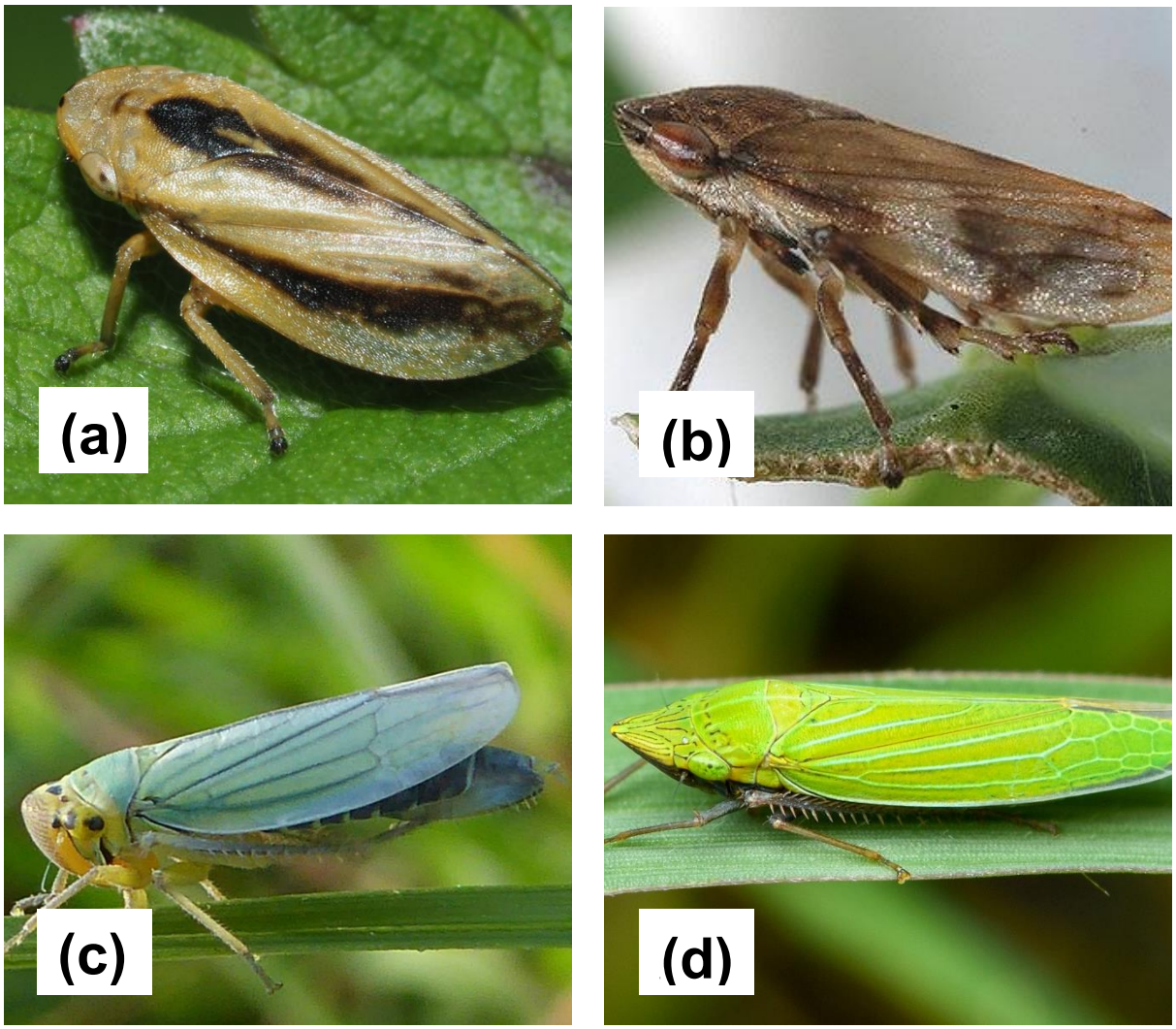

Figure 2: Europe insect vectors of *Xylella fastidiosa*: (a) *P. spumarius* (Biodiversity4all, 2020); (b) *Neophilaenus campestris* (British Bugs, 2008); (c) *Cicadella viridis* (Biodiversity4all,

2015); (d) *Draeculacephala robinsoni* (True Hoppers, 2022)

2.3 Volatile organic compounds

In recent years, biological control has emerged as an effective approach to pest management, becoming increasingly relevant as we seek to reduce the use of chemical products in agriculture (Huang et al., 2024).

Since the dawn of agriculture around 10,000 years ago, plant pests and diseases have been ongoing challenges to agricultural productivity. This problem remains a central concern, affecting food security and crop sustainability (El Bey et al., 2025). VOCs are substances with a boiling point below 250°C at 1 atm and are widely known for their easy evaporation at ambient temperatures (Anju et al.,2024; Lin et al., 2025).

These properties make them evaporate easily, being present both in the environment and emitted by living organisms, such as humans, plants and animals. The emission of these compounds can vary depending on the age and health of the organisms, among other factors (Lin et al., 2025).

2.3.1 Volatile organic compounds in the plant kingdom

Plants are complex organisms that produce a wide variety of secondary metabolites, both volatile and non-volatile. Among these, plant VOCs have gained prominence, being compounds that include tens of thousands of substances. These VOCs have shown great potential as a strategy to strengthen inducible plant defenses against biotic and abiotic stresses (Murali-Baskaran et al., 2022).

Volatile organic compounds are released by plants as part of their interaction with the environment, whether biotic or abiotic. They perform several essential ecological functions, such as attracting pollinators, repelling herbivores and also serving as responses to environmental stresses (Losch et al., 2024). Furthermore, they serve as warning signals to other plants of the same species, indicating that a plant is being attacked by herbivores (Zheng et al., 2025). Additionally, VOCs also help plants protect themselves. By releasing these compounds, plants can defend themselves from damage caused by stress, either by altering their chemical responses or interfering with the behavior of organisms around them (Liu et al., 2023).

2.3.2 Volatile organic compounds in the animal kingdom

Volatile organic compounds emitted by animals and microorganisms are produced by essential metabolic pathways and act in signaling resources, habitats and social interactions.

They function as chemical signals captured by organisms that have specific sensory receptors. In animals, olfactory receptors allow selective detection of these compounds, filtering out those with ecological and behavioral importance in the complex chemical environment (Frey et al., 2022).

Furthermore, they are highly dynamic and multifunctional, providing valuable information about various biological processes. Its detailed analysis, especially in environmental and respiratory studies, plays a crucial role in diagnosing diseases, monitoring ecosystems, and understanding the biochemical mechanisms underlying these interactions (Sadaka et al., 2024).

2.3.2.1 Volatile organic compounds sin insects

The insect environment is rich in VOCs, essential for searching for food, choosing substrates for oviposition, identifying reproductive partners and detecting threats. (Koutoumpa, Jacquin-Joly, 2014).

Furthermore, insects use olfactory signals to communicate with each other, whether to locate reproductive partners or to form colonies or social groupings. This chemical communication capacity facilitates organization and interaction within populations, contributing to the structuring of communities and collective survival strategies (Venkateswaran et al., 2023).

Among the most important chemical signals are sexual pheromones, which help in recognizing species, differentiating between males and females and evaluating the quality of partners. In many insect species, both males and females can act as senders and receivers of these signals. (Jacquin-Joly,Groot 2024). Pheromones are the main mediators of this intraspecific communication, playing an essential role in regulating reproduction (Wyatt, 2017).

2.4 Techniques for analyzing and characterizing volatile organic compounds

The techniques used to measure VOCs have different levels of sensitivity, ranging from more accessible methods, such as the electronic nose (e-nose), to combined approaches, such as the integration of the e-nose with gas chromatography (Schincaglia et al., 2024) and Fourier Transform Infrared Spectroscopy (Li et al., 2023). However, many of these methodologies require complex infrastructure or have high costs, in addition to being subject to uncertainties in both qualitative and quantitative precision.

Existing work on the characterization of volatile compounds from insects presents some limitations in their methodologies. For example, one study removed volatiles directly

from the glands of *Thyrinteina arnobia* (an insect from the *Geometridae* family), which were dead, which could compromise the accuracy of the results, since the insect's metabolic activity, essential for the release of volatile compounds, is no longer present after death (Almeida et al., 2021). This procedure can generate flaws in the characterization of compounds, since the same natural conditions of interaction between living individuals are not found. In this study, extracts from wings and legs of virgin female *T. arnobia* were analyzed by gas chromatography with flame ionization detector (GC-FID), but no significant difference was observed in the responses of male antennae to any of the stimuli offered, suggesting that *T. arnobia* does not use cuticular compounds for short-distance recognition (Almeida et al., 2021).

A study conducted by Senthilkumar et al. (2012) analyzed volatile compounds released by insects in the environment, identifying emissions from *Tribolium castaneum* (red flour beetle) and *Cryptolestes ferrugineus* (rusty grain beetle) using the headspace technique. However, this method has limitations, as environmental factors and surrounding plants can interfere with the results by mixing insect-emitted volatiles with other compounds present in the environment. The study also found that extreme temperatures significantly increased volatile emissions. In *T. castaneum*, the concentrations of methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, and 1-tridecene released by ten adults were 8.5, 9.1, and 10.6 mg/100 mL, respectively, compared to 7, 8, and 4.2 mg/100 mL for five adults. *T. castaneum* larvae did not produce volatile compounds under either normal or extreme conditions (Senthilkumar et al., 2012).

Furthermore, there are still no studies on the characterization of volatile compounds emitted by *X. fastidiosa* vectors, which represents an important gap in research. Although some approaches, such as the analysis of volatile compounds in other insects, can be adapted, there is no specific protocol that directly applies to *X. fastidiosa* vectors.

For these reasons, we propose a new methodology that seeks to overcome the flaws identified in previous studies. Our approach aims to optimize the process of extracting volatile compounds from live insects, as there is still no standardized protocol for this purpose. With this optimization, we hope to obtain more accurate and representative results of natural interactions, minimizing external interference and contributing to a deeper understanding of the chemical ecology involved in communication between insects.

2.5 Impact of volatile organic compounds characterization on the control of *Xylella fastidiosa*

Understanding the behavioral interactions of insects with different semiochemicals can

contribute to the improvement of pest monitoring and capture strategies (Baroffio et al., 2018). Insects use chemical communication through pheromones to locate food and hosts, in addition to playing essential roles in alert, defense, aggregation and reproduction (Guo et al., 2023). Identifying the compounds involved in this process makes it possible to create more efficient traps for population control and surveillance.

However, despite the potential of pheromones in pest management, the use of pesticides is still widely adopted in agriculture over time, mainly for the control of weeds, pests and plant diseases. However, excessive application of these organic compounds has contributed to the contamination of water resources, affecting both wastewater and aquatic ecosystems (Punniyakotti et al., 2024).

Given this scenario, traps that use sexual pheromones are an efficient and environmentally sustainable strategy for monitoring and controlling insect pests. This method takes advantage of insects' chemical communication, employing synthetic versions of these pheromones to attract and capture specific targets. Its application allows for early detection, population estimation and definition of the ideal time for management actions (Alnafisah, El-Shahed, 2024). Furthermore, creating controlled release systems for volatiles to attract and capture vectors can indirectly reduce environmental impacts associated with conventional control methods. This approach has great potential in sustainable pest management and biological control. (Punniyakotti et al., 2024). The adoption of ecological strategies is essential to minimize damage to plant health and preserve the balance of ecosystems.

4. MATERIALS AND METHODS

4.1 Insect capture and rearing

From mid-October to November 2024, adults of *P. spumarius* and *C. viridis,* were captured in an olive grove located in the Trás-os-Monte's region (Portugal) (41°48′10.1″ N, 6°44′50.9″ W) using entomological sweeping nets. From March to April 2025, fifth-instar nymphs were manually collected from the same olive grove. Both adults and nymphs were reared under controlled laboratory conditions of 23 °C, 60–79% relative humidity, and a photoperiod of 16 hours of light and 8 hours of darkness, on *Lavandula* sp. and *Medicago sativa* plants. To ensure virginity, newly emerged males and females, in the spring, were kept in separate rearing cages.

4.2 Optimization of HS-SPME conditions for the extraction of volatile organic compounds from *Philaenus spumarius*

The collection of VOCs emitted by *P. spumarius* was carried out using the headspace solid-phase microextraction (HS-SPME) technique coupled with gas chromatography-mass spectrometry (GC-MS). A series of preliminary assays were conducted to optimize the VOC extraction procedure. These tests were performed with adult females of *P. spumarius* and *C. viridis* collected in October 2024, with the objective of establishing the optimal experimental conditions for HS-SPME.

Only females of *P. spumarius* were used in these assays, as previous studies have demonstrated that males exhibit behavioral and electrophysiological responses to volatiles released by conspecific females, but not the opposite. According to Sevarika et al. (2022), unmated males are significantly attracted to female-emitted headspace volatiles, suggesting the existence of female-derived semiochemicals potentially involved in intraspecific chemical communication. Different experimental parameters were systematically evaluated, including the number of insects per vial and the fiber exposure time, to identify the combination that produced the most consistent and representative volatile profiles.

During optimization, insects were individually or collectively introduced into 20 mL glass vials. Two types of vials were compared: those cleaned but not sterilized, and those subjected to dry-heat sterilization at 90 °C for 8 hours, allowing assessment of the influence of vial cleanliness on chromatographic background and compound recovery. Each vial was sealed with a Teflon film that permitted insertion of the SPME fiber without altering the internal headspace conditions.

The vials were partially submerged (approximately three-quarters of their volume) in

a thermostatic water bath maintained at a constant temperature of 26 °C. After a 5-minute acclimation period, a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm; Supelco, Bellefonte, USA) fiber, previously conditioned in the GC injector, was inserted through the film and exposed to the headspace for the designated extraction time. Following extraction, the fiber was immediately introduced into the GC injector for thermal desorption and subsequent chromatographic analysis.

After systematic evaluation of all experimental variables, a standardized extraction protocol was established. The optimized conditions determined were as follows: two adult insects per sterilized 20 mL vial, 3-hour fiber exposure time at 26 °C. These parameters ensured efficient volatile recovery, minimized contamination, and provided reproducible chromatographic profiles suitable for comparative analyses among biological conditions.

4.3 GC–MS analysis and data processing during optimization

All preliminary assays performed for the optimization of HS-SPME conditions were analyzed by GC–MS under the same analytical parameters.

After each extraction, the SPME fiber was immediately introduced into the injector of a Shimadzu GC-2010 Plus gas chromatograph coupled to a Shimadzu GC/MS-QP2010 SE mass spectrometer (Shimadzu, Kyoto, Japan). Thermal desorption of volatiles was carried out directly in the injector port at 220 °C for 1 minute, followed by an additional 10 minutes for fiber cleaning and conditioning prior to subsequent analyses.

Chromatographic separation was achieved using a TRB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Teknokroma, Spain). The injector operated in splitless mode at 220 °C, using helium (Praxair, Portugal) as the carrier gas at a linear velocity of 30 cm s^{-1} and total flow of 24.4 mL min^{-1}. The oven temperature program was: 40 °C (1 min), followed by a ramp of 2 °C min^{-1} up to 220 °C, and held for 30 min. The ion source was maintained at 250 °C, operating in electron ionization (EI) mode at 70 eV with a scan range of m/z 35–500.

Each chromatogram obtained from the optimization assays was processed using GCMSolution software (Shimadzu). All visible peaks were integrated, and compounds were tentatively identified by comparison of their mass spectra and retention times with those available in the NIST database. Only compounds with a similarity index equal to or greater than 80% were considered for further evaluation. Peaks associated with fiber-related or laboratory contaminants were excluded.

The retention index (Kovats index) of each compound was calculated according to the

equation proposed by van Den Dool and Kratz (1963):

$$I = 100z + 100 * \frac{\log(t_R(i)) - \log(t_R(z))}{\log(t_R(z+1))\log(t_R(z))}$$

where $t_R(i)$is the retention time of the compound, and $t_R(z)$and $t_R(z+1)$correspond to the retention times of the n-alkanes eluting immediately before and after the compound, respectively, with $z$being the carbon number of the preceding alkane.

Theoretical Kovats indices were verified using the NIST, PubChem, and ChemSpider databases, ensuring compatibility with the column specifications used. Compounds were then organized according to their retention times, and all chromatographic data were compiled in Excel spreadsheets for later statistical analysis and comparison among optimization assays.

4.4 Characterization of volatile organic compounds profiles from unmated males, unmated females, and couples of *Philaenus spumarius*

In the spring of 2025, corresponding to the emergence of *P. spumarius* adults, the optimized HS-SPME–GC–MS procedure described above was applied to characterize the volatile profiles emitted by unmated males, unmated females, and male-female couples. The aim was to compare the volatile emissions from each biological condition and determine whether the presence of both sexes influenced the overall composition or intensity of emitted compounds. Active, healthy adults were selected from the laboratory colony and placed in sterilized 20 mL glass vials sealed with Teflon film. Each vial was maintained at 26 °C in a thermostatic water bath, and the SPME fiber was exposed to the headspace for 3 hours. The experimental design included three conditions: two unmated males per vial, two unmated females per vial, and one male–female pair per vial. Each condition was performed in five independent replicates under identical environmental and analytical conditions, with an empty sterilized vial serving as the control. After extraction, the fibers were desorbed and analyzed under the same GC–MS conditions previously established in 4.3. Data processing and compound identification followed the same procedures described for the optimization assays, including spectral comparison with the NIST database, calculation of Kovats indices, and verification in external chemical databases. All chromatographic and identification data were compiled for subsequent comparative analyses.

4.5 Analysis of results and writing of the master's dissertation

The GC–MS data were organized into a matrix containing the area of each identified VOC per replicate and typology. Only compounds consistently detected in at least four replicates per group were retained for further analyses. Prior to statistical analysis, data were log-transformed and standardized (Z-score normalization) to reduce the influence of scale differences among variables.

To assess differences in the overall composition of VOCs among typologies, a Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray-Curtis dissimilarities was conducted using the adonis2 function from the vegan package (Oksanen et al., 2019), with 999 permutations. Homogeneity of multivariate dispersion among groups was tested using the BETADISPER procedure (Anderson, 2006), implemented in the same package.

To visualize general trends and identify the VOCs contributing most to group separation, a Principal Component Analysis (PCA) was performed using the PCA function from the FactoMineR package (Le et al., 2008). The correlation biplot of the first two principal components was generated using the fviz_pca_biplot function from the factoextra package (Kassambara & Mundt, 2020).

To identify the compounds responsible for compositional dissimilarities among groups, a Similarity Percentage (SIMPER) analysis was conducted in vegan (Oksanen et al., 2019). A heatmap representing standardized abundances (Z-scores) was produced using the pheatmap package to illustrate differences in VOC profiles across groups, while a Venn diagram generated with the VennDiagram package (Chen & Boutros, 2011) summarized shared and unique VOCs among typologies.

All analyses were performed in R software (version 4.3.0; R Core Team, 2023). Graphical visualizations were produced using ggplot2 (Wickham, 2016), pheatmap, and factoextra.

5. RESULTS AND DISCUSSIONS

5.1 Optimization of HS-SPME conditions

A series of thirteen preliminary assays were performed to establish the optimal parameters for the extraction of VOCs emitted by *P. spumarius*. The tests evaluated the influence of several experimental factors, including vial sterilization, number of insects, and fiber exposure time, on the quality and reproducibility of chromatographic profiles.

The firsts preliminary assays were designed to determine the minimum number of insects and the appropriate fiber exposure time required to obtain a detectable and representative chromatographic signal of VOCs. In an initial test, a single adult female of *P. spumarius* was placed individually in a vial, and the SPME fiber was exposed to the headspace for thirty-minute. The resulting chromatogram showed only a few low-intensity peaks, corresponding to nineteen identifiable VOCs (Table 1)**,** indicating that the emission from a single insect during this short extraction period was insufficient to produce a consistent volatile profile.

Table 1 : Volatile compounds identified from the exposure of one female *Philaenus spumarius* for thirty minutes.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
2,4-dimethylhept-1-ene	9,702	51729	5,24	850,00	Pubchem
Hexanoic acid	17,947	32732	3,32	1013,00	Pubchem
2,2,4,6,6-pentamethylheptane	18,516	150182	15,23	1003,00	Pubchem
4-methyldecane	20,164	22778	2,31	1060,40	Pubchem
2,2-dimethyldecane	20,886	81359	8,25	1118,00	Pubchem
3,7-dimethylnonane	21,553	87884	8,91	1042,00	Pubchem
5-ethyl-2,2,3-trimethylheptane	22,920	189844	19,25	1001,00	Chemspider
3,7-dimethylnonane	23,295	40319	4,09	1042,00	Pubchem
2,2-dimethyldecane	24,104	52710	5,34	1113,00	Pubchem
2,6-dimethylundecane	24,104	31779	3,22	1210,00	Pubchem
Nonanal	27,416	25661	2,60	1102,00	Nist
2,2,4,4,6,8,8-heptamethylnonane	41,607	39597	4,01	1332,64	Pubchem
2,6,11-trimethyldodecane	41,949	5007	0,51	1320,00	Chemspider

2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,504	14531	1,47	1350,70	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,925	17468	1,77	1331,00	Chemspider
Dodecan-1-ol	51,335	10991	1,11	1474,00	Pubchem
3,5-bis(1,1-dimethylethyl)phenol	53,728	18928	1,92	1555,00	Chemspider
1,1'-oxybis(octane)	62,363	11538	1,17	1688,00	Chemspider
Di-n-octyl benzene-1,2-dicarboxylate	103,931	101318	10,27	2696,30	Pubchem

To evaluate the effect of insect number on VOCs emission, subsequent assays were performed by increasing the number of females per vial. When four adult females were analyzed simultaneously under the same thirty-minute exposure time sixteen VOCs were identified (Table 2), the areas of the identified peaks were larger than those recorded in test 1 (Figure 3). This result confirmed that the emission of volatiles was directly related to the number of insects confined in each vial, and that multiple individuals were required to obtain reproducible and representative chromatographic profiles.

Table 2 : Volatile compounds identified from the exposure of four female *Philaenus spumarius* for thirty minutes.

COMPOUND	**RT(MIN)**	**AREA**	**%RA**	**TK**	**DB**
2,2,4,6,6-pentamethylheptane	18,476	160440	16,77	1003,00	Pubchem
2,2,4,6,6-pentamethylheptane	21,286	98289	10,27	1003,00	Pubchem
3,7-dimethylnonane	21,550	136086	14,22	1042,00	Pubchem
2,6,8-trimethyldecane	21,870	30496	3,19	1121,00	Chemspider
5-ethyl-2,2,3-trimethylheptane	22,914	275603	28,80	1001,00	Chemspider
2,2-dimethyldecane	23,918	33052	3,45	1113,00	Pubchem
2,6,8-trimethyldecane	24,104	39944	4,17	1121,00	Chemspider
5-(2-methylpropyl)nonane	25,355	73634	7,70	1185,00	Chemspider
5-butylnonane	25,737	13232	1,38	1204,00	Chemspider
4-methylundec-1-ene	26,426	16375	1,71	1140,00	Chemspider
3,7-dimethyldecane	26,787	12365	1,29	1133,00	Pubchem

2,2,11,11-tetramethyldodecane	27,958	55427	5,79	1143,00	Chemspider
tridecane	33,345	20274	2,12	1300,00	Pubchem
2,2,4,4,6,8,8-heptamethylnonane	41,616	116877	12,22	1332,64	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,930	22020	2,30	1331,00	Chemspider
1,1′-oxybis(octane)	62,379	13136	1,37	1688,00	Chemspider

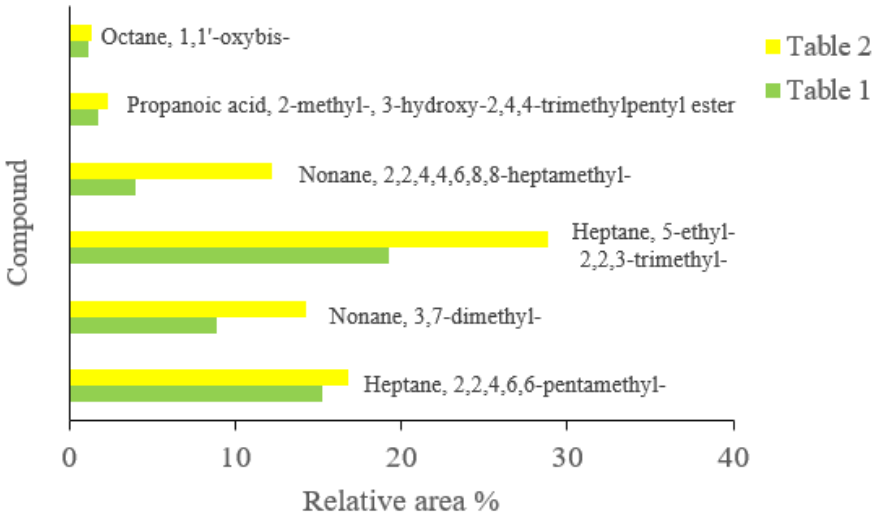

Figure 3 : Comparison of the relative area (%) of volatile compounds emitted by *Philaenus spumarius* that are repeated between Tables 1 and 2.

Based on the results obtained in the previous two tests, a third experiment was conducted to investigate whether increasing the fiber exposure time could result in a greater release of VOSs. For this new test, it was decided to return to using a single female *P. spumarius*, but with a modified fiber exposure time. In this experiment, the female was placed in the vial, where she remained for one hour, with the fiber exposed to the insect's environment. Twenty-one VOCs were identified in this test environment (Table 3).

Table 3 : Volatile compounds identified from the exposure of one female *Philaenus spumarius* for one hour.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
2,4-dimethylhept-1-ene	9,686	121738	8,70	850,00	Pubchem

Hexanoic acid	17,967	36315	2,60	1013,00	Pubchem
2,2,4,6,6-pentamethylheptane	18,508	79529	5,68	1003,00	Pubchem
2,2,4,6,6-pentamethylheptane	21,332	126977	9,08	1003,00	Pubchem
3,7-dimethylnonane	21,574	183216	13,10	1042,00	Pubchem
5-ethyl-2,2,3-trimethylheptane	22,941	364228	26,04	1001,00	Chemspider
2,2-dimethyldecane	23,933	42795	3,06	1113,00	Pubchem
3,6-dimethylundecane	24,124	55202	3,95	1210,00	Nist
5-butylnonane	25,370	94210	6,73	1204,00	Chemspider
5-(2-methylpropyl)nonane	25,750	24207	1,73	1185,00	Chemspider
2,3,4-trimethyldecane	26,126	75908	5,43	1121,00	Chemspider
3,7-dimethyldecane	26,436	16105	1,15	1133,00	Pubchem
3,7-dimethyldecane	26,810	28875	2,06	1133,00	Pubchem
4,6-dimethyldodecane	38,892	23020	1,65	1285,00	Chemspider
2,2,4,4,6,8,8-heptamethylnonane	41,633	31869	2,28	1332,64	Pubchem
2,6,11-trimethyldodecane	41,976	7980	0,57	1320,00	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,503	16854	1,20	1350,70	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,943	21901	1,57	1331,00	Chemspider
Tetradecane	46,763	9498	0,68	1400,00	Pubchem
Dodecan-1-ol	51,338	14494	1,04	1488,00	Pubchem
3,5-bis(1,1-dimethylethyl)phenol	53,739	24015	1,72	1555,00	Chemspider

The comparison between Tables 1 and 3 allowed us to identify the compounds present in both experimental conditions. From this selection, only the compounds that recurred in both analyses were considered, which served as the basis for the corresponding graph (Figure 4). It was found that, with increasing exposure time and maintaining the same number of females, there was no clear pattern in the variation in the relative peak area, some compounds increased, while others decreased. Therefore, additional tests are necessary for a more accurate interpretation of the results.

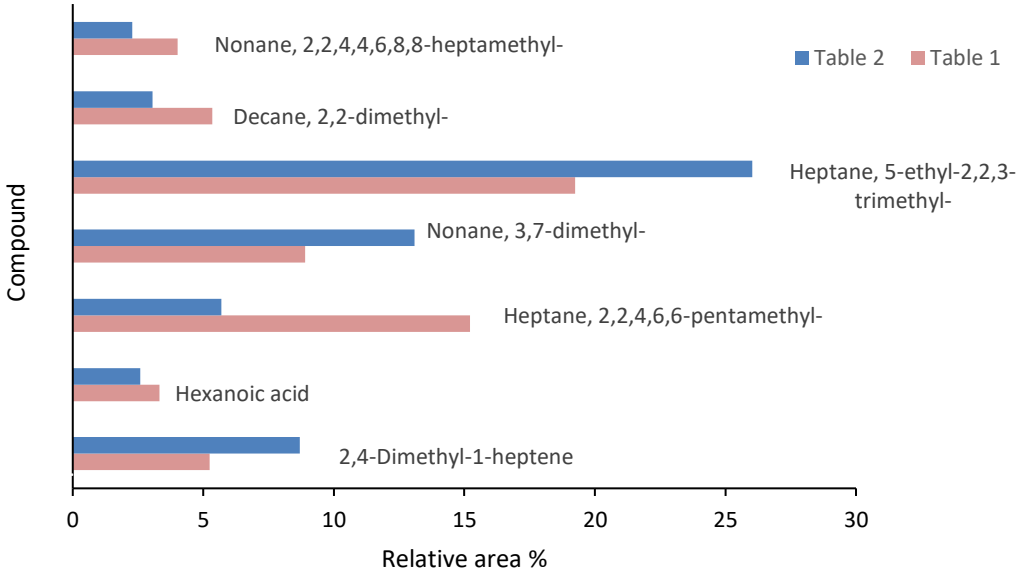

Figure 4 : Comparison of the relative area (%) of volatile compounds emitted by *Philaenus spumarius* that are repeated between Tables 1 and 3.

To ensure the reliability of the results and eliminate any possibility of contamination, two control tests were performed, the results of which are presented in Tables 4 and 5.

The test in Table 4 was conducted without the presence of the insect, reproducing all the steps of the experimental procedure. The vial was kept under the same temperature and water bath conditions, with the fiber exposed to the environment for one hour, to verify whether any compounds could be released or absorbed due to external contamination. Comparison of the chromatograms indicated that some peaks observed in the insect tests were also present in this control, suggesting possible interference from the environment or the vial itself.

To eliminate this hypothesis, the test in Table 5 was conducted using a vial that had been washed with soap and water, followed by drying in an oven at 90°C for eight hours. This procedure aimed to completely remove any residue and ensure the absence of contaminants. After sterilization, the empty vial was again exposed to the fiber for one hour and analyzed under the same experimental conditions.

The results showed that the chromatogram obtained from the sterilized vial contained one fewer compound compared to the previous control, demonstrating the effectiveness of cleaning and oven drying in eliminating external contamination. Therefore, it was decided that, from this point forward, all tests would be performed exclusively using previously sterilized vials, ensuring greater reliability and reproducibility of the results.

Table 4 : Volatile compounds identified from exposure of the empty bottle for one hour.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
2,6,11-trimethyldodecane	38,895	8002	18,07	1320,00	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,520	16870	38,10	1350,70	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,944	19414	43,83	1331,00	Pubchem

Table 5 ; Volatile compounds identified from the exposure of the empty bottle coming from the oven for one hour.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
1-ethoxy-2-(2-ethoxyethoxy)ethane	25,122	38282	73,77	1058,00	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,941	13612	26,23	1331,00	Chemspider

Given this conclusion, it was necessary to restart the experiments to ensure data reliability. Therefore, the procedure was resumed using a single female *P. spumarius*, but this time exclusively employing vials previously sterilized in an oven at 90 °C for eight hours, with the SPME fiber exposed to the headspace for thirty minutes. This new assay aimed to ensure that the peaks recorded in the chromatogram were exclusively from the release of compounds by the insect, eliminating any interference caused by external contamination. The Table 6 shows the eleven identified VOS in this assay.

Table 6: Volatile compounds identified from the exposure (empty bottle coming from the oven) of one female *Philaenus spumarius* for thirty minutes.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
1-(2-methoxy-1-methylethoxy)propan-2-ol	20,146	50163	8,68	981,00	Pubchem
2-(propan-2-yloxy)propane	20,536	24264	4,20	989,70	Chemspider
Nonanal	26,426	7714	1,33	1102,00	Nist
2-(2-butoxyethoxy)ethan-1-ol	32,396	53266	9,21	1167,70	Pubchem

2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,457	76073	13,16	1350,70	Pubchem
Propane-1,2,3-triyl triacetate	43,571	154568	26,74	1306,00	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,893	105071	18,17	1331,00	Chemspider
Di(propan-2-yl) hexanedioate	50,112	9422	1,63	1430,00	Pubchem
Hexadecane	52,649	13926	2,41	1600,00	Pubchem
Isobutyl 2,2,4-trimethyl-3-(carboxypropan-2-yl)pentanoate	58,617	48305	8,36	1605,00	Chemspider
Methyl (3-oxo-2-pentylcyclopentyl)acetate	61,809	19602	3,39	1657,00	Chemspider

Continuing the experiments and aiming to assess whether a larger number of insects would influence the quantity and intensity of the volatile compounds detected, the seventh test was performed. In this test, the same procedure as the previous test was followed, however, instead of a single *female P. spumarius*, four females were used.

The insects were placed in a vial previously sterilized in an oven at 90°C for eight hours. Initially, the insects remained in the vial for five minutes to acclimate to the environment. Then, the fiber was introduced and left exposed for thirty minutes, while the vial was kept in a water bath to preserve thermal stability throughout the process. The Table 7 shows the ten determined VOCs

Table 7: Replicate one – Volatile compounds identified from the exposure (empty bottle coming from the oven) of four female *Philaenus spumarius* for thirty minutes.

COMPOUND	**RT(MIN)**	**AREA**	**%RA**	**TK**	**DB**
2-ethylhexan-1-ol	21,222	16507	8,97	1064,00	Pubchem
Nonanal	26,434	9707	5,28	1102,00	Nist
Decanal	33,626	4675	2,54	1207,00	Nist
Nonadecane	38,837	12578	6,84	1310,00	Chemspider
2,2,4,4,6,8,8-heptamethylnonane	41,566	25853	14,06	1332,64	Pubchem
2,6,11-trimethyldodecane	41,924	11180	6,08	1320,00	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,454	31000	16,85	1350,70	Pubchem

3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,882	35808	19,47	1331,00	Chemspider
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,608	25474	13,85	1605,00	Chemspider
Methyl 3-oxo-2-pentylcyclopentaneacetate	61,797	11151	6,06	1657,00	Chemspider

Analysis of the generated chromatograms allowed us to determine whether increasing the number of females would result in significant differences in the intensity or composition of the detected volatile compounds, comparing the results obtained with those of the test performed with a single female. The same conclusion (Figure 3) was reached: with an increase in the number of females, while maintaining the same exposure time, there was an increase in the relative area (Figure 5).

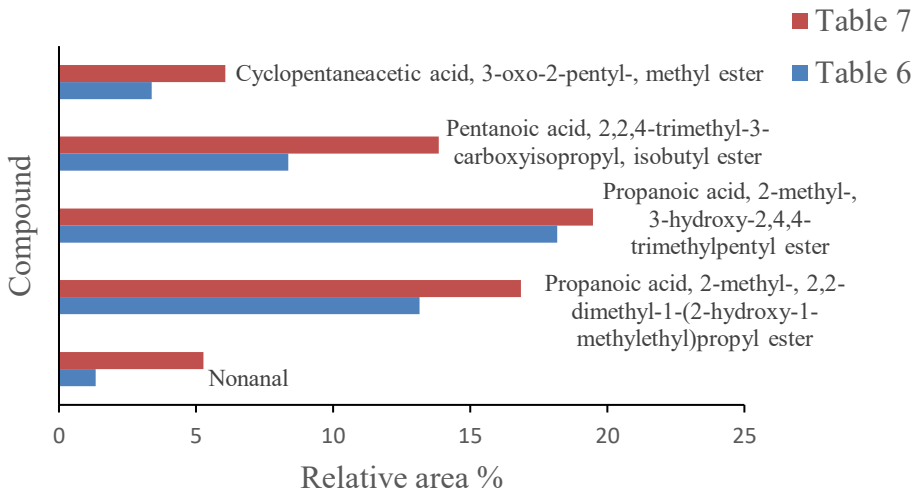

Figure 5 : Relative abundance (%) comparison of volatile organic compounds emitted by *Philaenus spumarius*, showing the compounds identified in both Table 6 and Table 7**.**

To ensure the reproducibility of the results, as the peaks observed in test 7 were consistent, test 8 was performed, repeating exactly the same experimental conditions. As in the previous test, four female *P. spumarius* insects, recently captured and kept in a small, insulated container, were used. The experimental procedure was maintained: the insects were placed in a previously sterilized vial, underwent a five-minute acclimation period, and then the fiber was exposed for thirty minutes, with the vial kept in a water bath. After fiber collection, analysis was performed in a gas chromatograph following the same temperature protocol. Thirteen volatile organic compounds were identified (Table 8).

Table 8: Replicate two – Volatile compounds identified from the exposure (empty bottle coming from the oven) of four female *Philaenus spumarius* for thirty minutes.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
1-(2-methoxy-1-methylethoxy)propan-2-ol	20,226	71021	15,66	981,00	Pubchem
2-ethylhexan-1-ol	21,277	27755	6,12	1037,00	Pubchem
6-methylidene-2-methyloctan-2-ol	24,240	20165	4,45	1074,00	Chemspider
Nonanal	26,489	4908	1,08	1102,00	Nist
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,368	6471	1,43	1180,78	Pubchem
2-(2-butoxyethoxy)ethan-1-ol	32,477	8789	1,94	1169,00	Pubchem
4-(tert-butyl)cyclohexyl ethanoate	39,786	10404	2,29	1315,80	Pubchem
2,2,4,4,6,8,8-heptamethylnonane	41,670	45620	10,06	1332,64	Pubchem
2,6,11-trimethyldodecane	42,089	19188	4,23	1320,00	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,553	82954	18,30	1350,70	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,978	118144	26,06	1331,00	Chemspider
6,10-dimethylundeca-5,9-dien-2-one	50,049	14518	3,20	1455,00	Pubchem
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	51,737	23456	5,17	1494,00	Pubchem

The comparison between Tables 7 and 8, knowing that they used the same experimental conditions, allowed the creation of Table 9, which represents the comparison of volatile organic compounds. It was observed that some compounds were identified only in one of the conditions and not in the other, even when the analyses were performed in duplicate. This variation reinforces the importance of experimental repetition, since the emission of volatile compounds by insects may not occur consistently, at certain times, the insect may release the compound, while at others, due to its biological and behavioral nature.

Table 9: Comparison of Tables 7 and 8 (replicate – four females for thirty minutes) of volatile compounds emitted by *Philaenus spumarius*.

COMPOUND	PRESENT IN TABLE 7	PRESENT IN TABLE 8
1-(2-methoxy-1-methylethoxy)propan-2-ol		x
2-ethylhexan-1-ol	x	x

6-methylidene-2-methyloctan-2-ol		x
Nonanal	x	x
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol		x
2-(2-butoxyethoxy)ethan-1-ol		x
Decanal	x	
Nonadecane	x	
4-(tert-butyl)cyclohexyl ethanoate		x
2,2,4,4,6,8,8-heptamethylnonane	x	x
2,6,11-trimethyldodecane	x	x
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	x	x
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	x	x
6,10-dimethylundeca-5,9-dien-2-one		x
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one		x
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	x	
Methyl 3-oxo-2-pentylcyclopentaneacetate	x	

Based on the conclusions obtained in the previous tests, test 9 was conducted using a single female of the species *C. viridis,* also considered a potential vector of *X. fastidiosa*. This new test aimed to confirm that the detected compounds were, in fact, emitted by insects and not from external contamination. Furthermore, because the test involved a different species, it was expected to observe the formation of a distinct chromatographic profile, representing compounds characteristic of *C. viridis*.

The same experimental protocol was maintained: the insect was placed in a previously sterilized vial, acclimated for five minutes, and then the fiber was exposed for one hour, with the vial kept in a water bath. After the exposure period, the fiber was collected and analyzed in a gas chromatograph under the same conditions as the previous tests. Table 10 below shows the twenty identified volatile organic compounds.

Table 6 : Volatile compounds identified from the exposure of one female *Cicadella viridis* for one hour.

COMPOUND	RT(MIN)	AREA	%RA	TK	DB
Methoxy(phenyl)methanimine	13,155	282572	69,01	1301,00	Chemspider
1-(2-methoxy-1-methylethoxy)propan-2-ol	19,551	160011	39,08	1011,00	Pubchem
1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane	20,188	19862	13,87	1016,00	Nist

2-ethoxyethyl 2-methoxyethyl carbonate	20,551	82820	20,23	1211,00	Chemspider
1-methyl-2-(propan-2-yl)benzene	20,856	32521	22,72	1027,00	Nist
2-propylpentan-1-ol	21,291	19924	4,87	1052,80	Pubchem
6-methylidene-2-methyloctan-2-ol	24,262	10897	2,66	1074,00	Chemspider
1-methyl-4-(1-methylethylidene)cyclohexene	25,353	7728	5,40	1097,00	Nist
3,7-dimethylocta-1,6-dien-3-ol	26,170	8441	5,90	1112,00	Nist
Nonanal	26,487	6970	4,87	1108,00	Nist
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,348	4225	1,03	1180,78	Pubchem
2-(2-butoxyethoxy)ethan-1-ol	32,486	21161	5,17	1169,00	Pubchem
2-methylprop-2-en-1-yl 2-methylpropanoate	34,207	34883	8,52	1004,00	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,546	79517	19,42	1350,70	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,981	122425	29,90	1331,00	Chemspider
Dodecanal	47,279	34997	24,45	1420,00	Nist
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	51,751	9618	2,35	1494,00	Pubchem
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,699	27504	6,72	1605,00	Chemspider
Tetradecane oxide	59,504	5124	3,58	1702,00	Chemspider
2-(propan-2-yl) tetradecanoate	71,046	5020	3,51	1837,00	Pubchem

Furthermore, a comparison was made between Table 3, which presents the results for the volatile compounds emitted by *P. spumarius*, and Table 9, corresponding to the compounds detected in *C. viridis*. Based on this analysis, Table 11 was prepared, excluding the compounds present in the control vial. It was observed that distinct compounds were emitted between the species, indicating that the method employed was efficient in collecting and detecting volatiles, since the change in species resulted in the identification of different chemical profiles.

Table 7 : Comparison of Tables 3 and 9 of volatile compounds emitted by *Philaenus spumarius* and *Cicadella viridis.*

COMPOUND	PRESENT IN TABLE 3	PRESENT INTABLE 9

2,4-dimethylhept-1-ene	x	
Hexanoic acid	x	
2,2,4,6,6-pentamethylheptane	x	
1-(2-methoxy-1-methylethoxy)propan-2-ol		x
1-methyl-4-(1-methylethyl)-7-oxabicyclo[2.2.1]heptane		x
2-ethoxyethyl 2-methoxyethyl carbonate		x
1-methyl-2-(propan-2-yl)benzene		x
2-propylpentan-1-ol		x
2,2,4,6,6-pentamethylheptane	x	
3,7-dimethylnonane	x	
5-ethyl-2,2,3-trimethylheptane	x	
2,2-dimethyldecane	x	
3,6-dimethylundecane	x	
6-methylidene-2-methyloctan-2-ol		x
1-methyl-4-(1-methylethylidene)cyclohexene		x
5-butylnonane	x	
5-(2-methylpropyl)nonane	x	
2,3,4-trimethyldecane	x	
3,7-dimethylocta-1,6-dien-3-ol		x
3,7-dimethyldecane	x	
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol		x
2-(2-butoxyethoxy)ethan-1-ol		x
2-methylprop-2-en-1-yl 2-methylpropanoate		x
4,6-dimethyldodecane	x	
2,2,4,4,6,8,8-heptamethylnonane	x	
2,6,11-trimethyldodecane	x	
Tetradecane	x	
Dodecanal		x
Dodecan-1-ol	x	
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one		x
3,5-bis(1,1-dimethylethyl)phenol	x	
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate		x
Tetradecane oxide		x

Based on all the results obtained in the previous steps, a final test was performed in triplicate, in which the fiber remained exposed for 3 hours, using four females. From this step, Tables 12, 13, and 14 were generated, which present the compounds identified in each replicate.

Table 8 : Replica one - volatile compounds identified from the exposure of four female *Philaenus spumarius* for three hours.

COMPOUND	RT(MIN)	AREA	%RA
Hexane	3,179	126732	2,10
Acetic acid	3,569	114365	1,89
Cyclohexane	4,410	40891	0,68
2,2,4-trimethylpentane	4,490	1136720	18,80
Butyl ethanoate	8,461	63912	1,06
1,3-dimethylbenzene	11,067	583645	9,65
Methoxy(phenyl)methanimine	13,400	1952798	32,29
2,2,4,6,6-pentamethylheptane	18,528	76418	1,26
2,5-hexanediol	20,264	41601	0,69
2-ethoxyethyl 2-methoxyethyl carbonate	20,568	45427	0,75
2-ethylhexan-1-ol	21,240	243540	4,03
Phenylacetaldehyde	22,059	92720	1,53
2-butyl-1-octanol	23,658	17705	0,29
(Z)-4-tridecene	24,206	14120	0,23
2,4-dimethylpentanal	25,635	11084	0,18
2,6-dimethylheptadecane	26,282	20571	0,34
Nonanal	26,498	44372	0,73
2-phenylethanol	27,037	232466	3,84
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,344	11412	0,19
Hexadecane	33,366	38999	0,64
Decanal	33,711	9415	0,16
2-methylprop-2-en-1-yl 2-methylpropanoate	34,202	18300	0,30
5-(2-methylpropyl)nonane	38,908	21519	0,36
4-(tert-butyl)cyclohexyl ethanoate	39,757	18478	0,31
Tridecane	40,242	24051	0,40
Tetracosan-1-ol	40,654	7855	0,13
(E)-3-eicosene	41,203	6997	0,12
2,2,4,4,6,8,8-heptamethylnonane	41,652	126988	2,10
2,6,11-trimethyldodecane	41,993	18386	0,30
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,541	153977	2,55
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,966	173452	2,87
1-chlorohexadecane	45,265	15940	0,26
Tetradecane	46,776	61269	1,01

Longifolene	47,127	5231	0,09
Dodecanal	47,288	19585	0,32
3,8-dimethylundecane	50,651	15727	0,26
1-decanol	51,379	12153	0,20
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	51,727	17153	0,28
Hexadecane	52,720	39530	0,65
2,6-di-tert-butyl-4-methylphenol	53,755	143186	2,37
2,6,11,15-tetramethylhexadecane	55,406	23501	0,39
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,695	128355	2,12
2,2,3,3,5,6,6-heptamethylheptane	58,820	11208	0,19
Cyclododecanol	59,516	6210	0,10
2-[(1R,2S,4as,5R,8ar)-1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphthalen-1-yl]methanol	62,787	14514	0,24
Eicosane	64,950	20985	0,35
2-(propan-2-yl) tetradecanoate	71,055	3773	0,06
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	73,129	20014	0,33

Table 9: Replica two - volatile compounds identified from the exposure of four female *Philaenus spumarius*for three hours.

COMPOUND	**RT(MIN)**	**AREA**	**%RA**
Acetic acid	3,373	33631	1,24
1-methyl-2-(propan-2-yl)benzene	11,065	468290	17,29
Methoxy(phenyl)methanimine	13,242	731308	27,00
4,4-dimethylhexan-3-ol	20,224	30371	1,12
2-ethoxyethyl 2-methoxyethyl carbonate	20,537	33381	1,23
2-ethylhexan-1-ol	21,235	78116	2,88
3-methyl-5-propylnonane	21,568	43506	1,61
Phenylacetaldehyde	22,048	55478	2,05
5-ethyl-2,2,3-trimethylheptane	22,940	71185	2,63
3-methyldodecane	23,291	15024	0,55
2,5,6-trimethyloctane	23,656	87330	3,22
1-nonanol	24,175	7981	0,29
Decyl pentyl sulfite	24,475	37937	1,40
2,4-dimethyl-1-decene	24,767	15450	0,57
Decyl pentyl sulfite	26,105	22169	0,82

Nonanal	26,485	8105	0,30
2-phenylethanol	27,006	91465	3,38
2,2,4-trimethyldecane	27,983	18360	0,68
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,331	3956	0,15
Decanal	33,660	3030	0,11
2-methylprop-2-en-1-yl 2-methylpropanoate	34,167	17557	0,65
2,6,11-trimethyldodecane	38,885	16610	0,61
4-(tert-butyl)cyclohexyl ethanoate	39,746	10305	0,38
Tridecane	40,209	16714	0,62
2-hexyl-1-decanol	40,634	10401	0,38
2,2,4,4,6,8,8-heptamethylnonane	41,636	61707	2,28
11-methyldodecan-1-ol	41,762	8710	0,32
2,6,11-trimethyldodecane	41,981	8317	0,31
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,509	96186	3,55
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,945	121991	4,50
Tetradecane	46,754	25766	0,95
5-undecyne	51,440	9486	0,35
7-tetradecene	51,755	14535	0,54
Cyclododecane	51,897	30608	1,13
2,6,10,15-tetramethylheptadecane	52,379	8010	0,30
Heptadecane	52,707	27728	1,02
Pentadecane	52,948	18969	0,70
2,6-di-tert-butyl-4-methylphenol	53,744	23057	0,85
Heptadecane	55,406	18035	0,67
2-hexyl-1-dodecanol	56,001	8919	0,33
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,675	116020	4,28
6-methyltridecane	58,815	13416	0,50
Tetracosane	64,934	11959	0,44

Table 10 : Replica three - volatile compounds identified from the exposure of four female *Philaenus spumarius* for three hours.

COMPOUND	**RT(MIN)**	**AREA**	**%RA**
Acetic acid	3,400	37333	1,00
Methylbenzene	6,619	98283	2,63
Butyl ethanoate	8,514	93124	2,49
1,3-dimethylbenzene	11,099	436932	11,67
1-methyl-2-(propan-2-yl)benzene	12,394	123198	3,29

Methoxy(phenyl)methanimine	13,302	698401	18,66
3-octen-1-ol	17,875	48662	1,30
6-methylheptane-1,6-diol	20,293	23680	0,63
2-ethoxyethyl 2-methoxyethyl carbonate	20,607	21575	0,58
5-ethyl-2,2,3-trimethylheptane	20,963	17053	0,46
2-ethylhexan-1-ol	21,302	137247	3,67
Phenylacetaldehyde	22,134	190765	5,10
2,2,5-trimethylhexane	23,008	33355	0,89
5-ethyl-2,2,3-trimethylheptane	23,722	41822	1,12
1-octanol	24,254	9455	0,25
Decyl pentyl sulfite	24,537	18644	0,50
4,8-dimethyl-1-nonanol	24,854	4997	0,13
1-dodecene	25,701	8054	0,22
Nonanal	26,554	16096	0,43
2-phenylethanol	27,090	576221	15,40
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,396	10661	0,28
(E)-2-decen-1-ol	33,741	5101	0,14
2-methylprop-2-en-1-yl 2-methylpropanoate	34,229	12614	0,34
Phenylacetic acid	37,105	640076	17,10
Hexadecane	38,963	13907	0,37
Tetradecane	40,285	16083	0,43
3,7,11-trimethyldodecan-1-ol	40,699	9133	0,24
11-methyldodecan-1-ol	41,272	12156	0,32
2,2,4,4,6,8,8-heptamethylnonane	41,697	74828	2,00
3-ethyl-6-(trifluoroacetoxy)octane	41,818	8899	0,24
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,590	42597	1,14
Pentadecane	44,486	11208	0,30
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45,015	53204	1,42
Pentadecane	46,832	29883	0,80
Dodecanal	47,327	6356	0,17
6,10-dimethylundeca-5,9-dien-2-one	50,093	14521	0,39
2,6,10-trimethyldodecane	50,723	10756	0,29
7-tetradecyne	51,522	11770	0,31
8-heptadecene	51,829	19368	0,52
Cyclododecane	51,963	15326	0,41
2-ethyl-2-methyltridecan-1-ol	52,460	8466	0,23
4,6-dimethyldodecane	52,783	22029	0,59
Tetradecane	53,016	19994	0,53
2,6-di-tert-butyl-4-methylphenol	53,808	24607	0,66
Nonadecane	55,458	14369	0,38

Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,749	43994	1,18
Heptadecane	58,873	12995	0,35
Eicosane	65,012	14737	0,39

The same procedure was performed for the control vial (without the presence of insects) in order to identify possible interferences or compounds originating from the environment and the experimental material. The results obtained for this condition are presented in Table 15.

Table 11 : Volatile compounds identified from the exposure of the empty bottle coming from the oven for three hours.

COMPOUND	RT(MIN)	AREA	%RA
Methoxy(phenyl)methanimine	13,075	180235	37,60
2,2,5-trimethylhexane	23,626	19182	4,00
2-(propan-2-yl) tetradecanoate	61,720	9156	1,91
Hexadecanoic acid	77,689	9284	1,94
Dotriacontane	83,104	44045	9,19
Heneicosane	83,582	121852	25,42
Tetratetracontane	102,368	95590	19,94

After applying the entire methodological procedure, the following table presents the compounds detected in the three replicates of the experiment with females collected in the fall, resulting in the final identification of fifty-eight volatile organic compounds. For each representative compound, the mean experimental retention time, the mean theoretical Kovats number, and the mean peak area were calculated, composing the final Table 16 with the results.

Table 12 : Volatile compounds detected in females of *Philaenus spumarius* in autumn, after the characterization process and final filtration.

COMPOUND	RT(MIN)	AREA	KOVATS CALC	KOVATS TEORICO	BD
Acetic acid	6,61 ± 0,01	177398,33 ± 119478,52	831,39 ± 0,2	660,40	Pubchem
Methylbenzene	2,32 ± 0,12	50566 ± 54469,85	489,38 ± 1,79	763	Nist
Butyl ethanoate	8,47 ± 0,04	59384 ± 36216,92	878,93 ± 0,74	818	Pubchem
1,3-dimethylbenzene	11,08 ± 0,02	496289 ± 77260,17	916,99 ± 0,24	900	Nist
1,4-dimethylbenzene	12,36 ± 0,03	140322,67 ± 15363,49	932,48 ± 0,37	908	Nist
3-octen-1-ol	17,85 ± 0,02	28961,67 ± 17133,49	1018,34 ± 0,22	979	Nist

1,2,4-trimethylbenzene	18,65 ± 0,11	36080,33 ± 34938,71	1027,01 ± 1,19	993,9	Nist
6-methylheptane-1,6-diol	20,26 ± 0,03	31884 ± 9055,8	1061,12 ± 0,48	1151	chemspider
2,2,4,6,6-pentamethylheptane	20,9 ± 0,06	20542,67 ± 12246,26	1069,81 ± 0,78	1003	Pubchem
2-ethylhexan-1-ol	21,26 ± 0,04	152967,67 ± 83824,99	1074,54 ± 0,49	1064	Pubchem
3-methyl-5-propylnonane	21,57 ± 0,05	22469 ± 19980,34	1078,58 ± 0,61	1052	Pubchem
Phenylacetaldehyde	22,08 ± 0,05	112987,67 ± 69883,67	1085,12 ± 0,59	1049	Pubchem
5-ethyl-2,2,3-trimethylheptane	22,97 ± 0,04	38014,67 ± 31103,39	1096,12 ± 0,43	1001	Chemspider
1-octanol	24,21 ± 0,04	10518,67 ± 3204,74	1110,83 ± 0,46	1078	Nist
2,4-dimethyl-1-decene	24,82 ± 0,05	7440,33 ± 7110,16	1117,72 ± 0,51	1117	Chemspider
1-dodecene	25,7 ± 0,07	7818,67 ± 3389,13	1127,49 ± 0,72	1180	Pubchem
Nonanal	26,51 ± 0,04	22857,67 ± 19055,55	1148,7 ± 0,52	1108	Nist
2-phenylethanol	27,04 ± 0,04	300050,67 ± 249344,86	1156,17 ± 0,59	1121	Nist
2,2,4-trimethyldecane	28 ± 0,03	10626,67 ± 7764,69	1169,28 ± 0,41	1165	Chemspider
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	31,36 ± 0,03	8676,33 ± 4105,14	1211,77 ± 0,41	1180,78	Pubchem
Decanal	33,7 ± 0,04	5848,67 ± 3257,5	1250,82 ± 0,6	1188	Nist
2,6,11-trimethyldodecane	38,92 ± 0,04	17345,33 ± 3858,91	1321,45 ± 0,51	1320	Chemspider
4-(tert-butyl)cyclohexyl ethanoate	39,78 ± 0,04	12528 ± 5207,43	1332,15 ± 0,54	1352	Pubchem
Tetradecane	40,25 ± 0,04	18949,33 ± 4429,42	1341,24 ± 6,19	1413	Chemspider
2,2,4,4,6,8,8-heptamethylnonane	41,66 ± 0,03	87841 ± 34531,23	1369,17 ± 0,47	1330,8	Pubchem
2,6,11-trimethyldodecane	42,01 ± 0,04	10730,33 ± 6779,21	1374,28 ± 0,52	1320	Chemspider
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43,55 ± 0,04	97586,67 ± 55703,21	1396,55 ± 0,58	1350,7	Pubchem
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44,98 ± 0,04	116215,67 ± 60331,68	1416,53 ± 0,49	1331	Chemspider
2,6,10-trimethyldodecane	45,28 ± 0,04	10238,67 ± 4943,98	1420,63 ± 0,59	1392	Chemspider
6,10-dimethylundeca-5,9-dien-2-one	50,05 ± 0,04	14056 ± 405,49	1500,99 ± 0,62	1440	Pubchem

(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	51,77 ± 0,05	17018,67 ± 2419,3	1526,62 ± 0,77	1494	Pubchem
Heptadecane	52,74 ± 0,04	29762,33 ± 8926,09	1540,61 ± 0,58	1612	Pubchem
2,6-di-tert-butyl-4-methylphenol	53,77 ± 0,03	63616,67 ± 68913,42	1565,8 ± 0,57	1519	Pubchem
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58,71 ± 0,04	96123 ± 45564,38	1644,92 ± 0,59	1605	Chemspider
Heptadecane	58,84 ± 0,03	12539,67 ± 1172,31	1649,56 ± 5,1	1711	Chemspider
Tetradecanal	59,52 ± 0,03	3421,33 ± 3362,75	1667,02 ± 0,52	1618	Nist
2-(propan-2-yl) tetradecanoate	71,06 ± 0,04	3345,33 ± 387,37	1886,62 ± 0,68	1837	Pubchem
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	73,13 ± 0,03	14881,33 ± 4623,99	1926,05 ± 0,61	1868	Pubchem

In tests performed with *P. spumarius* females, variations in the profile of VOCs emitted were observed depending on the fiber exposure time. When four females were exposed for three hours (Table 16), fifty-eight compounds were identified, while exposure of the same number of females for thirty minutes (Table 8) resulted in the detection of thirteen compounds. Table 17 presents a comparison between the two experimental conditions, highlighting the compounds exclusive to each exposure time and those common to both situations.

Table 13 : Comparison of Tables 16 and 8 of volatile compounds **emitted by** *Philaenus spumarius.*

COMPOUND	PRESENT IN TABLE 16	PRESENT IN TABLE 8
Methylbenzene	x	
Acetic acid	x	
Butyl ethanoate	x	
1,3-dimethylbenzene	x	
1,4-dimethylbenzene	x	
3-octen-1-ol	x	
1,2,4-trimethylbenzene	x	
1-(2-methoxy-1-methylethoxy)propan-2-ol		x
6-methylheptane-1,6-diol	x	
2,2,4,6,6-pentamethylheptane	x	
2-ethylhexan-1-ol	x	x
3-methyl-5-propylnonane	x	
Phenylacetaldehyde	x	
5-ethyl-2,2,3-trimethylheptane	x	
1-octanol	x	
6-methylidene-2-methyloctan-2-ol		x
2,4-dimethyl-1-decene	x	
1-dodecene	x	
Nonanal	x	x
2-phenylethanol	x	
2,2,4-trimethyldecane	x	
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	x	x
2-(2-butoxyethoxy)ethan-1-ol		x
Decanal	x	
2,6,11-trimethyldodecane	x	
4-(tert-butyl)cyclohexyl ethanoate	x	x
Tetradecane	x	
2,2,4,4,6,8,8-heptamethylnonane	x	x
2,6,11-trimethyldodecane	x	x
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	x	x
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	x	x
2,6,10-trimethyldodecane	x	
Tetradecane	x	
6,10-dimethylundeca-5,9-dien-2-one	x	x

(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	x	x
Heptadecane	x	
2,6-di-tert-butyl-4-methylphenol	x	
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	x	
Heptadecane	x	
Tetradecanal	x	
2-(propan-2-yl) tetradecanoate	x	
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	x	

Among the identified compounds, ten were common to both tests: 1-hexanol, 2-ethyl-; nonanal; levomenthol; 4-tert-butylcyclohexyl acetate; nonane, 2,2,4,4,6,8,8-heptamethyl-; dodecane; 2,6,11-trimethyl-, propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester; propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester; 5,9-undecadien-2-one, 6,10-dimethyl-; and α-isomethyl ionone. The remaining compounds were identified exclusively in samples obtained with the fiber exposed for three hours, indicating that the longer collection time favored the detection of a wider number of volatiles emitted by *P. spumarius* females. Based on these results, the experimental parameters for the next stage of the study were defined. The increase in the number of females generated chromatographic peaks of greater intensity; however, this variation did not necessarily reflect an increase in the quantity of compounds released. Therefore, increasing the number of individuals did not provide a qualitative gain in the characterization of volatiles.

With four females, there was greater physical interaction between the insects and the adsorbent fiber, likely due to the higher density of individuals in the vial. This direct contact with the fiber caused enough friction to release compounds from the adsorbent material, not the insects, resulting in chromatograms with numerous undesirable peaks, which required additional effort during the analysis to eliminate them.

The use of three insects was not feasible, as an odd number would make it difficult to form couples for testing with males and females. The exposure time of the fibers did not show a direct relationship with the peak area, but it did influence the quantity of compounds identified in each replicate.

Therefore, the ideal number of insects per test was set at two individuals, with an exposure time of three hours. Each experimental condition was repeated five times, including tests with females, males and couples, ensuring reliability and reproducibility of the results.

Table 18 presents a summary of the optimization tests, describing the experimental conditions used in the collection of volatile organic compounds, as well as the main results obtained.

Table 14 : Summary of optimization tests, including the experimental conditions adopted for the collection of volatile organic compounds and the results obtained

Assay	Experimental Condition	Species / No. of Insects	Exposure Time	Sterilized Vial	Main Observations / Results
1	Preliminary test	*P. spumarius* ♀ (1)	30 min	No	Low-intensity peaks;
2	Increased number of insects	*P. spumarius* ♀ (4)	30 min	No	A greater number of females increases the intensity of the compound peaks.
3	Increased exposure time	*P. spumarius* ♀ (1)	1 h	No	No clear pattern; variation in the intensity of the compound peaks.
4	Control (empty vial)	—	1 h	No	Possible environmental contamination detected.
5	Control (sterilized vial)	—	1 h	Yes	Fewer compounds; cleaning proved effective.
6	Repeat assay one with sterilized vial	*P. spumarius* ♀ (1)	30 min	Yes	Clean chromatogram; no contamination observed.
7	Increased number of insects	*P. spumarius* ♀ (4)	30 min	Yes	Stronger peaks; emission intensity increases with more insects.
8	Repeat of Assay 7	*P. spumarius* ♀ (4)	30 min	Yes	Reproducibility confirmed; biological variation noted.
9	Species substitution	*Cicadella viridis* ♀ (1)	1 h	Yes	Distinct VOC profile confirms biological origin of emissions.
10–12	Triplicate assays	*P. spumarius* ♀ (4)	3 h	Yes	58 compounds identified; consistent and reproducible profiles.
13	Control (empty sterilized vial)	—	3 h	Yes	Residual background contamination evaluated.

5.2 Characterization of volatile organic compounds profiles from unmated males, unmated females, and couples of *Phialenus spumarius*

Volatile compounds characteristic of males, females, and couples of *P. spumarius* were identified, with identification reliability equal to or greater than 80%. The results regarding peak areas and retention times are presented in Tables A1 to A16 in the appendix.

After data consolidation, the final compounds from each experimental group were summarized into three categories, presenting mean retention times, Kovats indices, and relative areas, which represent the profile of VOCs emitted by males, females, and couples. Table 19 presents the identification of two volatile organic compounds from males, Table 20 shows the identification of nine compounds from females, and Table 21 describes twenty compounds identified from couples.

Table 15 : Volatile compounds identified in males of *Philaenus spumarius* after the characterization and final filtration stage.

COMPOUND	RT(MIN)	AREA	KOVATS CALC	KOVATS TEORICO	BD
2-ethylhexan-1-ol	21,31 ± 0,02	12629,6 ± 3798,92	1075,18 ± 0,28	1048	PubChem
2,2,4,4,6,8,8-heptamethylnonane	41,7 ± 0,02	8348,4 ± 4371,92	1369,72 ± 0,33	1332,64	PubChem

Table 16 : Volatile compounds identified in females of *Philaenus spumarius* after the characterization and final filtration stage.

COMPOUND	RT(MIN)	AREA	KOVATS CALC	KOVATS TEORICO	BD
1-hydroperoxy-1-methylpentane	11,28 ± 0,05	31332,6 ± 7427,19	919,6 ± 0,62	914,00	Chemspider
2-ethylhexan-1-ol	21,32 ± 0,01	16423,8 ± 5966,4	1075,39 ± 0,18	1064,00	Pubchem
Benzyl alcohol	21,57 ± 0,02	19147,4 ± 10075,13	1078,59 ± 0,24	1082,00	Pubchem
2,2,4,4,6,8,8-heptamethylnonane	41,7 ± 0,01	13635,4 ± 5912,74	1369,72 ± 0,12	1332,64	Pubchem
1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene	60,24 ± 0,27	5892 ± 2100,39	1671 ± 4,79	1567,00	Pubchem
Methyl 3-oxo-2-pentylcyclopentaneacetate	61,94 ± 0,01	10389,2 ± 5480,79	1708,92 ± 0,17	1657,00	Chemspider

Acetic acid	3,39 ± 0,13	268835,5 ± 92854,53	733,24 ± 1,41	660,40	Pubchem
1-(2-methoxy-1-methylethoxy)propan-2-ol	20,28 ± 0,03	28320,75 ± 16834,01	1061,37 ± 0,36	981,00	Pubchem
5-methyloctadecane	65,01 ± 0,01	4230,75 ± 2393,73	1768,69 ± 0,19	1853,00	Pubchem

Table 17 : Volatile compounds identified in couples *Philaenus spumarius* after the characterization and final filtration stage.

COMPOUND	RT(MIN)	AREA	KOVATS CALC	KOVATS TEORICO	BD
1-methoxy-2-propyl ethanoate	11,24 ± 0,01	67319,4 ± 26860,7	919,05 ± 0,09	857,30	Pubchem
1-(2-methoxy-1-methylethoxy)propan-2-ol	20,26 ± 0,02	48767,6 ± 30937,49	1061,11 ± 0,27	981,00	Pubchem
2-ethylhexan-1-ol	21,3 ± 0,01	14487,4 ± 5289	1075,03 ± 0,17	1111,00	Chemspider
Benzyl alcohol	21,54 ± 0,03	31468,2 ± 4873,51	1078,27 ± 0,34	1081,00	Pubchem
2-butoxyethyl ethanoate	25,71 ± 0,02	9956,6 ± 4032,38	1127,54 ± 0,21	1061,00	Pubchem
2-(2-butoxyethoxy)ethan-1-ol	32,55 ± 0,08	8403,6 ± 5765,63	1225,79 ± 0,91	1193,00	Pubchem
4-(tert-butyl)cyclohexyl ethanoate	39,83 ± 0,03	5135,4 ± 1759,22	1332,82 ± 0,43	1322,00	Pubchem
2,2,4,4,6,8,8-heptamethylnonane	41,69 ± 0,01	24341 ± 8182,47	1369,52 ± 0,22	1332,64	Pubchem
Tetradecane	46,82 ± 0,01	25324 ± 12719,19	1450,53 ± 0,23	1413,00	Chemspider
Dodecanal	47,32 ± 0,01	4951,2 ± 2546,59	1458,66 ± 0,23	1412,00	Nist
(Z)-6,10-dimethylundeca-5,9-dien-2-one	50,08 ± 0,01	9881,8 ± 1951,08	1501,54 ± 0,22	1431,00	Pubchem
Dodecan-1-ol	51,43 ± 0,01	9116,8 ± 7579,69	1521,62 ± 0,21	1488,00	Pubchem
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	51,79 ± 0,03	3924,4 ± 2339,47	1526,91 ± 0,48	1494,00	Pubchem
3,5-bis(1,1-dimethylethyl)phenol	53,82 ± 0,01	12235,6 ± 8330,19	1566,65 ± 0,15	1555,00	Chemspider

Diethyl benzene-1,2-dicarboxylate	58,53 ± 0,01	16049 ± 11850,68	1642,22 ± 0,11	1603,00	Nist
Methyl 3-oxo-2-pentylcyclopentaneacetate	61,93 ± 0,02	10538 ± 9756,79	1708,44 ± 0,26	1654,00	Chemspider
2,6,10,14-tetramethylheptadecane	67,36 ± 0,1	3620,2 ± 1804,83	1811,75 ± 1,77	1893,00	Pubchem
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	73,17 ± 0,01	19361,4 ± 18664,69	1926,79 ± 0,2	1916,00	Pubchem
Cis-5-ethenyl-2-(hydroxymethyl)-5,6,7-trimethyltetrahydrofuran	24,26 ± 0,02	9154,5 ± 2807,25	1111,35 ± 0,28	1089,00	Pubchem
2-phenoxyethanol	34,68 ± 0,06	13761,5 ± 11227,82	1264,83 ± 0,82	1210,00	Pubchem

The analysis of VOCs revealed strong differentiation among males, females, and couples of *P. spumarius* (Figure 6a). Across all samples, 24 VOCs were detected. The Venn partition showed that couples exhibited the highest chemical richness, with 15 unique VOCs, whereas females displayed five unique VOCs and males showed no unique compounds. Two VOCs were shared by all three groups (male-female-couple), and there were two additional VOCs shared only between females and couples. No pair-only overlap was detected for male-couple or male-female (i.e., those counts were zero) (Figure 6b).

The PERMANOVA based on Bray-Curtis dissimilarities confirmed statistically significant differences among typologies ($F = 25.43$; $R^2 = 0.809$; $p = 0.001$; 999 permutations), indicating that about 81 % of the total variance in volatile composition is explained by biological condition. No significant heterogeneity in dispersion was detected (BETADISPER: $F = 0.32$; $p = 0.746$), confirming that these differences are due to composition rather than within-group variance. The SIMPER analysis identified the compounds that contributed most to group differentiation. In the male-female contrast, 1-Hexanol, 2-ethyl- and Acetic acid explained more than 30 % of the pairwise dissimilarity. Differences between females and couples were mainly associated with Benzyl alcohol, Octadecane, 5-methyl-, and Dodecanal, while male-couple contrasts were dominated by Nonane, 2,2,4,4,6,8,8-heptamethyl- and 1-Methoxy-2-propyl acetate. The ten most influential VOCs collectively explained over 70 % of total dissimilarity, defining a small but chemically meaningful subset of markers that distinguish each group.

The PCA biplot (Figure 6c) clearly separated individuals by typology, confirming strong chemical segregation. The first two axes explained 86.5 % of total variance (Dim1 = 63.8 %, Dim2 = 22.7 %). Couples clustered separately, driven mainly by Dodecanal and other

long-chain aldehydes; females grouped with Acetic acid, Benzyl alcohol, and Octadecane, 5-methyl-; and males were characterized by 1-Hexanol, 2-ethyl- and Nonane, 2,2,4,4,6,8,8-heptamethyl-. These patterns confirm a sex- and context-specific chemical differentiation, consistent with the PERMANOVA and SIMPER outcomes.

The heatmap of standardized abundances (Figure 6d) further supported these results. Couples showed a broad and balanced emission spectrum, including compounds absent from isolated individuals, while males and females displayed narrower and more specialized profiles dominated by specific compound classes, short-chain alcohols in males and long-chain aldehydes or esters in females. Hierarchical clustering grouped samples according to typology, reinforcing the robustness of the observed patterns.

The distinct chemical signatures identified here likely reflect sex- and context-dependent regulation of volatile biosynthesis in *P. spumarius*. Couples exhibited the richest and most diverse blend, suggesting that the presence of both sexes triggers interactive or synergistic modulation of emission pathways. For instance, Sevarika et al. (2022) demonstrated that *P. spumariu*s uses volatile compounds for intraspecific communication and that such emissions can mediate behavioral responses, supporting the idea that volatiles function as semiochemicals in this species.

Chemical dimorphism in volatiles is consistent with sex-dependent olfactory behavior documented in *P. spumarius*. A recent study by Rodrigues et al. (2025) found that olfactory responses to olive-tree volatiles were sex-dependent, indicating differences in sensory perception between males and females. Although that study did not directly address emission diversity or pair effects, it reinforces the hypothesis that olfactory dimorphism could underlie the differential VOC emission observed here. Electrophysiological work by Germinara et al. (2017) demonstrated that *P. spumarius* adults respond antennally to various plant VOCs, confirming the species' sensitivity to volatile cues.

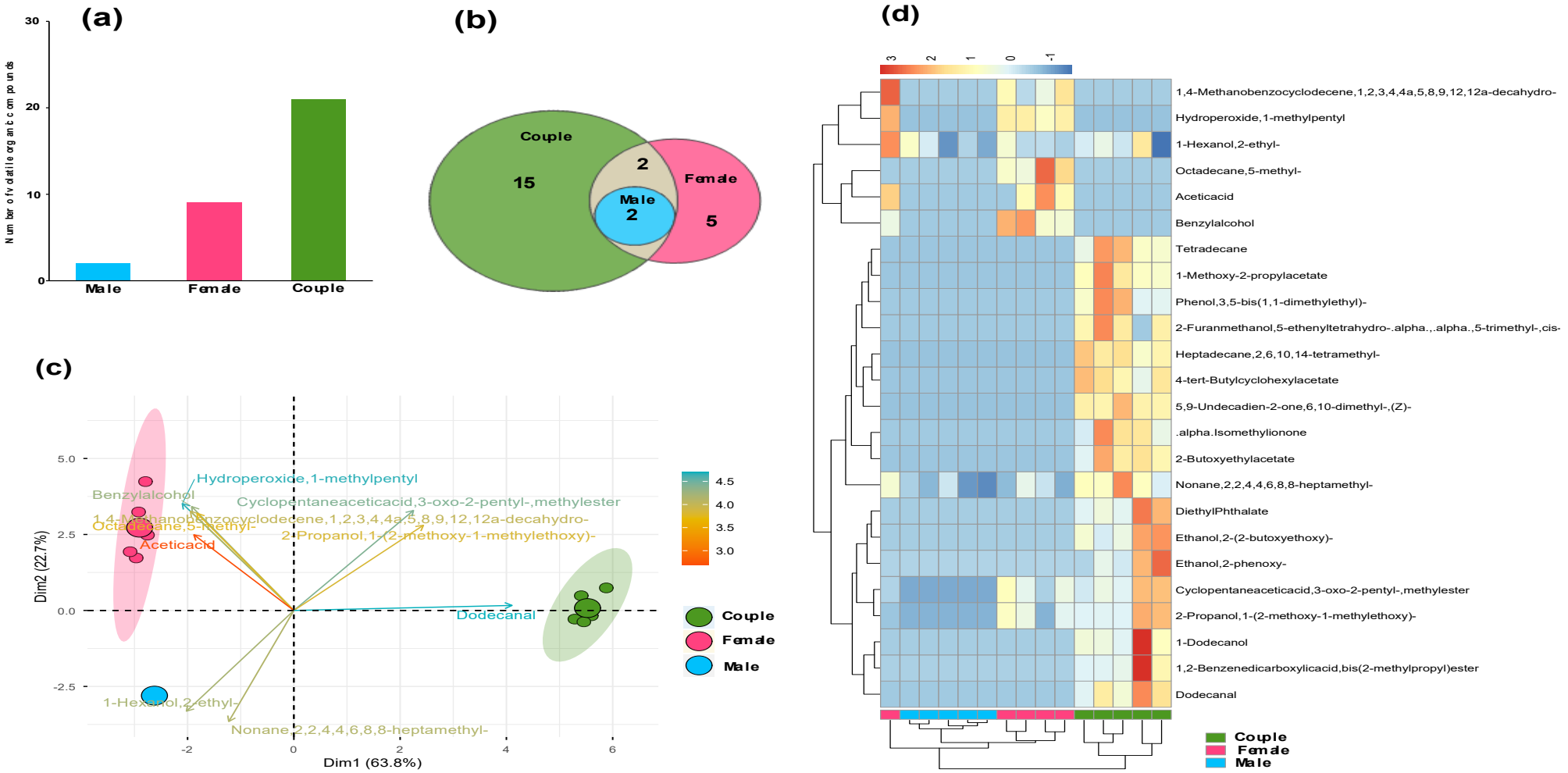

Figure 6 : Comparative analysis of volatile organic compounds (VOCs) emitted by males, females, and couples. (a) Total number of VOCs detected in each biological group. (b) Venn diagram showing the number of exclusive and shared VOCs among males, females and couples. (c) Principal Component Analysis (PCA) biplot based on the ten most contributing compounds. (d) Heatmap of standardized volatile abundances (Z-scores), illustrating distinct chemical signatures for each typology.

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Among the volatile compounds detected in *P. spumarius*, some stood out for their relevance. For example, the compound 2-ethylhexan-1-ol (Figure 7), which was identified in females, males, and groups of *P. spumarius* males, and groups of *P. spumarius*.

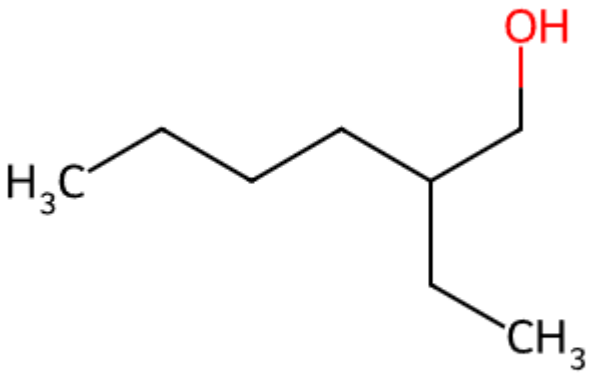

Figure 7 : Structural formula 2-ethylhexan-1-ol Source: ChemSpider (2025).

In studies conducted by Cruz et al. (2012), 2-ethylhexan-1-ol was detected in low concentrations in greenhouse-grown wheat leaves, describing it as a rare and toxic compound to both the *Fusarium* sp. fungus and tetraploid wheat itself. This toxicity suggests that the compound may act as a chemical defense, inhibiting the development of fungal pathogens. Furthermore, the authors observed genetic variation in the production of this VOC among different wheat genotypes, indicating that the emission of 2-ethylhexan-1-ol may be associated with natural resistance mechanisms.

Conversely, in a recent study by D'Isita et al. (2024) on the behavioral responses of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) to the odors of ancient and modern wheat genotypes, it was observed that alcohols were one of the most abundant chemical classes in the emitted volatiles, with 1-hexanol, 2-ethylhexan-1-ol, being one of the main constituents in genotypes such as Faridur and Mec. Wheat varieties with higher alcohol contents were also the most attractive to both insects, while those with lower levels of these compounds showed a lower olfactory response. These results indicate that 2-ethylhexan-1-ol and similar compounds can act as attractive signals in specific contexts, especially in plant-insect interactions and in stored grain environments.

Furthermore, recent studies conducted by Wang et al. (2025) showed that 2-ethylhexan-1-ol acts as a repellent for female Indian meal moths (*Plodia interpunctella*), a

stored product pest. Females significantly avoided substrates with higher concentrations of the compound, especially high-oleic peanuts. This result reinforces the potential of 2-ethylhexan-1-ol as a bioactive molecule with behavioral and defensive functions in chemical interactions between insects and their environment.

Similarly, the compound 2,2,4,4,6,8,8-heptamethylnonane (Figure 8), was identified in the volatile extracts of females, males, and mating couples of *P. spumarius.*According to Hera et al. (2024), Nonane presented a variable importance projection (VIP) score of 1.30, exceeding the critical threshold of 1.20 and indicating its strong association with wasp rejection behavior. This suggests that they may function as chemical signals involved in defense. Furthermore, according to Khan et al. (2024), in studies with *Bacillus sp*. LNXM12, this same compound was identified by GC-MS (Table 1), suggesting that it is also part of the volatile profile of microorganisms with bioactive potential. Although the article does not specify the direct antifungal activity of 2,2,4,4,6,8,8-heptamethylnonane,its presence indicates that it may contribute to biological effects.

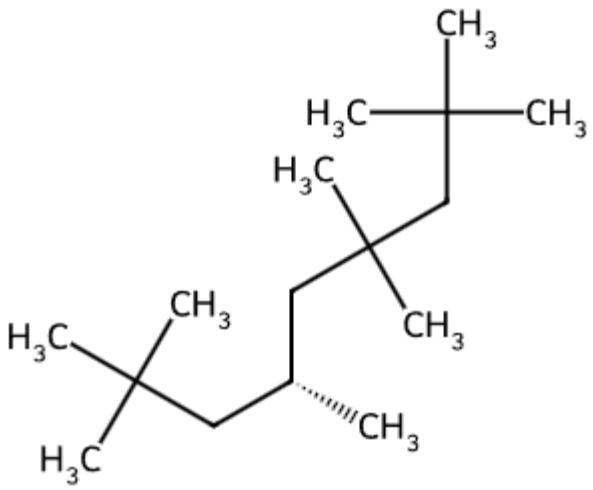

Figure 8 : Structural formula 2,2,4,4,6,8,8-heptamethylnonane - Source: ChemSpider (2025).

The VOC Benzyl alcohol (Figure 9) was identified only in tests with females. According to recent studies, Abdel-Baki et al. (2024), benzyl alcohol exhibits significant insecticidal activity against *Acanthoscelides obtectus*, the main pest of stored beans. Toxicity tests revealed its potential repellent and antifeedant effects.

Furthermore, Fang et al. (2018) also evaluated benzyl alcohol in the behavioral response of *Spodoptera litura* to sex pheromones. Electroantennary imaging results showed

that the presence of benzyl alcohol in the mixtures increased the neuronal response of males to olfactory stimuli, indicating a synergistic effect in pheromone detection. In the same context, Jiang et al. (2025) identified benzyl alcohol in oils extracted from *Cryptotympana* atrata and *Clanis bilineata tingtauica*, demonstrating its natural occurrence in insects.

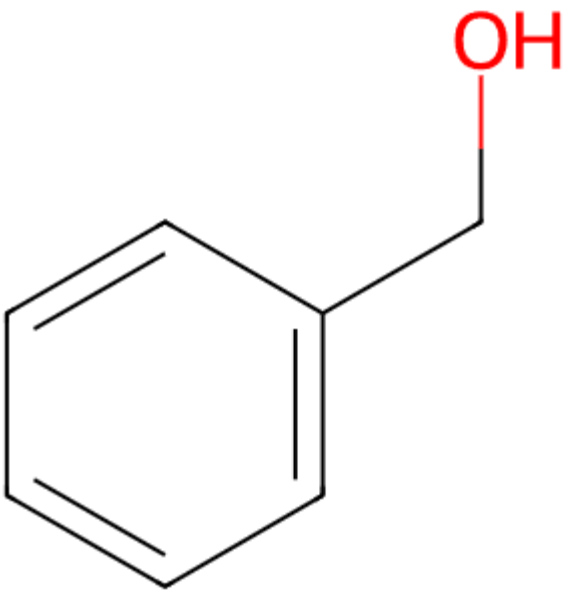

Figure 9 : Structural formula Benzyl alcohol Source: ChemSpider. (2025).

On the other hand, bis(2-methylpropyl) benzene-1,2-dicarboxylate (Figure 10) identified only in couple tests, second Jiang et al. (2018) reported the presence of VOC in the seed extract of *Robinia pseudoacacia L.*, demonstrating significant insecticidal activity against aphids, achieving mortality of over 95%.

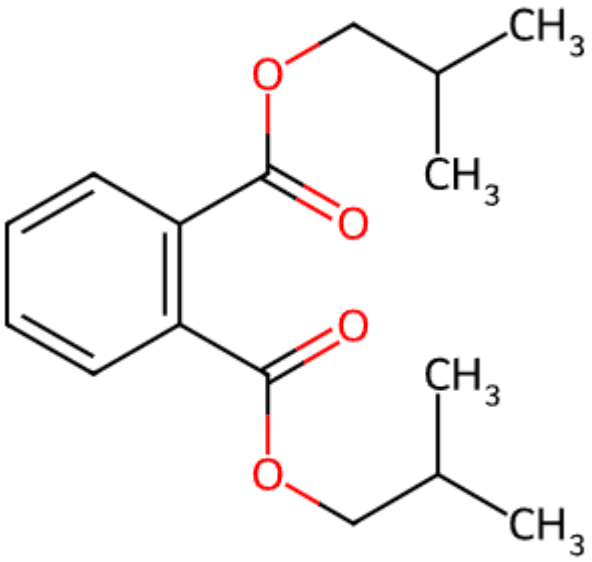

Figure 10 : Structural formula bis(2-methylpropyl) benzene-1,2-dicarboxylate Source: ChemSpider (2025).

Furthermore, 2-(2-butoxyethoxy)ethan-1-ol (Figure 11), was identified only in couples. Second Budenberg et al. (2009) also identified this compound by GC-MS in volatile emissions associated with larval trails of the aphidophagous predator *Episyrphus balteatus (Diptera: Syrphidae)*. In this study, females were observed to adjust their oviposition behavior according to the presence of chemical trails left by conspecific larvae, indicating that such trails contain semiochemicals capable of modulating oviposition site choice. Complementarily, Kong et al. (2025) reported the presence of the same VOC in the volatile emissions of Oolong tea leaves (*Camellia sinensis*) after piercing by green leafhoppers (Jacobiasca formosana), associating it with the plant's metabolic response to insect attack.

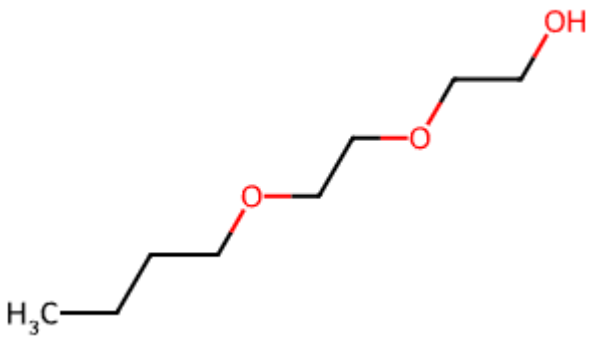

Figure 11 : Structural formula 2-(2-butoxyethoxy)ethan-1-ol Source: ChemSpider (2025).

Tetradecane was also identified only in paired tests.In the literature, Perez-Santaescolastica et al. (2025) identified VOCs in studies with edible insects of the order *Orthoptera, such as Acheta domesticus, Locusta migratoria*, and *Tenebrio molitor*, when evaluating the effect of heat treatments (blanching and sterilization). Similarly, Rana et al. (2024) reported VOCs in studies with the wheat aphid (*Sitobion avenae*), demonstrating their interaction with odorant-binding proteins (OBPs), especially SavOBP10. This protein exhibited high binding affinity to several volatiles emitted by wheat plants, including tetradecane. These compounds have been identified as important signaling molecules in chemical communication between insects and host plants.

Dodecanal was identified in a couple test, and previous studies have reported the presence of this compound in edible insect-enriched food products (E-INS). Sadeghi et al. (2025) observed that the incorporation of powdered buffalo worm larvae and insect flour into Italian-style breads leavened with mixed cultures *of Leuconostoc citreum, Levilactobacillus* brevis, and *Weissella cibaria* significantly influenced the profile of volatile compounds, including aldehydes and hydrocarbons, including dodecanal. Similarly, Gaglio et al. (2021) investigated the effect of adding powdered larvae of *T. molitor* and *Alphitobius diaperinus* to the production and fermentation of Italian sourdough breads, evaluating the impact of these ingredients on the physicochemical and microbiological properties, and the VOC identified in the final product.

1-Dodecanol detected only in tests with pairs in the study by Luo et al. (2023), was also identified in research with sexual pheromones of the Asian citrus psyllid (*Diaphorina citri*), where it was found in volatiles emitted by females, suggesting its role in chemical communication between insects.

Acetic acid (Figure 15) was one of the components detected only in isolated females. Similar results were reported by George et al. (2016), who observed that acetic acid, along with formic acid, was generated as a degradation product of monoterpenes (*β-ocimene and citral*) in contact with air. In their study, these acids elicited strong antennal responses and a significant increase in probing activity in the Asian citrus psyllid, a vector of citrus greening disease. The

detection of acetic acid suggests that simple molecules derived from oxidative processes may also play a relevant role in the olfactory communication of sap-sucking insects.

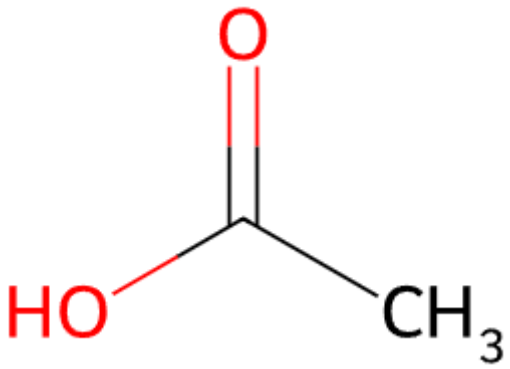

Figure 12 : Structural formula Acetic acid Source: ChemSpider. (2025).

4-(tert-butyl)cyclohexyl ethanoate(Figure 16) was identified among the volatile compounds emitted by P. spumarius pairs. According to the study by VanderGiessen et al. (2023), this VOC can significantly influence mosquito attraction to human hosts. The authors demonstrated that the use of soaps containing this compound alters the profile of volatiles emitted by the skin, modifying mosquitoes' host preferences. Thus, even at very low concentrations,

4-(tert-butyl)cyclohexyl ethanoate

acetate can affect the chemical behavior of insects, acting both as a natural semiochemical and as a natural stimulant.

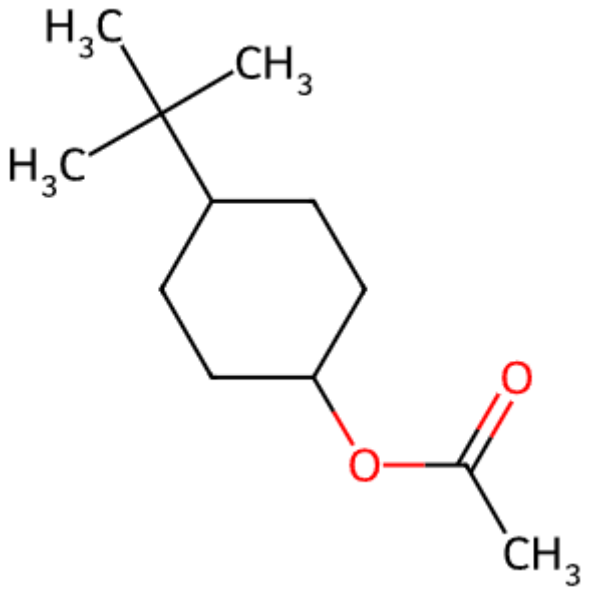

Figure 13 : Structural formula

4-(tert-butyl)cyclohexyl ethanoate

acetate Source: ChemSpider (2025).

Furthermore, cis-5-ethenyl-2-(hydroxymethyl)-5,6,7-trimethyltetrahydrofuran (Figure 17) was identified only in the *P. spumarius* pair assays. According to Li et al. (2022), the VOC is present in the essential oils of Jasminum sambac and other aromatic plants, and showed a significant effect on the behavior of RIFA (*Solenopsis invicta*) breeding ants. Behavioral assays in a Y-tube and CG-EAD indicated that this compound acts as an attractant, inducing approach responses, especially in virgin ants..

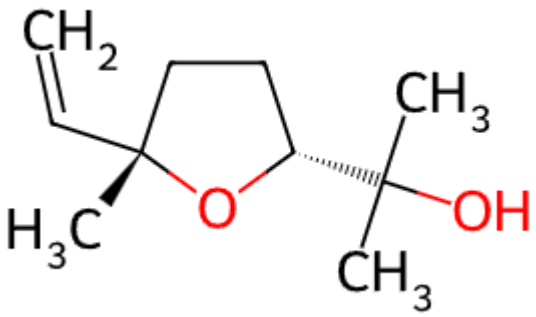

Figure 14 : Structural formula cis-5-ethenyl-2-(hydroxymethyl)-5,6,7-trimethyltetrahydrofuran Source: ChemSpider. (2025).

Another relevant aspect observed was the comparison between the tests carried out in September and May with females (Table 20).

5.3 Comparison of the insects' volatile profiles between spring and autumn

In the autumn 2024 assay (mated females), thirty-nine volatile organic compounds (VOCs) were identified, whereas in the spring 2025 assay (unmated females) only eight were detected, showing a marked reduction in the diversity of emitted compounds (Table 22). This variation in VOC profiles may reflect differences in the females' reproductive status as well as seasonal environmental factors that influence volatile production and emission.

Table 18 : Comparison of volatile compounds emitted by female *Philaenus spumarius* in autumn 2024 and spring 2025

COMPOUND	AUTUMN 2024	SPRING 2025
Methylbenzene	x	
Acetic acid	x	x
Butyl ethanoate	x	
1,3-dimethylbenzene	x	
1-hydroperoxy-1-methylpentane		x
1,4-dimethylbenzene	x	
3-octen-1-ol	x	
1,2,4-trimethylbenzene	x	
6-methylheptane-1,6-diol	x	
1-(2-methoxy-1-methylethoxy)propan-2-ol		x
2,2,4,6,6-pentamethylheptane	x	
2-ethylhexan-1-ol	x	x
3-methyl-5-propylnonane	x	
Benzyl alcohol		x
Phenylacetaldehyde	x	
5-ethyl-2,2,3-trimethylheptane	x	
1-octanol	x	
2,4-dimethyl-1-decene	x	
1-dodecene	x	
Nonanal	x	
2-phenylethanol	x	
2,2,4-trimethyldecane	x	
(1R,2S,5R)-2-isopropyl-5-methylcyclohexan-1-ol	x	
Decanal	x	
2,6,11-trimethyldodecane	x	
4-(tert-butyl)cyclohexyl ethanoate	x	

Tetradecane	x		
2,2,4,4,6,8,8-heptamethylnonane	x		x
2,6,11-trimethyldodecane	x		
2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	x		
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	x		
2,6,10-trimethyldodecane	x		
Tetradecane	x		
6,10-dimethylundeca-5,9-dien-2-one	x		
(3E)-3-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	x		
Heptadecane	x		
2,6-di-tert-butyl-4-methylphenol	x		
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	x		
Heptadecane	x		
Tetradecanal	x		
1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene			x
Methyl 3-oxo-2-pentylcyclopentaneacetate			x
5-methyloctadecane			x
2-(propan-2-yl) tetradecanoate	x		
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	x		

Among the compounds identified, three were common to both tests: acetic acid, 2-ethylhexan-1-ol and 2,2,4,4,6,8,8-heptamethylnonane. The presence of these compounds in both periods indicates that they are part of the basic volatile profile emitted by *P. spumarius* females, regardless of their physiological state.

On the other hand, the autumn test showed a more diverse volatile emission profile, including compounds such as 1,2,4-trimethylbenzene, 1-octen-3-ol, nonanal, 2-phenylethanol, levomenthol, 4-(tert-butyl)cyclohexyl acetate, and 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate, which were absent in unmated females. These compounds are commonly associated with behavioral processes such as sexual attraction and chemical communication, suggesting a possible relationship between the mating phase and the release of these volatiles.In contrast, the spring test revealed the presence of compounds such as benzyl alcohol, methyl 3-oxo-2-pentylcyclopentaneacetate, 5-methyloctadecane, and 1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene, which were not detected in the autumn samples. These compounds may reflect metabolic characteristics specific to unmated females.

The characterization of the volatile compounds emitted by *P. spumarius* revealed marked variations in chemical composition between samples collected in different seasons (Figure 18). The diagram allows visualizing the comparison between spring pairs and autumn females, showing that, in autumn, females already presented compounds that, in spring, were detected only when the insects were in pairs. The presence of six compounds common to autumn females and spring pairs was observed, suggesting that certain volatiles begin to be produced as females mature, regardless of the presence of a male.

Furthermore, when comparing spring pairs, spring females, and autumn females, only two compounds were found in common. These same compounds were detected in tests with isolated males (autumn), isolated females (autumn and spring), and pairs (autumn), indicating that these are VOCs characteristic of the species, present in both sexes and in all stages evaluated.

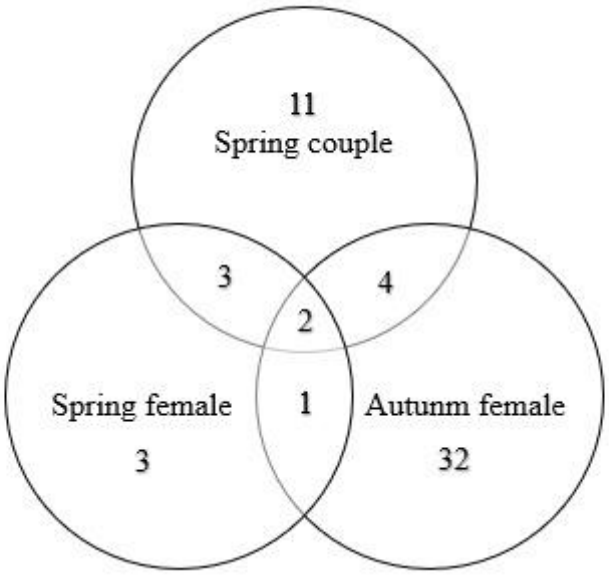

Figure 15 : Quantity of common volatile compounds identified between the spring pair, spring female and autumn female of the insect *Philaenus spumarius*.

These variations can be understood in light of the life cycle of *P. spumarius*. This species exhibits an annual cycle characteristic of foamy leafhoppers, classified as univoltine, in which oviposition occurs in late summer/early autumn, and the eggs remain in diapause during the winter, hatching in spring as temperatures rise (Lago et al., 2023; Gilioli et al., 2024). During this period, newly emerged nymphs go through five instars until reaching adulthood, which typically occurs between late spring and early summer (Bodino et al., 2019). Thus, at the time

of collections in spring, most individuals had not yet reached sexual maturity, which explains the low emission of volatile compounds observed in females and couples. In autumn, the population is predominantly composed of sexually mature and reproductively active adults, which may explain the greater number and diversity of compounds detected in females during this period.

6. CONCLUSION AND FUTURE PERSPECTIVES

In this study we successfully developed and optimized a headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC–MS) protocol for the extraction and analysis of VOCs emitted by *P. spumarius*.

The optimized analytical procedure ensured reproducibility and reliability, enabling consistent detection and quantification of volatiles released by both individual insects and couples.

For the first time, the volatile profile of *P. spumarius*, the primary European vector of *X. fastidiosa*, was characterized, providing a comprehensive chemical basis for understanding its intraspecific communication and behavioral ecology. This methodological advancement establishes a solid foundation for future studies on the species' semiochemical interactions and for the identification of compounds potentially involved in reproductive or ecological signaling.

Distinct volatile profiles were identified for unmated males, unmated females, and couples, revealing clear chemical differentiation among typologies. Males exhibited a simpler and more limited emission spectrum, whereas females released a broader and more complex blend of volatiles. Couples produced the most diverse chemical profile, suggesting that the interaction between sexes enhances or modulates VOC emission. These results indicate that physiological and social factors strongly influence the release of semiochemicals, potentially associated with mating and recognition behavior.

Seasonal observations further revealed that VOC emission patterns are affected by both physiological state and environmental conditions. During the reproductive period, females exhibited higher chemical activity and emitted a wider range of volatiles, while pre-reproductive individuals released only a few ubiquitous compounds likely associated with basal communication. These patterns highlight the dynamic nature of *P. spumarius*' chemical system, which varies according to reproductive stage and environmental context.

Overall, this work not only provides the first chemical characterization of volatiles emitted by *P. spumarius* but also delivers a validated and reproducible analytical protocol for VOC extraction and analysis.

Although this study provided clear evidence of chemical differentiation among typologies, further research is needed to verify the biological functions of the identified compounds. Behavioral assays-such as Y-tube olfactometer and field-trapping experiments-should be performed to assess whether these volatiles elicit attraction or recognition responses. Longitudinal studies across different seasons and reproductive stages will clarify how physiological and environmental factors shape emission dynamics. Comparative analyses

between isolated and paired individuals will also help distinguish basal emissions from those directly related to sexual signaling.

In the long term, VOCs confirmed to have behavioral activity could be integrated into pheromone-based monitoring or control strategies, contributing to the sustainable management of *P. spumarius* populations and reducing the dissemination of *X. fastidiosa*. Altogether, these findings provide an important step forward in understanding the species' chemical ecology and pave the way for future applied and fundamental research.

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ANNEXES

Table A 1 : Peak areas and retention times of volatile compounds identified in replicate 1, containing two male *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Methoxyphenyl oxime	13.214	1E+06
2-Ethyl-1-hexanol	21.331	14368
2,2,4,4,6,8,8-heptamethylnonane	41.689	11175
Isobutyl 3-hydroxy-2,2,4-trimethylpentanoate	43.578	12683
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.014	13526
Phenyl adamantane-3-carboxylate	56.625	12233
1,2,3,4,4a,5,8,9,12,12a-Decahydro-1,4-methanobenzocyclodecene	59.766	2494

Table A 2 : Peak areas and retention times of volatile compounds identified in replicate 2, containing two male *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Methoxyphenyl oxime	13.195	390233
Benzeneacetic acid	36.960	121395
2-Ethyl-1-hexanol	21.304	12351
2,2,4,4,6,8,8-heptamethylnonane	41.713	8174
Phenyl adamantane-3-carboxylate	56.656	14344
1,2,3,4,4a,5,8,9,12,12a-Decahydro-1,4-methanobenzocyclodecene	59.784	2642

Table A 3 : Peak areas and retention times of volatile compounds identified in replicate 3, containing two male *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Methoxyphenyl oxime	13.199	350158
2-Ethylhexyl butanoate	43.595	8396
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.024	10627
(S)-2-Methyldodecan-1-ol	21.305	8585
2,2,4,4,6,8,8-heptamethylnonane	41.711	4821
4,5-Dichloro-2-(adamantan-1-yl)pyridazin-3(2H)-one	56.615	2868

Table A4: Peak areas and retention times of volatile compounds identified in replicate 4, containing two male *Philaenus spumarius* insects, exposed for 3 h**.**

COMPOUND	RT(MIN)	AREA
Methoxyphenyl oxime	13.192	334431
2-phenylethanol	27.082	32699
Benzeneacetic acid	37.115	400420
2,2,4,4,6,8,8-heptamethylnonane	41.715	14059
2-Ethylhexyl cyanoacetate	21.324	9743

1-(Adamantan-1-yl)butan-1-ol	56.700	3324

Table A5: Peak areas and retention times of volatile compounds identified in replicate 5, containing two male *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Methoxyphenyl oxime	13.185	436035
2-Ethyl-1-hexanol	21.276	18101
Nonanal	26.518	12343
2-phenylethanol	27.080	32375
Methyl 3,3-dimethylbutanoate	41.664	3513

Table A 4 : Peak areas and retention times of volatile compounds identified in replicate 1, containing two female *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
(R)-Propane-1,2-diol	5.864	124949
Methoxyphenyl oxime	13.201	439502
Hexanoic acid	18.016	21886
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.241	46329
2-Ethoxyethyl 2-methoxyethyl carbonate	20.582	41705
Benzyl alcohol	21.556	28939
Nonanal	26.539	10205
2-(2-Butoxyethoxy)ethanol	32.538	8645
Decanal	33.740	4186
2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43.578	19526
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.023	21446
Heptyl 6-ethyloctan-3-yl oxalate	55.474	5839
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.744	19764
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.953	17874

Table A 5 : Peak areas and retention times of volatile compounds identified in replicate 2, containing two female *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
2,2,4-trimethylpentane	4.541	627753
1-Methoxy-2-propyl acetate	11.236	43456
Methoxyphenyl oxime	13.285	429283
2-Ethyl-1-hexanol	21.320	17401
Nonanal	26.531	9596
Levomenthol	31.418	2528
Decanal	33.735	6938
2,2,4,4,6,8,8-heptamethylnonane	41.687	20906

Isobutyl 2,2,4-trimethyl-3-hydroxypentanoate	43.579	9769
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.008	9248
2,6,11-trimethyldodecane	55.454	8377
4-Chlorooctahydro-2,4-methano-1H-indene	56.613	39712
Phenyl adamantane-3-carboxylate	56.919	23798
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.711	10737
1-chlorononadecane	58.865	7391
1,2,3,4,4a,5,8,9,12,12a-Decahydro-1,4-methanobenzocyclodecene	59.750	5892
Tetratetracontane	79.274	47076

Table A 6 : Peak areas and retention times of volatile compounds identified in replicate 3, containing two female *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
2,2-dimethylhexane	4.536	5E+06
(R)-Propane-1,2-diol	6.115	144097
2-Methylpropanoic acid	6.505	11671
Butane-2,3-diol	7.385	116078
N,N-dimethylformamide	7.520	11115
Butane-2,3-diol	7.754	32877
Methoxyphenyl oxime	13.343	572539
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.302	32959
2-Ethoxyethyl 2-methoxyethyl carbonate	20.616	12885
Benzyl alcohol	21.586	31243
Nonanal	26.535	8609
2-phenylethanol	27.089	20166
Decanal	33.733	2990
3-Methyl-2-butenyl 2-methylpropanoate	34.236	15506
2,2,4,4,6,8,8-heptamethylnonane	41.705	17897
2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43.585	24318
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.014	33915
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.744	31460
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.934	11386

Table A 7 : Peak areas and retention times of volatile compounds identified in replicate 4, containing two female *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
2,2,3,3-tetramethylbutane	4.560	1E+06
Methoxyphenyl oxime	13.207	337636
4-Ethyl-1-octyn-3-ol	21.340	26454
Benzyl alcohol	21.584	13064

Nonanal	26.547	9582
2-phenylethanol	27.092	30335
Decanal	33.752	3350
Benzeneacetic acid	37.356	2E+06
2,2,4,4,6,8,8-heptamethylnonane	41.696	13782
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.008	6460
5-(2-Methylpropyl)nonane	52.784	6581
5-Methyl-2-(propan-2-yl)hexan-1-ol	55.474	5238
2-Bromododecane	58.876	8038
Eicosane	65.014	7312

Table A 8 : Peak areas and retention times of volatile compounds identified in replicate 5, containing two female *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Acetic acid	3.317	197439
(R)-Propane-1,2-diol	5.965	49423
Methoxyphenyl oxime	13.262	605690
Hexanoic acid	18.022	11547
2-methoxybutane	20.286	28131
2-Ethoxyethyl 2-methoxyethyl carbonate	20.577	25052
Benzyl alcohol	21.545	12237
Nonanal	26.550	10615
1,5-Dimethyl-7-oxabicyclo[4.1.0]heptane	34.254	15646
Isobutyl 2,2,4-trimethyl-3-hydroxypentanoate	43.598	48768
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.021	47230
4-Chlorooctahydro-2,4-methano-1H-indene	56.634	25132
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.754	69017
1,2,3,4,4a,5,8,9,12,12a-Decahydro-1,4-methanobenzocyclodecene	60.372	3544
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.939	12656

Table A 9 : Peak areas and retention times of volatile compounds identified in replicate 1, containing one mating pair of *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
Acetic acid	3.506	146513
Propane-1,2-diol	6.105	66499
1-Methoxy-2-propyl acetate	11.246	111851
Methoxyphenyl oxime	13.326	485777
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.290	27187
2-Ethoxyethyl 2-methoxyethyl carbonate	20.612	16662

2-Ethyl-1-hexanol	21.308	21478
Benzyl alcohol	21.575	33584
2-Methyl-6-methyleneoctan-2-ol	24.282	13313
2-Butoxyethyl acetate	25.707	14887
Nonanal	26.531	11489
2-Phenylethanol	27.077	12656
Levomenthol	31.394	4136
Decanal	33.754	4881
1,5-Dimethyl-7-oxabicyclo[4.1.0]heptane	34.244	20670
Hexyl octyl sulfite	38.956	3728
4-(tert-Butyl)cyclohexyl acetate	39.806	6156
2,2,4,4,6,8,8-Heptamethylnonane	41.687	22435
2,6,11-Trimethyldodecane	42.040	5391
2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43.586	37703
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.008	45716
Nonadecane	46.825	41027
Dodecanal	47.325	5259
Heptyl 6-ethyloctan-3-yl oxalate	50.725	8317
(E)-Tetradec-3-ene	51.435	5310
α-Isomethyl ionone	51.778	6930
2,6,11-Trimethyldodecane	52.430	5330
5-(2-Methylpropyl)nonane	52.778	12914
2-Methyloctadecane	53.021	11078
3,5-Di-tert-butylphenol	53.833	22571
2-Methyltetracosane	55.481	13436
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.738	40659
Hexacosane	58.886	24625
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.955	12847
Nonadecane	64.444	4498
2-Isopropyl-5-methylheptan-1-ol	65.006	5161

Table A 10 : Peak areas and retention times of volatile compounds identified in replicate 2, containing one mating pair of *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	**RT(MIN)**	**AREA**

Hexane	3.290	940322
1-Methoxy-2-propyl acetate	11.248	54389
Methoxyphenyl oxime	13.202	438221
Octamethylcyclotetrasiloxane	19.500	269611
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.259	25661
(E)-Dec-2-en-1-ol	21.297	14386
Benzyl alcohol	21.568	28570
Nonanal	26.536	18882
Levomenthol	31.384	3693
Decanal	33.753	6750
Hexyl octyl sulfite	38.970	8464
2,2,4,4,6,8,8-Heptamethylnonane	41.702	23234
Nonadecane	42.052	12111
2,6,11-Trimethyldodecane	42.645	5801
Triacetin	43.686	68301
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.023	31317
2-Methyltetracosane	46.827	12605
4,6,8-Trimethylnon-1-ene	50.103	9250
2-Methyltetracosane	50.713	11104
(E)-Octadec-3-ene	51.449	5953
7-Hexyleicosane	52.433	9199
Tetracosane	52.777	20679
Nonadecane	53.009	14282
3,5-Di-tert-butylphenol	53.822	9009
2-Methyltetracosane	55.470	11216
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.744	34357
Pentadecane	58.881	8485
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.933	8443
2-Isopropyl-5-methylheptan-1-ol	65.004	4794

Table A 11 : Peak areas and retention times of volatile compounds identified in replicate 3, containing one mating pair of *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	**RT(MIN)**	**AREA**
Propan-2-ol	5.796	128653
1-Methoxy-2-propyl acetate	11.234	50858
Methoxyphenyl oxime	13.192	680413
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.236	84589
2-Ethoxyethyl 2-methoxyethyl carbonate	20.548	60696
4-Ethyl-1-octyn-3-ol	21.274	16059
Benzyl alcohol	21.519	27427

2,5-Dimethylhexane-2,5-diol	23.169	9256
2-Butoxyethyl acetate	25.685	11556
Nonanal	26.525	10868
2,4-Dimethylpentane-2,3-diol	29.708	7203
2-(2-Butoxyethoxy)ethanol	32.494	14172
Decanal	33.725	5216
1,5-Dimethyl-7-oxabicyclo[4.1.0]heptane	34.209	45660
2-Phenoxyethanol	34.628	20141
3,5,5-Trimethyl-2(5H)-furanone	40.212	27921
Dodecan-3-yl acetate	41.294	20316
2,2,4,4,6,8,8-Heptamethylnonane	41.676	22973
11-(1-Ethylpropyl)heneicosane	42.039	14870
2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	43.568	92128
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	45.001	136391
Tetradecane	46.804	19043
Dodecanal	47.298	8507
Dodecan-1-ol	51.415	22383
Eicosane	52.756	16210
Nonadecane	53.002	10155
Hexyl octyl sulfite	55.438	22916
2-Methyltetracosane	56.017	6638
2,6,11-Trimethyldodecane	57.177	6853
Diethyl phthalate	58.525	31607
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.728	253493
Methyl 3-oxo-2-pentylcyclopentaneacetate	61.922	28318
2-Isopropyl-5-methylheptan-1-ol	64.984	8300
2-Methyloctacosane	67.302	6193
Diisobutyl phthalate	73.156	51240
Oct-1-ene	38.379	3800
Pentyl undecyl sulfite	38.950	3017
3-(Octadecyloxy)propyl stearate	39.886	2415
Diisopropyl adipate	50.206	5724
(1,3-Dimethylbutyl)cyclohexane	50.682	2805
α-Isomethyl ionone	51.756	4644
3,3,5-Trimethylheptane	52.421	4249
3,5-Di-tert-butylphenol	53.816	4717
4-Methyl-1-(propan-2-yl)bicyclo[3.1.0]hexan-3-ol	54.991	2199
1-Bromo-2-methyldecane	64.425	3036
Isobutyl 2-pentyl sulfite	69.737	929

Table A 12 : Peak areas and retention times of volatile compounds identified in replicate 4, containing one mating pair of *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
R-(-)-1,2-propanediol	5.811	189392
Oxime-, methoxy-phenyl-_	13.197	605500
Hexanoic acid	18.016	20967
2-Propanol, 1-(2-methoxy-1-methylethoxy)-	20.263	80645
Carbonic acid, 2-ethoxyethyl 2-methoxyethyl ester	20.579	56403
Benzyl alcohol	21.542	39084
Cyclotrisiloxane, hexamethyl-	25.156	138011
2-Butoxyethyl acetate	25.711	9110
Nonanal	26.536	12976
2,3-Pentanediol, 2,4-dimethyl-	29.752	8736
Ethanol, 2-(2-butoxyethoxy)-	32.527	14972
Decanal	33.741	5401
Propanoic acid, 2-methyl-, 3-methyl-2-butenyl ester	34.234	27603
Ethanol, 2-phenoxy-	34.634	26231
Nonanoic acid	38.304	7272
3-Ethyl-3-methylheptane	38.955	7773
2(5H)-Furanone, 3,5,5-trimethyl-	40.234	21402
3-Acetoxydodecane	41.288	14705
Nonane, 5-(1-methylpropyl)-	42.033	8132
Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	43.580	48961
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	45.009	73796
Tridecane	46.825	17127
Dodecanal	47.334	5883
Heptadecane, 2,6,10,14-tetramethyl-	50.705	9995
1-Dodecanol	51.432	8148
2-Bromotetradecane	52.775	13192
Nonadecane	55.458	18902
Sulfurous acid, octadecyl 2-propyl ester	56.049	8721
Sulfurous acid, hexyl octyl ester	57.196	4558
Diethyl Phthalate	58.537	25928
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	58.742	75009
Heptadecane	58.878	19088
Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	61.935	28069
Heptadecane, 2,6,10,15-tetramethyl-	64.447	3963
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	73.174	20819

Table A 13 : Peak areas and retention times of volatile compounds identified in replicate 5, containing one mating pair of *Philaenus spumarius* insects, exposed for 3 h.

COMPOUND	RT(MIN)	AREA
(R)-Propane-1,2-diol	5.880	125486
Methoxyphenyl oxime	11.235	72981
Hexanoic acid	13.177	471350
1-(2-Methoxy-1-methylethoxy)propan-2-ol	20.250	25756
2-Ethoxyethyl 2-methoxyethyl carbonate	20.554	11403
Benzyl alcohol	21.520	28676
Hexamethylcyclotrisiloxane	25.691	10379
2-Butoxyethyl acetate	26.528	8522
Nonanal	31.376	2779
2,4-Dimethylpentane-2,3-diol	33.732	5179
2-(2-Butoxyethoxy)ethanol	38.927	4950
Decanal	41.665	37745
3-Methyl-2-butenyl 2-methylpropanoate	42.014	4874
2-Phenoxyethanol	43.563	20077
Nonanoic acid	44.989	28563
3-Ethyl-3-methylheptane	46.797	36818
3,5,5-Trimethyl-2(5H)-furanone	50.071	13358
Dodecan-3-yl acetate	50.706	12844
5-(1-Methylpropyl)nonane	51.415	3790
2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl 2-methylpropanoate	52.395	8542
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	52.746	18552
Tridecane	52.989	17092
Dodecanal	53.809	19642
2,6,10,14-Tetramethylheptadecane	54.426	4729
Dodecan-1-ol	54.985	14057
2-Bromotetradecane	55.436	19051
Nonadecane	56.049	8719
Octadecyl propan-2-yl sulfite	58.717	31638
Hexyl octyl sulfite	58.868	46968
Diethyl phthalate	61.915	10538
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	64.417	5018
Heptadecane	64.972	5580
Methyl 3-oxo-2-pentylcyclopentaneacetate	69.728	4355
2,6,10,15-Tetramethylheptadecane	21.305	6739
Diisobutyl phthalate	32.496	4550
(R)-Propane-1,2-diol	34.216	15480

Methoxyphenyl oxime	40.255	3221
Hexanoic acid	39.804	4539
1-(2-Methoxy-1-methylethoxy)propan-2-ol	41.286	5027
2-Ethoxyethyl 2-methoxyethyl carbonate	47.320	3200
Benzyl alcohol	51.845	4901
Hexamethylcyclotrisiloxane	57.158	4160
2-Butoxyethyl acetate	58.523	6876
Nonanal	67.306	3393
2,4-Dimethylpentane-2,3-diol	73.173	9971

Table A 14 : Peak areas and retention times of volatile compounds identified in control samples (empty vials).

COMPOUND	RT	AREA
Methoxyphenyl oxime	13.175	262448
Nonanal	26.509	9929
2-phenylethanol	27.052	29300
Levomenthol	31.348	2616
Decanal	33.695	3539
Isobutyl 2,2,4-trimethyl-3-hydroxypentanoate	43.547	17160
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	44.960	19612
Nonadecane	52.968	6245
Isobutyl 2,2,4-trimethyl-3-(propan-2-ylcarboxy)pentanoate	58.710	12972
2-Bromododecane	58.845	7397