INSTITUTO POLITÉCNICO DE BRAGANÇA
Escola Superior Agrária

# Host-plant preference of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*, and their effect on the insect development

**Nour Ksouri**

*Dissertation submitted to Escola Superior Agrária de Bragança*
*to obtain the Degree of Master in Biotechnological Engineering*

Supervised by
**José Alberto Cardoso Pereira**
**Isabel Cristina de Sousa Rodrigues**

**Bragança**
**2023**

This work was supported by the Foundation for Science and Technology (FCT, Portugal) by national funds FCT/MCTES to CIMO (UID/AGR/00690/2020) and SusTEC (LA/P/0007/2020).

## Acknowledgements

I would like to express my sincere thanks to the following people who contributed to the elaboration of this work.

First, Professor Paula Baptista and Professor José Alberto Pereira, for the opportunity to be a part of this project, for the guidance and all the support.

Special thanks to Isabel Rodrigues for availability, revisions and suggestions, assistance with statistical analysis and great advice throughout the whole process.

## Remerciement

Je remercie dieux le tout pissant de m'avoir accordé le pouvoir, la santé et la volanté d'entamer et de finir ce travail.

Je tiens aussi à remercier toute ma famille pour le support qu'ils m'ont apporté spécialement Leila Zaibi, Gilles Bernard, Sofiane Cherif et tous ceux qui ont croyez en moi.

Je remercie également ma chère Ibtihel Kacem pour toute la joie qu'elle a apporté dans ma vie et pour tous les bons moments partagés durant ces temps difficiles, je t'aime.

"Vous faites partie de mon histoire, de ma mémoire et de mon paysage, merci." – Kim taehyung

## Abstract

*Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae), commonly known as the meadow spittlebug, has recovered scientists' attention for decades and has been extensively studied for its biological and ecological interests. However, this species has been rarely associated with significant economic or agricultural damage until the detection of *Xylella fastidiosa* (Wells et al. 1987) (Xathomonadales: Xanthomonadaceae), a plant pathogenic bacterium, in Europe for the first time in Italy in 2013. *Philaenus spumarius* has been identified to have a key role in the pathogen's propagation, being its essential transmission vector in Europe. Since currently, there is no cure for *X. fastidiosa*, vector control is perceived as the main tool to limit the spread of this pathogen. Therefore, understanding the choice of host plants and how they affect vector insect development can be fundamental for implementing approaches to manipulate the behavior of the vector and implement sustainable control strategies. In this sense, this work aims to evaluate the selection of host plants by nymphs and adults of *P. spumarius*, and their effect on the development of the insect vector. For that, we studied, in field conditions, the effect of four host plants, namely *C. myconis S. tenerrimus, C. arvensis*, and *C. segetum*, in the development of *P. spumarius.* We assessed the olfactory response of nymphs, at different soil humidity (10%, 50%, and 70%), and adults of *P. spumarius* towards the four plants (and the olive tree, only for the adults). Additionally, the volatile profile of the plants was also assessed, and we developed models to predict the effect of the volatiles produced by these plants on insect behavior. The plant species generally does not significantly affect the insect's morphological parameters. The choice made by *P. spumarius* nymphs was significantly affected by the plant species and the soil's percentage of water content. *Sonchus tenerrimus* and *C. myconis* were the two most chosen plants by the nymphs. The olfactory response of the adults of *P. spumarius* revealed that the sex of the insects did not influence the choice of plants and that *S. tenerrimus* was the most preferred plant by females when compared to *C. segetum*, *C. arvensis*, and the control. The models suggested that the nymphs and adults of *P. spumarius* are repelled by D-Limonene. Understanding how these insects interact with their environment is crucial to create effective control strategies.



**Keywords:** The meadow spittlebug, Vector, Cover-ground vegetation, OQDS, Olfactometer, Gram-negative.

# Resumo

*Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae) vulgarmente conhecido como cigarrinha-das-espumas, tem despertado a atenção dos cientistas ao longo de décadas, dedicando-se ao estudo da sua biologia e ecologia. Contudo, apesar, deste inseto raramente ser associado a prejuízos económicos ou agrícolas, em 2013, em Itália, foi identificado como o principal vetor de *Xylella fastidiosa* (Wells Raju et al. 1986) (Xathomonadales: Xanthomonadaceae). Uma vez que não existe cura para *X. fastidiosa*, o controlo dos seus vetores é considerado como a ferramenta mais eficaz para limitar a disseminação deste patógeno. Assim, compreender a preferência de *P. spumarius* pelas diferentes plantas hospedeiras e a forma como afetam o desenvolvimento do inseto vetor é fundamental para a implementação de abordagens integradas capazes de manipular o comportamento do vetor e implementar estratégias de controlo sustentáveis. Neste sentido, o objetivo principal deste trabalho é avaliar a selecção de plantas hospedeiras por ninfas e adultos de *P. spumarius*, e o seu efeito no desenvolvimento do vector. Para isso, em condições de laboratorio, o efeito de quatro plantas hospedeiras, nomeadamente *C. myconis, S. tenerrimus, C. arvensis*, e *C. segetum*, no desenvolvimento de *P. spumarius* foi estudado. A resposta olfactiva das ninfas, em diferentes humidades do solo (10%, 50%, e 70%), e dos adultos de *P. spumarius* perante as quatro plantas (e oliveira, apenas para os adultos), foi avaliada. Adicionalmente, foi também avaliado o perfil volátil das plantas, e foram desenvolvidos modelos para prever o efeito dos voláteis produzidos por estas plantas no comportamento dos insectos. No geral, as plantas escolhidas não afetaram significativamente o desenvolvimento morfológico do insecto. A escolha feita pelas ninfas foi significativamente afectada pela espécie da planta e pela percentagem de água no solo. *S. tenerrimus* e *C. myconis* foram as duas plantas mais escolhidas pelas ninfas. A resposta olfactiva dos adultos de *P. spumarius* revelou que o sexo dos insectos não influenciou a escolha das plantas e que *S. tenerrimus* foi a planta mais escolhida pelas fêmeas quando comparada com *C. segetum*, *C. arvensis*, e o controlo. Os modelos sugeriram que tanto as ninfas como os adultos de *P. spumarius* são repelidos por D-Limonene. Compreender como estes insetos interagem com o seu ambiente é crucial para criar estratégias de controlo eficazes.



**Palavras-chaves:** cigarrinha-das-espumas, vetor, Vegetação de cobertura, sindrome do declineo rápido da oliveira, *Gram-negativo.*

# General Index

# Index of figures

# Index of tables



# List of abbreviation

ANOVA: Variance Analysis
GC-MS: Gas Chromatography-Mass Spectrometry
GLM: Generalized Linear Model
OQDS: Olive Quick Decline Syndrome
PCA: Principal Component Analyses
VIF: Variance Inflation Factor
VOC: Volatil Organic Compound







General introduction

# CHAPTER1

and objectives

*Xylella fastidiosa* (Xanthomonadales: Xanthomonadaceae) is a xylem-limited plant pathogenic bacterium and is considered one of the most severe emerging plant diseases worldwide (Retchless et al., 2014). This Gram-negative, rod, slow growing bacterium was described over a century ago by wells et al., (1987). *Xylella fastidiosa* was detected in Europe (Italy) for the first time in the autumn of 2013. After this first outbreak, this bacterium was reported in other European counties (Saponari et al., 2017), namely France in 2015, Spain in 2016, and Portugal in 2019 (EPPO, 2022). Several studies have shown that *X. fastidiosa* has a host plant range of more than 500 species, which may be one of the causes of its propagation all over the world (Martelli et al., 2016; EFSA, 2018; Cornara et al., 2020). This bacterium has been associated with many diseases affecting agricultural plants such as grapevine, peach, almond, citrus, and olive (Goheen et al., 1973; Hopkins, 1982). So far, *X. fastidiosa* remains incurable, and only a few approaches are set to protect plants such as the eradication of infected plants and vector control (Colella et al., 2019).

Olive Quick Decline Syndrome (OQDS) is one of many diseases caused by *X. fastidiosa*, it first appeared a few years ago in a small area of southern-east Italy (Martelli et al., 2016). As the name implies, it is a fatal disease that begins with foliage desiccation on olive trees and quickly progresses to tree death (Asteggiano et al., 2021; Martelli et al., 2016). Since its discovery, the OQDS outbreak has expanded; in 2020, approximately 6 million olive trees in Puglia (Italy) showed symptoms of complete or partial decline (Nutti et al., 2021).

The application of control measures, such as eradication of infected plants and vector control, has proven efficient in reducing the spread of the disease. (Colella et al., 2019), particularly the biological control of the main vector of the causing agent, *Philaenus spumarius* (Hemiptera: Aphrophoridae) (Liccardo et al., 2020). This spittlebug species of the Aphrophoridae family (Yurtsever, 2000) is the most abundant vector of *X. fastidiosa*, in Europe, with the highest transmission rate among the other vectors (Cavalieri et al., 2019; Dongiovanni et al., 2019; Saponari et al., 2014). *Philaenus spumarius* is well known for scientists for being a high polymorphic insect, its body coloration differs widely (about 20 distinct colors are known). They are usually yellowish, brownish, or black in color, with brighter patches on a dark background, but they can also have dark markings on a lighter background (Dicke, 1962; Seabra et al., 2020; Halkka, 1964; Yurtsever, 2000). *Philaenus spumarius* have a hemimetabolous annual life cycle, starts with the stage "egg" from November to January (Cornara et al., 2018; Yurtsever,

2000) which will develop after over five nymphal instars from N1 to N5 (Yurtsever, 2000), this stage is characterized by the nymphs occupation of the ground cover vegetation. All the nymphal stages "N1 to N5" occur beneath a thick layer of foam, formed by the nymph itself as a byproduct of feeding on the plant's xylem, that will provide protection from predators, climate factors and other threats to the nymph. This nymphal stage lasts from the end of March to June, and finally, the emergence of adults begins from June and lasts to November, this stage is manly characterized by the mating of the insects and the oviposition, where females will again lay their eggs, which will mark the beginning of another cycle (Cornara et al., 2018; Yurtsever, 2000).

The harmful effects of this insect on crops are established on two main levels, the first during the nymphal stage by sucking approximately three hundred times their own weight of plant sap in only 24 hours (Horsfield, 1978), and the second during the adult stage by transporting diseases from one plant to another (Delong & Severin, 1950) it became necessary to investigate it's relation and means of communication, with each other and with their environment, to establish strategies to diminish its population and by consequence diminishing the spread of the associated diseases.

Given the significance of the olive groves in Portugal and the devastating consequences of *X. fastidiosa* in Italy, the implementation of adequate management measures is needed to prevent and limit the spread of this pathogen. Therefore, knowledge about the main vector biology and survival development in native plants from Europe is crucial to establishing ground cover management strategies in olive orchards to diminish the disease spreading.

Considering the above, this study aims to evaluate the selection of host plants by nymphs and adults of *P. spumarius*, and their effect on the development of the insect vector.

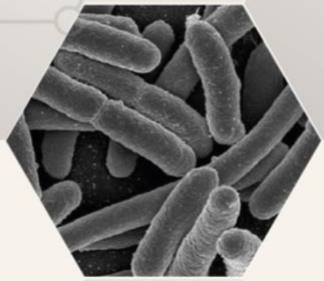
Henceforward, the main objectives of this work are the following:

i. Evaluation of the effect of the host plant in the *P. spumarius* development
ii. Evaluation of the host plant selection by nymphs at different soil humidity levels.
iii. Evaluation of the olfactory preferences of *P. spumarius* adults towards plants of the ground cover vegetation.
iv. Characterization of the volatile compounds of host plants of *P. spumarius*.









# CHAPTER2

*Literature review*

## 2.1 *Xylella fastidiosa*

The developments and advancements in transportation and communication technology have increased globalization since the 18th century. This increase in global interactions has caused a growth in international trade and the exchange of ideas, beliefs, and cultures with a considerable positive effect on the world. However, while increasing worldwide travels, humans do not only bring the "material trappings of our culture" but also transport life materials, sometimes intentionally and other times accidentally. The introduction of foreign species into new habitats can have devastating ecological and economic consequences (Vitousek et al., 2014). An important example with high impact is the recent introduction and emergence of *Xylella fastidiosa* (Wells et al., 1987) in Europe, North Africa, and the Middle East (Cornara et al., 2017; Hopkins, 1989; Saponari et al., 2014).

*Xylella fastidiosa* is a xylem-limited plant pathogenic bacterium considered one of the most severe emerging plant disease threats worldwide (Retchless et al., 2014). This bacterium can develop in more than 500 plant species, including ornamental and agricultural plants (Martelli et al., 2016; EFSA, 2018, Cornara et al., 2020). This species was isolated and described for the first time in 1987 in the United States of America by Wells et al., (1987). Initially, this bacterium was only a threat in the United States of America, but in the same year, it was associated with a disease that decimated citrus orchards, the citrus variegated chlorosis, in Brazil (Janse & Obradovic, 2015). Simultaneously, an epidemic of Pierce's disease, an important vine disease, occurred in Southern California, contributing to an increase in research on this bacterium. In 1993, it was detected in Taiwan and was designated as *X. taiwnese* (Almeida & Nunney, 2015). In 2014, the bacterium was detected in almond trees on the Asian continent (Iran) (Janse & Obradovic, 2015).

In Europe until 2013, only sporadic records of *X. fastidiosa* were made, however, they have not been independently confirmed (Sicard et al., 2018). Thus, the first official detection of *X. fastidiosa* in Europe was in 2013 in olive groves in the region of Bari (South Italy), (Saponari et al., 2013). After this first report, this pathogen was already registered in other European countries, namely: France in 2015, Switzerland in 2015, Germany in 2016 (according to EPPO of Switzerland (2015-10) and EPPO of Germany

(2016-07), it was detected in few plants, and it was eradicated immediately), Spain in 2016, and Portugal in 2019 (EPPO, 2022) (Figure 1).

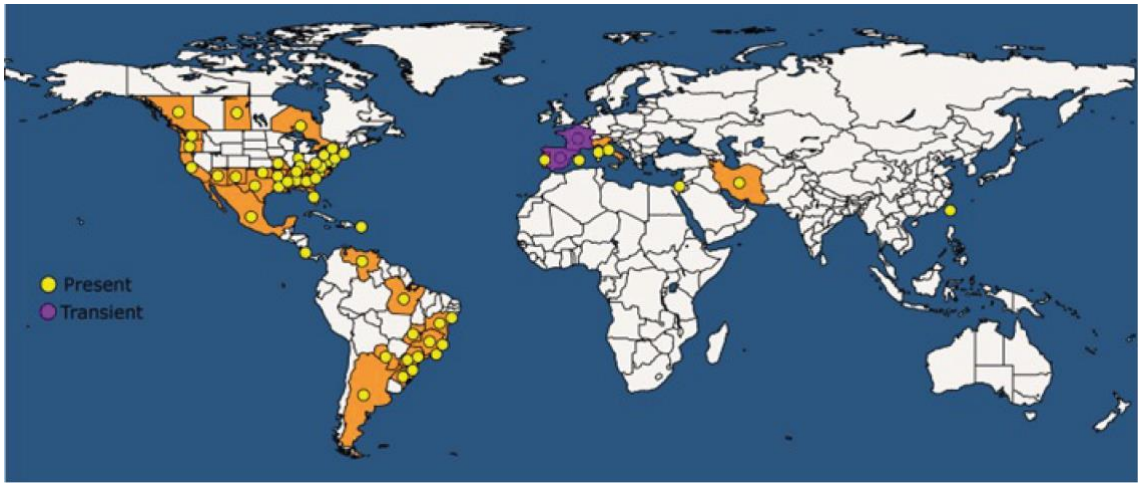



**Figure 1.** *Xylella fastidiosa* world distribution (EPPO, 03-06-2022).

The main dispersal pathway of *X. fastidiosa* over long distances is the movement of infected plant material or insect vectors from areas where the pathogen occurs (Denancé et al., 2017). However, the bacterium is transmitted and disseminated over short distances by xylem sap-feeding specialists (EFSA, 2015). In Europe, all xylem sap-feeding insects are considered potential vectors (Serio et al., 2019). These insects belong to the sub-order Cicadomorpha (Hemiptera: Auchenorrhyncha) and include all spittlebugs/froghoppers (Cercopoidea), all cicadas (Cicadoidea), and sharpshooter leafhoppers (Membracoidea: Cicadellidae: Cicadellinae) (Stancanelli et al., 2015). In Europe, three Aphrophoridae species have been confirmed as vectors, *P. spumarius* (L., 1758) (Saponari et al., 2014), *P. italosignus* Drosopoulos & Remane (2000), and *Neophilaenus camprestris* (Fallén, 1805) (Saponari et al., 2014; Cavalieri et al., 2019)).

*Xylella fastidiosa* colonizes two environments, the mouthparts of its insect vectors and the plant host xylem (Martelli et al., 2016; Sabella et al., 2019). The transmission mediate insect vectors occur as follows: (i) the insect vector acquires the pathogen by feeding on an infected plant, (ii) the bacterium attaches and retains itself in the vector's foregut cuticle; (iii) when the insect feeds on another (healthy) plant, there is detachment and inoculation of the bacteria in the xylem of the new host plant (a representative scheme is shown in Figure 2) (Janse & Obradovic, 2010).

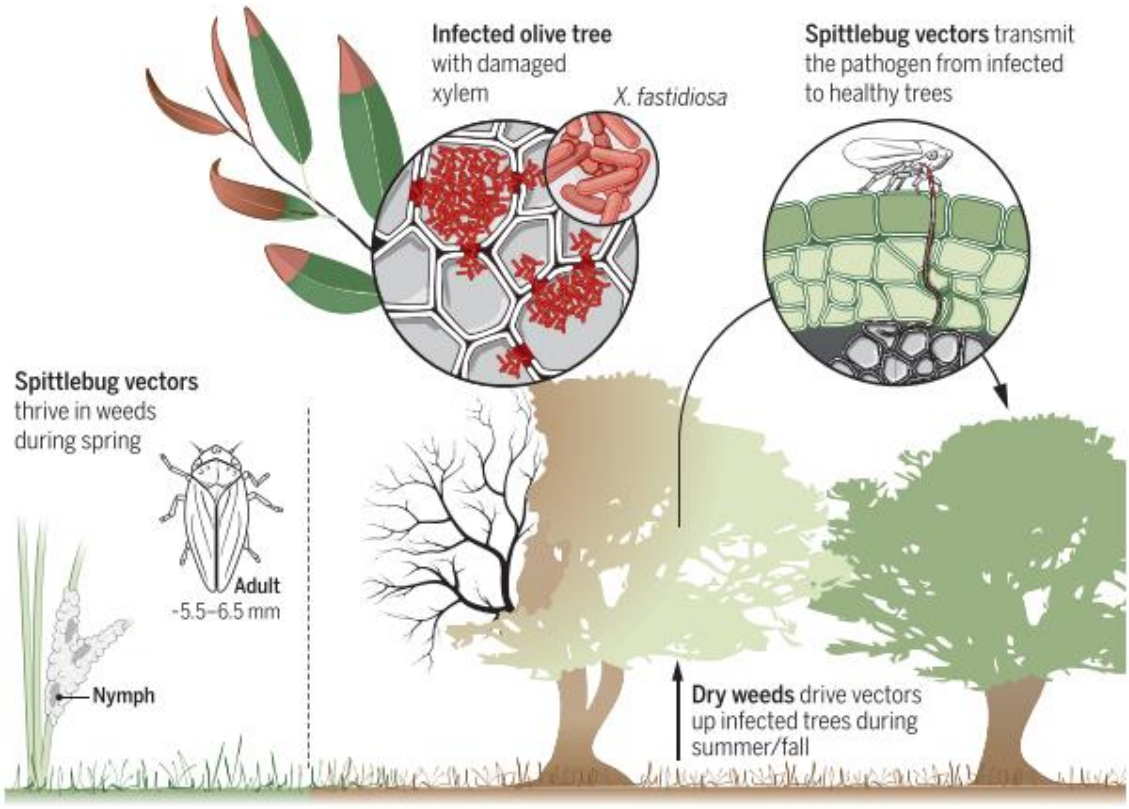



**Figure 2.** *Xylella fastidiosa* transmission mode, tree to tree, by its European main vector *Philaenus spumarius* (Almeida, 2016).

In the insect vector, *X. fastidiosa* multiplies and persists in the adult vector's intestine throughout its lifetime (Almeida et al., 2005). However, the colonization of the bacteria is restricted only to the alimentary canal of the vector. Once infected with *X. fastidiosa*, the insect can inoculate the pathogen into healthy plants immediately after acquisition since the bacterium does not need a latency period (Almeida et al., 2005). The bacterium is not transmitted transovarially to the vector's progeny (Janse & Obradovic, 2010). Nevertheless, like insect adults, nymphs can also transmit *X. fastidiosa* from infected to healthy plants (EFSA, 2013). However, nymphs lose the ability to transmit bacteria after molting (EFSA, 2013).

Plant colonization is a progressive process. *Xylella fastidiosa* moves and multiplies actively on the xylem vessel wall, forming a biofilm (Figure 3). As the bacterial population grows, blockage of the xylem vessels can occur and, consequently, the passage of water and soluble mineral nutrients (Janse & Obradovic, 2010). Thus, symptoms of

this harmful organism are associated with bacterial blockage of xylem fluid transport. The most observable symptom is the dehydration (burning) of leaves or shoots. A green part of the plant suddenly dries out and turns brown, while the adjacent tissues turn red or yellow as plants show drying and eventually followed by plant death (Figure 4) (EFSA, 2013). These symptoms can vary according to the host plants, the subspecies involved, and the climatic conditions (EFSA, 2013). Many wild plants, such as grasses, sedges, and trees, may carry the pathogen but rarely show symptoms (Janse & Obradovic, 2010; Leu, 1993; Saponari et al., 2014).

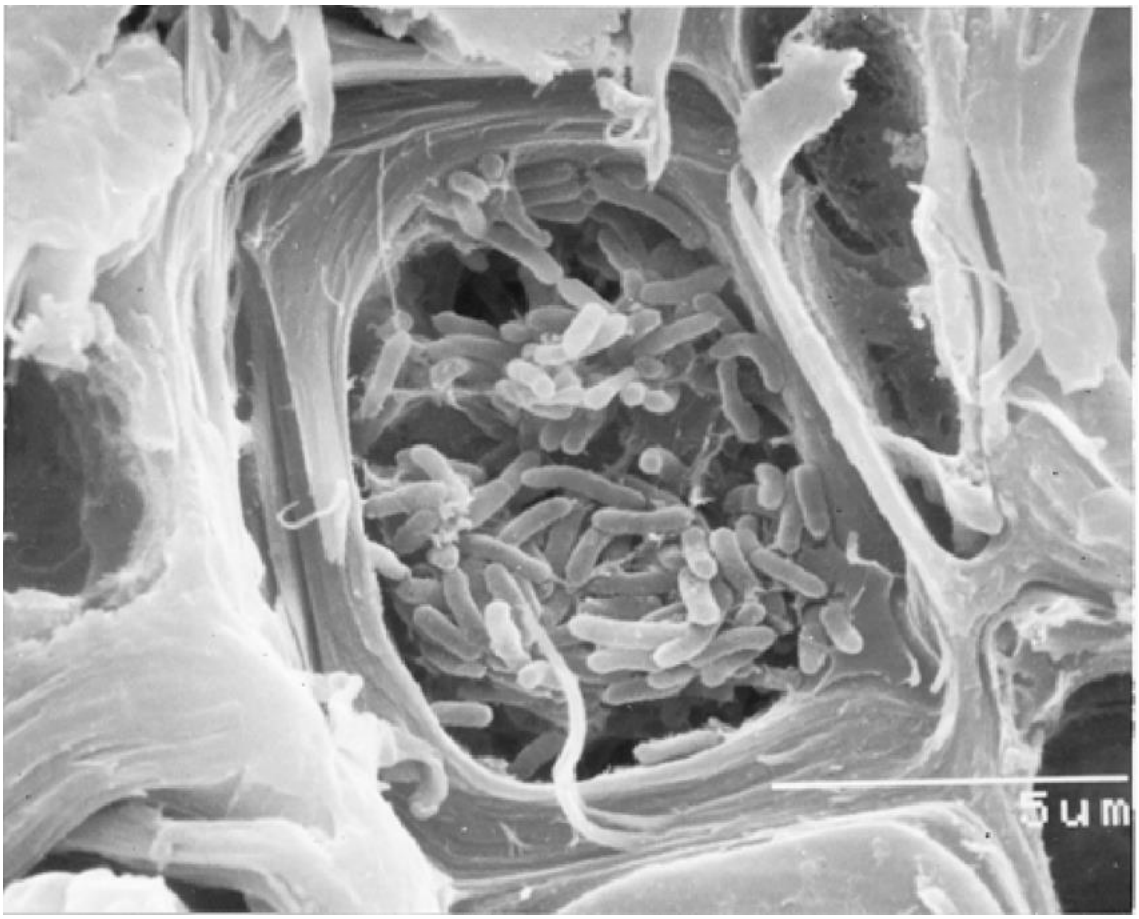



**Figure 3.** *Xylella fastidiosa* in a cross-section of petiole xylem vessel seen by scanning electron microscopy (De Lima et al., 1998).

*Xylella fastidiosa* has been related to many plant diseases that lead to severe economic losses, such as "Pierce's disease" (PD) of the grapevine, the "Almond Leaf Scorch", the "Olive Quick Decline Syndrome", and "Citrus Variegated Chlorosis" (CVC) among others (Janse & Obradovic, 2015).

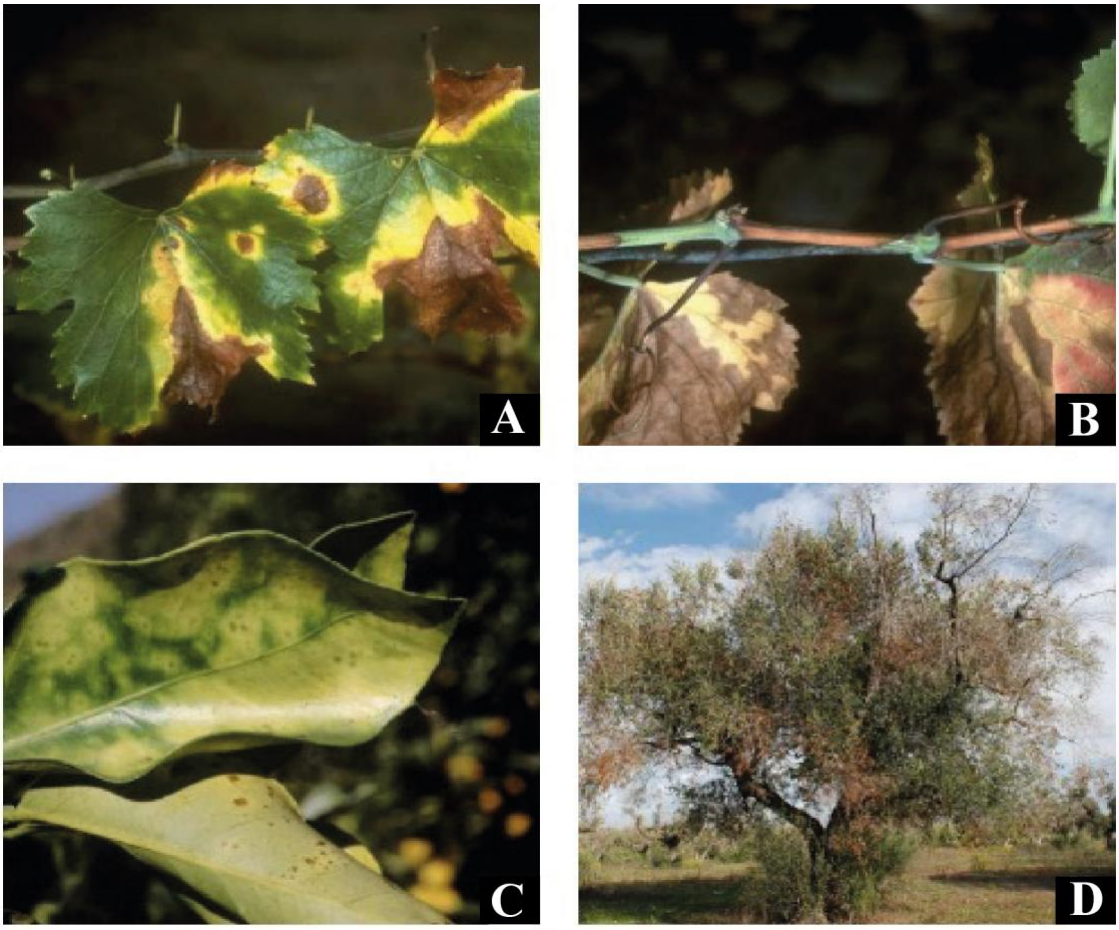



**Figure 4.** Symptoms and damage in different hosts of *Xylella fastidiosa*: (A) Marginal dryness and wilting in vineyard leaves; (B) Irregular maturation of the bark in the vineyard; (C) Interveinal chlorosis and mottling in orange leaves; (D) Dried leaves and branches of olive tree (Scortichini, 2004).

## 2.2 Olive Quick Decline Syndrome

Olive trees are among the most cultivated plants in the world, with 10.2 million hectares of planted trees in 2014 (Cruz et al., 2017). In the European Union, Portugal is one of the top four producers of olive oil, alongside Spain, Italy, and Greece (European commission, 2020).

The olive quick decline syndrome is a disease that appeared in a small area near Gallipoli a few years ago (Ionian coast of the Salento Peninsula, Southern-East Italy) (Martelli et al., 2016). Olive trees have been affected by a progressive disease commences with foliage desiccation that quickly progresses to tree death. This situation was observed and occurred speedily and spread through lower Salento's heavily olive-grown countryside (Asteggiano et al., 2021; Martelli et al., 2016). *Xylella fastidiosa* has already

infected approximately 200,000 hectares of olive orchards and destroying most olive trees in its path, including 1,000-year-old olive trees (Abbott, 2016 ). Over the past years, the disease's area has expanded, raising the risk of spreading to neighboring nations in the Mediterranean basin, where 95 per cent of the world's olives are produced (Abbott, 2016).

The first visual symptoms in an infected tree occur between three and 18 months after initial infection, depending on the time of year, tree age, and variety. OQDS is an incurable disease, and one of the treatments is to reduce the spread of both the pathogen and its insect vectors through eradication, containment, and vector control (Colella et al., 2019). The presence of leaf scorch and scattered desiccation of twigs and small branches are the main symptoms of the OQDS in the early stages of infection. As time passes, these symptoms get worse and more severe and spread to the rest of the crown, which takes on a burned appearance like it, as shown in Figure 5 below (Colella et al., 2019; Luvisi et al., 2017; Martelli et al., 2016).


**Figure 5.** The olive quick decline syndrome: (A) initial, (B) intermediate, and (C) final stages (Martelli et al., 2016).

The European Commission published an audit on May 31, 2016, alleging that the national and regional authorities in Puglia have planned to spend less than half of the €10 million (US$11.2 million) set aside for OQDS containment measures (Abbott, 2018). Furthermore, the Paglian government has set aside €1.8 million (US$2 million) for tracking *X. fastidiosa*, which causes OQDS, because if it is not contained, it could endanger the entire European olive industry sector (Abbott, 2017).

## 2.3 *Philaenus spumarius*

*Philaenus spumarius* was identified as the main and the most abundant vector of *X. fastidiosa* in Europe (Dongiovanni et al., 2019; Saponari et al., 2014). It is also known as the meadow froghopper or meadow spittlebug. This species is a spittlebug of the *Aphrophoridae* family (Yurtsever, 2000). The genus name *Philaenus* comes from the Greek *philein* ("love"), while the species name *spumarius* is from the Latin *spuma* ("sparkling"), referring to the foam nests; the binomial *P. spumarius* can be translated as "foam lover".

*Philaenus spumarius* have an annual and hemimetabolous life cycle (McGavin, 2001). Adults emerge around the end of April and beginning of May, with a high population abundance in late spring to early summer, after that a movement from herbaceous plants to olive trees occurs; indeed, *P. spumarius* adults have been observed moving from the shrubby vegetation of olive orchards to the canopy of olives and other evergreen or deciduous trees and shrubs (Di Serio et al., 2019; Bodino et al., 2017). This movement can also be observed when the vegetation cover persists throughout the summer, indicating that this movement from ground cover vegetation to the trees is not caused only by the herbaceous hosts drying out. At the end of summer and beginning of autumn, adult females return to herbaceous vegetation for oviposition (Cornara et al., 2018). Females lay 10 to 50 spittlebug eggs on underneath plants in the litter at the end of the summer season, ensuring a hatch in the spring season (Hamilton 1982).

*Philaenus spumarius* reaches a body length of 5–7 millimeters. Generally, females are slightly larger than males (Saponari et al., 2014). The body coloration of these polymorphic insects differs widely (about 20 different colors are known) (Figure 6). They are usually yellowish, brownish, or black in color, with brighter patches on a dark background, but they can also have dark markings on a lighter background (Dicke, 1962; Seabra et al., 2020; Halkka, 1964; Yurtsever, 2000).

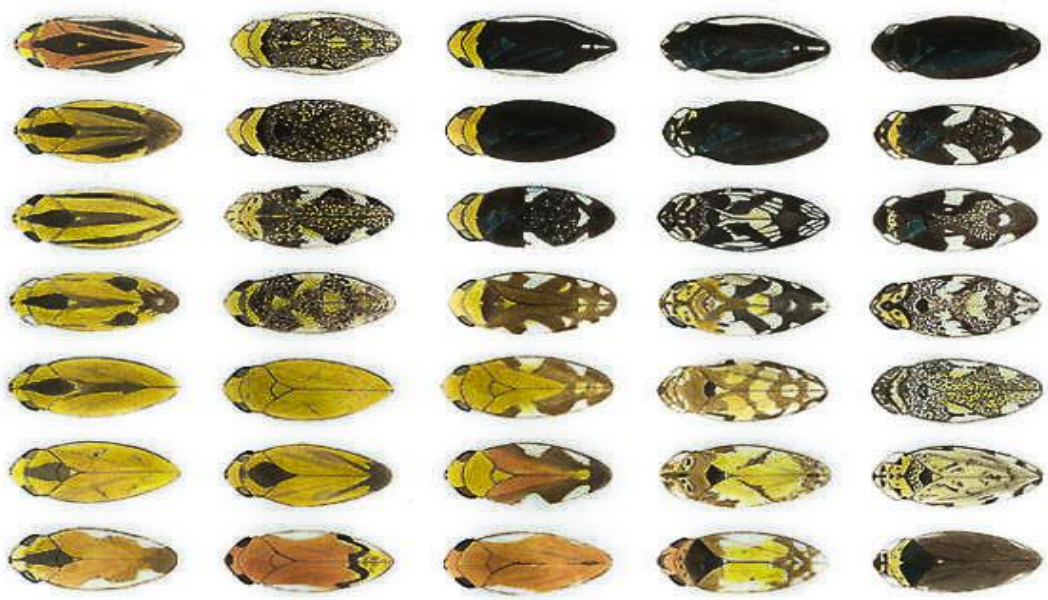


**Figure 6.** *Philaenus spumarius* color varieties (Bugguide, 2006).

## 2.4 *Philaenus spumarius* life stages

### Eggs

The *P. spumarius* eggs are approximately 1 mm long and 0.35 mm wide, with an ovoid, tapering shape (Figure 7A). When the egg is first oviposited, it is yellowish-white with a dark orange pigmented spot in the shell at one end. If the egg is fertilized, the orange spot grows larger and a black, lid-like formation forms on it in about 90 days (Yurtsever, 2000). This black lid-like formation indicates that the eggs are ready to hatch. The young nymph leaves the egg via the black lid. If the egg is not fertilized or is unhealthy, the black lid does not develop, and the orange spot remains, but the egg turns brown and eventually shrivels(Cornara et al., 2018; Yurtsever, 2000).

### Nymphs

There are five nymphal instars of *P. spumarius*. When the first instar emerges, it is light orange. From the late first to the fifth instar, this orange color fades to green. Other morphological changes occur as the nymph develops. For instance, as the body length increases with each larval stage, the length of the legs increases in proportion to the body length, and the abdomen flattens dorso-ventrally. Wing pads also start to appear in the

third instar and become more visible in the fourth and fifth instar (Cornara et al., 2018; Yurtsever, 2000).

**First instar (N1)**

When newly hatched, the body length is approximately 1.35 mm. It is orange in color and produces little foam on the host plant (Figure 7B). Wing pads, and external genitalia are absent. It's delicate and moves slowly (Cornara et al., 2018; Yurtsever, 2000).

**Second instar (N2)**

The body length of the N2 nymph is about 2.25 mm, which is slightly longer than the first instar, but they can be distinguished by their yellowish-orange color (Figure 7C). Wing pads and external genitalia have yet to be created (Cornara et al., 2018; Yurtsever, 2000).

**Third instar (N3)**

The body is about 3 mm long and more greenish than yellow. It is easily distinguished from previous larval stages (Figure 7D). Furthermore, wing pads and external genitalia develop, but the nymphs cannot be sexed yet (Cornara et al., 2018; Yurtsever, 2000).

**Fourth instar (N4)**

The body is about 4.75 mm long, and its color is greenish. External genitalia and yellowish wing pads are clearly visible (Figure 7E). Under x10 magnification, the fourth instar can be sexed with difficulty (Cornara et al., 2018; Yurtsever, 2000).

**Fifth instar (N5)**

The body is about 6.25 mm long and green. External genitalia can be seen under magnification, and the yellowish wing pads are quite well developed (Figure 7F). At this life stage the nymph produces a lot of foam on the host plant (Cornara et al., 2018; Yurtsever, 2000).

**Adults**

Adults emerge approximately 50 days after the nymphal stages. Adults typically remain in the foam mass until the cuticle is hard and becomes fully pigmented (Figure 7G). They may, however, leave their foam mass earlier on occasion. Adults reach sexual maturity about ten days after leaving their foam, and females may mate several times thereafter (Cornara et al., 2018; Yurtsever, 2000).

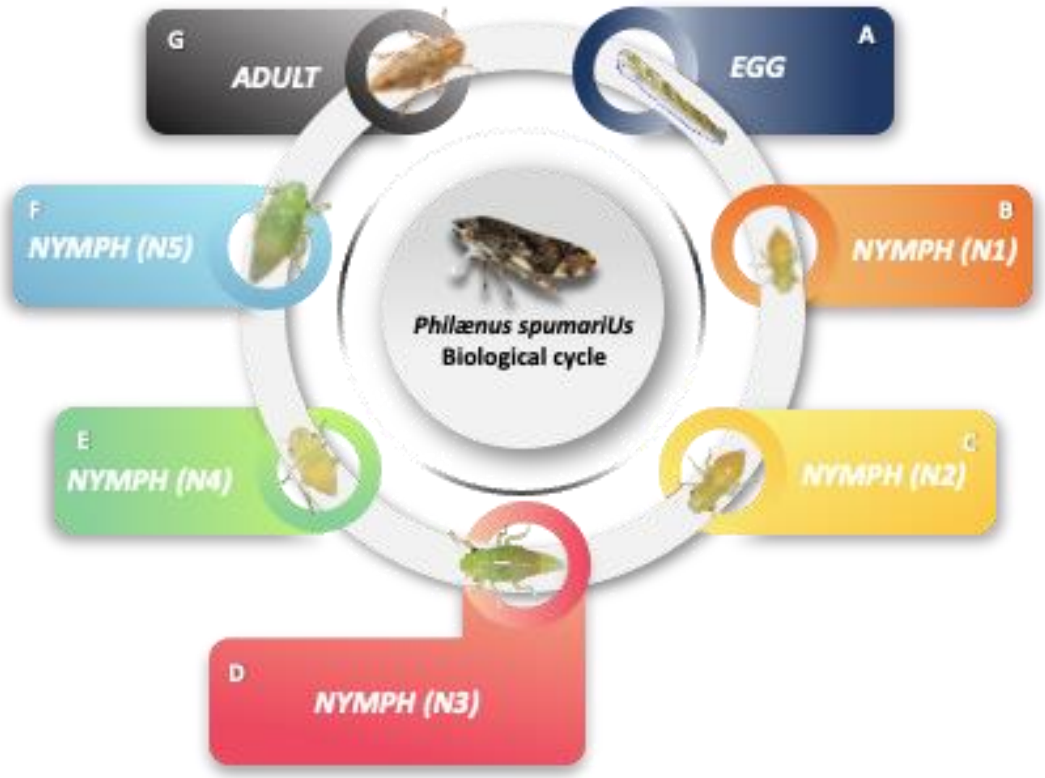



**Figure 7.** Biological cycle of Philaenus spumarius (Nour Ksouri).

## 2.5 Host plant preference of *P. spumarius*

In recent decades, the Common Agricultural Policy has recommended and promoted the maintenance and implementation of cover crops for sustainable agriculture (Guzmán et al., 2019). Nevertheless, due to the high range of hosts of the vector, this practice can increase the number of vectors in the crops. However, suppression of cover crops may have negative consequences, such as diminishing natural enemies by reducing their important resources and decreasing their potential as biocontrol agents of the bacterium vectors and other pests. So, identifying the vector host plant preference and identifying compounds that regulate host plant choice could open new control strategies. Furthermore, this information will contribute to developing and applying control strategies such as push and pull, attract and kill techniques. For this, specific targets in the crops, for example, trapping plants or traps that release compounds, are used to attract the insects and maintain that away from cultivated crops limiting the bacterium's spread (Cascone et al., 2022).

*Philaenus spumarius* has host plant preferences (Villa et al., 2020) for both adults and nymphs due to its ability to communicate with its environment (Avosani et al., 2020). Studies have also shown that *P. spumarius* communicate by emitting vibrational signals, which play specific roles, for example, in mating behavior (Avosani et al., 2020).

Ganassi et al., (2020) showed that some essential oils (EOs) evoked antennal responses of *P. spumarius* (males and females), demonstrating the ability of both sexes' peripheral olfactory systems to perceive volatile compounds in these EOs.

Females of *P. spumarius* were also attracted to volatile organic compounds (VOCs) emitted by plants, at low concentrations, according to Rodrigues et al., (2022), which are detected by olfactory sensilla found on insect antennae (Ranieri et al., 2016). Plant-emitted VOCs serve as important signals for insects looking to find hosts for host recognition/non-host prevention and host plant selection (Bruce & Pickett, 2011; Tumlinson, 2014).

Male and female antennae are sensitive to changes in EO concentrations; this fact was demonstrated by dose-dependent Electroantennograph (EAG) responses evoked by an increase in the concentration of each EO (Ganassi et al., 2020). This finding supports the theory that EO volatiles can serve as long-distance cues for *P. spumarius* adults (Sevarika, 2022). Furthermore, differences in male and female perception of some concentrations of the EOs proved the ability of the olfactory system of *P. spumarius* to perceive them selectively (Ganassi et al., 2020). All these observations in different studies support the fact that the adult *P. spumarius* antennal sensibility responds to a wide range of stimuli, and it is the main used way for the location and the selection of the host plants (Rodrigues et al., 2022).

# CHAPTER3

Material & methods

## 3.1 Material and methods

### 3.1.1 Selection and preparation of the plant species

Four plant species in Portuguese olive groves were selected to conduct this study (Figure 8): *Coleostephus myconis* (L.), *Sonchus tenerrimus* L., *Calendula arvensis* L., and *Chrysanthemum segetum* L. The selection of these four species was based on a survey conducted by Villa et al. (2020), where the host plants of nymphs of spittlebugs were studied in the vegetation ground cover of olive groves in North-eastern Portugal. In this work, *C. myconis* and *S. tenerrimus* were the main host plants of *P. spumarius* nymphs. However, on the opposite side, the same work indicated that *C. arvensis* and *C. segetum*, presented a very low colonization rate by nymphs. Therefore, the four species were identified in the field and transplanted individually into pots one month before beginning the assays (Host plant preference by nymphs of P. spumarius at different soil humidity, Olfactory response of P. spumarius adults towards different host plants …) to adapt the plants to the laboratory conditions. Fifteen plants of each species were kept under light, temperature, humidity, soil characteristics, and photoperiod conditions.


**Figure 8.** Selected plants: (A) *Coleostephus myconis*, (B) *Sonchus tenerrimus*, (C) *Calendula arvensis*, (D) *Chrysanthemum segetum* (Flora-on, 2022).

### 3.1.2 Effect of the host plant on the morphological parameters of *P. spumarius*

The effect of the host plant on the development of *P. spumarius* was evaluated in the natural ground cover vegetation of an olive grove located in Suçães, Mirandela (41°28'43.0"N 7°14'39.8"W). To complete this study, fifteen plants naturally colonized by nymphs of *P. spumarius* of the plant species selected in the section "3.1.1. Plant selection and preparation" were used. On each plant, only one 2$^{nd}$ instar nymph was kept

(if more were found in the same plant, only one was kept); plants were then protected with a tule chamber to prevent the nymphs from leaving the plant (Figure 9). Finally, the emerging adults were killed, and the following parameters were recorded: (i): Sex of adults, (ii): Body width (iii): Body length, (iv): Eye-Eye, (v): Eye-Head, (vi): Head length, (vii): Head width, (viii): Leg, (ix): Total length, (x): Pronotum length, (xi): Pronotum width, (xii): Wing width, (xiii): Wing length, (xiv): Aedeagus length.

These parameters were measured by taking scaled photographs of the entire insect with a binocular microscope, "Leica". After imaging the entire specimen, the males last abdominal segments were removed with forceps under a binocular magnifier. The soft tissue was put in hot 10% potassium hydroxide (KOH) for 3 minutes, and the remaining calcified parts were removed to obtain the aedeagus, which was stored in glycerin. Each aedeagus was imaged by placing it between coverslips filled with glycerin, which helped to keep the aedeagus in the correct orientation. Morphometric measurements were taken from photographs taken by the binocular microscope.

**Figure 9.** The selected plants in the field to study the effect of the host plant on the development of *Philaenus spumarius.*

### 3.1.3 Host plant preference by nymphs of *P. spumarius* at different soil humidity

The xylem is the tissue that transports mainly water and salt from the soil to the plant's upper membranes. Given that *P. spumarius* is an insect that feeds on the xylem and water is the primary component of this tissue (Huberty & Denno, 2004), it is

important to investigate how different soil humidity levels affect insects' preferences for particular plants. Under laboratory conditions, the four selected plant species were planted in the same pot at equal distances from a central point (Figure 10). The soil in which the plants were transplanted had different water content percentages of 10%, 50%, and 70%, respectively. Five nymphs in the 3$^{rd}$ instar stage (to ensure the mobility and survival of the nymphs), collected in the same locations mentioned previously, were released at the central point of the pot. After 2, 12, 24, and 72 hours of the nymphal release: (i) the host plant selected by the nymphs, and (ii) the number of nymphs per plant, were recorded. For each water content, five repetitions were performed.

**Figure 10.** Container used for humidity level assays (50 x 40 x 40 cm).

### 3.1.4 Olfactory response of *P. spumarius* adults towards different host plants

An eight-chamber olfactometer was utilized to assess the olfactory response of females and males of *P. spumarius* towards the four different host plants previously selected and the olive tree. (Figure 11).

Three healthy and fresh leaves from olive and the four plants were placed in the small chambers in the center arena of the olfactometer device (the remaining chambers served as controls). Three insects were placed in each corner of the main arena (40 x 40

x 7 cm). The bottom part was a 40 x 40 cm white opaline plate, the chambers located in its center.

Each chamber had a dimension of 6 x 5 cm with an entrance hole of 1 x 1 cm, allowing insects to enter. Perforated from below and linked to 250 ml washing bottles containing activated charcoal (AppliChem, Panreac ITW) dissolved in 100 ml of distilled water to purify and humidify the airflow provided by pumps with a 12 cm3/min airflow.

The top layer was a transparent plate (40 x 40 cm) with entrances in its 4 corners allowing the entry of 12 insects (3 in each corner). For each sex, 15 repetition were performed and the results were recorded after 30 minutes of insect release.

The chambers were cleaned after each experiment.

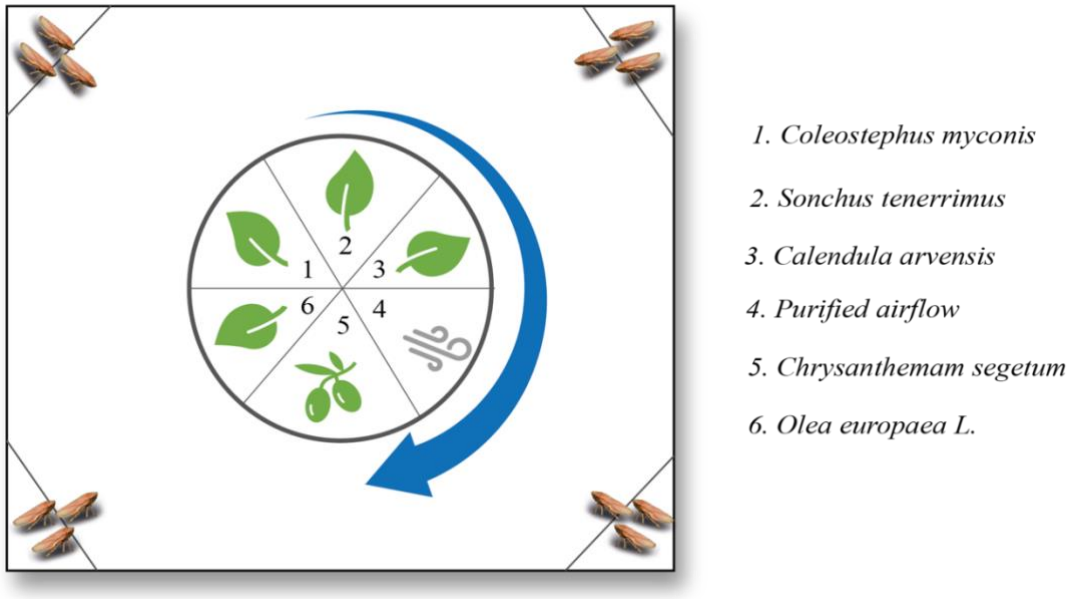



**Figure 11.** Schematic drawing of the placement of the insects and plants in the arena of the six-chamber olfactometer.

### 3.1.5 Characterization of the volatile profile of the host plants

The volatile profiles of *C. myconis*, *S. tenerrimus*, *C. arvensis*, and *C. segetum* leaves were analyzed utilizing HS-SPME (headspace solid-phase microextraction) and GC/MS (gas chromatography with mass spectrometry detector). The data used for the olive's Volatil Organic Compounds (VOC), (*Olea europea)* were collected from Rodrigues et al. (2020), study.

For the HS-SPME, approximately 0.5 g of healthy leaves were placed in 50 ml individual vials for each plant species, and the leaf petioles were covered with aluminum

foil to avoid any possible registration of oxidation volatiles. After sealing the vials with a polypropylene cap, 5 µl of 4-methyl-2-pentanol (0.127 mg/ml) (Sigma Aldrich, USA) was added as an internal standard. In a water bath, the volatiles were released at 40 °C for 5 min. Then, a fiber coated with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/ PDMS 50/30 µm) (Supelco, Bellefonte, USA) was exposed to the headspace for volatile adsorption for 30 min. For each plant species, five replicates of HS-SPME analysis were performed.

Chromatographic analysis was evaluated with a Shimadzu GC- 2010 Plus with a Shimadzu GC/ MS- QP2010 SE mass spectrometer (gas chromatography coupled with mass spectrometry detector). Thermal desorption for 1 min in the injection port of the chromatography system (220°C) will elute the volatile chemicals from the HS-SPME fiber, was used to detect the volatiles (Figure 12). After that, the fiber was kept in the injector port for additional 10 minutes for the cleaning and conditioning process before being used again. The employed column was a TRB-5MS (30 m, 0.25 mm, 0.25 m) from Teknokroma (Spain). The injector was set to 220°C, and the injections were manual, in splitless mode with helium (Praxair, Portugal) at 30 cm/s and a total flow of 24.4 ml/min as the mobile phase. The oven temperature was set at 40°C (1 min) and 2°C/min to 220°C (30 min). The full scan MS spectra fragments were compared with those obtained from the NIST 69 Library (National Institute of Standards and Technology, Gaithersburg, MD, USA), PubChem of the NIH (National Center for Biotechnology information) and the ChemSpider data base. The areas of the chromatographic peaks were determined by integrating the reconstructed chromatogram from the full scan chromatogram using the ion base (m/z intensity 100%) for each compound.

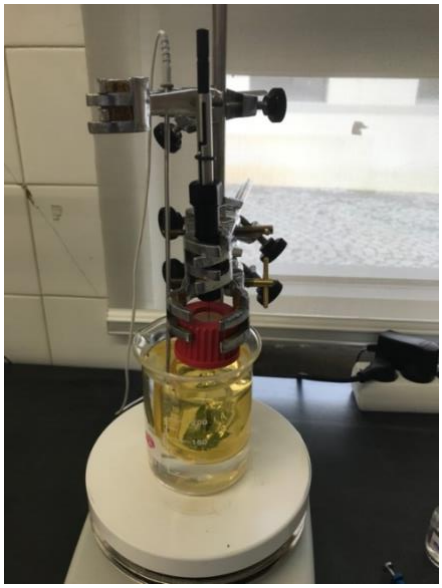


**Figure 12.** HS-SPME fiber placed in 50 ml vial for 30 minutes for volatile organic compounds adsorption.

## 3.2 Data analysis

### 3.2.1 Effect of the host plant on the morphological parameters of *P. spumarius*

To assess the effect of the *C. myconis*, *S. tenerrimus*, *C. arvensis*, and *C.segetum* on the morphological parameters of *P. spumarius* an Analysis Of Variance (ANOVA) was performed using the software PAST v.4.10 ( (Hammer et al., 2001)). Tukey's test ($\alpha$ <0.05) was used as a subsequent multiple-comparison post-hoc tests, in the same software.

### 3.2.2 Host plant preference by nymphs of *P. spumarius* at different soil humidity

Generalized Estimating Equations ($\alpha = 0.05$) with Poisson distribution were used to compare the choice of the plants by *P. spumarius* nymphs at different humidity soil levels. Choses plants, time and humidity level were considered as response variables. Tukey's test ($\alpha$ <0.05) was used as a subsequent multiple-comparison post-hoc test. Dead insects and insects that did not choose any of the chambers were excluded from the statistical analysis.

### 3.2.3 Olfactory response of *P. spumarius* adults towards different host plants

The same model described in the section "3.2.2. Host plant preference by nymphs of *P. spumarius* at different soil humidity" was used to assess the olfactory response of *P. spumarius* towards different host plants. Sex, plant species, and the interaction between these two terms (*C. myconis*, *S. tenerrimus*, *C. arvensis*, and *C. segetum*, *O. europea* and the control) were considered as response variables. A post-hoc Tukey's tests ($\alpha$= 0.05) was used as a subsequent multiple comparison for males and females separately. Insects that did not make any choice were excluded from the statistical analysis.

### 3.2.4 Characterization of the volatile profile of the host plants

To compare the volatile profiles of the plants, an ANOVA test was performed using the software PAST v.4.10 (Hammer et al., 2001), and a Tukey's test ($\alpha < 0.05$) was used as a subsequent multiple-comparison post-hoc tests. Additionally, a Principal Component Analysis using the function PCA from the 'FactoMineR' package (Lê et al., 2008), PCA was performed in R software v.3.5.1 (R Core Team, 2020). The fviz pca biplot function from the 'factoextra' package was used to create the correlation biplot of the two first PCs (Kassambara & Mundt, 2020).

### 3.2.5 The potential effect of the VOCs on the behaviour of nymphs and adults of *P. spumarius*

The potential effect of the VOCs on the behaviour of nymphs and adults of *P. spumarius* was assessed with a generalized linear model (Poisson distribution). VOCs were used as explanatory variables. The explanatory variables were checked for multicollinearity prior to executing the models. For that, principal component analyses (PCA) and the Pearson correlations were calculated. The PCA function was used to visualize the contribution to the variance of the explanatory variables and their relations, and the Pearson correlations were calculated using the function cor from base R (Figure S1 and S2). To further assess multicollinearity, the variance inflation factor (VIF) was also calculated: The highest VIF scores were below three (the common threshold for VIF is usually $> 10$; (Dormann et al. 2013)). When multicollinearity among explanatory variables was found, the models maintained the VOCs that contributed most to the plants' characterization. For the nymphs and adults, models were selected by comparing the Akaike information criterion (Akaike 2011). The models were checked for overdispersion and residual distribution using the "DHARMa" package (Hartig 2017).

The final model used for *P. spumarius* nymphs includes the volatiles: 1-Heptanol , 1,7-Dioxaspiro[5.5]undecane , D-Limonene . And the final model used for *P. spumarius* adults was: 1,7-Dioxaspiro[5.5]undecane, 2-Tolyloxirane , 3-Hexen-1-ol, (Z)-, Butanoic acid, 3-hexenyl ester, (E)-, D-Limonene, Indane, Oxime-, methoxy-phenyl-_ and β-Cubebene.







# CHAPTER4

Results

## 4.1 The effect of the host plant on the development of *P. spumarius*

Based on our findings, the plant species on which the insect is found has a significant impact on certain parameters, such as body width, head length, head width, and pronotum length. Indeed, insects on Sonchus tenerrimus have the largest average body width (2.56 ± 0.03 mm) and those on Calendula arvensis have the smallest (2.43 ± 0.03 mm). The same is true for head length and head width; the largest values are found in insects associated with Sonchus tenerrimus, and the smallest with Calendula arvensis.

Pronotum length also appears to be significantly affected by the plant species. Insects from Chrysanthemum segetum have the shortest pronotum length (0.54 ± 0.01 mm), while those from Calendula arvensis have the longest (0.68 ± 0.03 mm).

Other parameters such as body length, eye-eye distance, eye-head distance, leg length, total length, pronotum width, wing width, wing length, and aedeagus length show no significant difference among the insects from different plants. This suggests that these aspects of insect morphology are not influenced by the plant species.

**Table 1.** The measurements of the different morphological characters (Mean±SE) of *Philaenus spumarius* adults.

| Measurements (mm) | *Calendula arvensis* | *Coleostephus myconis* | *Chrysanthemum segetum* | *Sonchus tenerrimus* | P-value |
|---|---|---|---|---|---|
| | Mean ± S. E | Mean ± S. E | Mean ± S. E | Mean ± S. E | |
| Body width | 2.43 ± 0.03 | 2.54 ± 0.04 | 2.44 ± 0.04 | 2.56 ± 0.03 | 0.02 |
| Body length | 5.20 ± 0.07 | 5.34 ± 0.07 | 5.13 ± 0.17 | 5.31 ± 0.09 | 0.47 |
| Eye-Eye | 1.14 ± 0.03 | 1.15 ± 0.02 | 1.20 ± 0.03 | 1.16 ± 0.03 | 0.48 |
| Eye-Head | 1.46 ± 0.02 | 1.45 ± 0.02 | 1.43 ± 0.04 | 1.45 ± 0.01 | 0.88 |
| Head length | 1.91 ± 0.03 | 2.00 ± 0.02 | 1.98 ± 0.03 | 2.02 ± 0.02 | 0.04 |
| Head width | 0.51 ± 0.02 | 0.45 ± 0.01 | 0.49 ± 0.01 | 0.51 ± 0.02 | 0.02 |
| Leg | 3.74 ± 0.12 | 4.02 ± 0.09 | 3.84 ± 0.08 | 4.01 ± 0.06 | 0.09 |
| Total length | 5.88 ± 0.07 | 6.02 ± 0.06 | 5.78 ± 0.08 | 6.04 ± 0.08 | 0.06 |

| | | | | | |
|---|---|---|---|---|---|
| Pronotum length | 0.68 ± 0.03 a | 0.62 ± 0.01 a | 0.54 ± 0.01 | b 0.61 ± 0.02 ab | <0.001 |
| Pronotum width | 1.98 ± 0.03 | 2.00 ± 0.03 | 1.94 ± 0.03 | 2.00 ± 0.03 | 0.5 |
| Wing width | *1.50 ± 0.25* | *1.28 ± 0.04* | *1.16 ± 0.03* | *1.59 ± 0.23* | 0.28 |
| Wing length | 4.40 ± 0.23 | 4.79 ± 0.04 | 4.53 ± 0.07 | 4.57 ± 0.24 | 0.47 |
| Aedeagus length | 0.48 ± 0.03 | 0.51 ± 0.01 | 0.49 ± 0.00 | 0.54 ± 0.03 | 0.05 |
| Sex: Males | 3 | 3 | 1 | 4 | - |
| Sex: Females | 12 | 12 | 14 | 11 | - |

## 4.2 Host plant preference by nymphs at different soil humidity

The choice made by *P. spumarius* nymphs was found to be significantly influenced by two factors: the plant species and the percentage of water content in the soil. Additionally, the effect of time was also observed to have an impact on the choice of plants (Table 2).

Among the four plant species studied, *S. tenerrimus* and *C. myconis* were the two most preferred plants by *P. spumarius* nymphs (Figure 13A). This suggests that these two plants have a higher attractiveness or suitability for the nymphs compared to the other plants.

Interestingly, the results indicate that the percentage of water content in the soil has a significant effect on the nymph's choice of plants. As the soil water level increases, there is a noticeable decrease in the preference for certain plants by the nymphs (Figure 13B). This suggests that the availability or abundance of water in the soil plays a role in shaping the nymph's plant selection behavior.

However, for *S. tenerrimus* specifically, the preference of *P. spumarius* nymphs for this plant increases with higher soil water levels (Figure 13C). This finding suggests that the nymphs exhibit a stronger attraction or affinity towards *S. tenerrimus* as the soil humidity increases.

**Table 2.** The results of the GEE developed for the effect of the humidity levels in time on the choice of nymphs of *Philaenus spumarius*.

| | Df | $X^2$ | P-value |
|---|---|---|---|
| Plant | 3 | 40.3 | <0.001 |
| Water content (%) | 1 | 9.9 | <0.001 |
| Hour | 1 | 1.1 | 0.29 |
| Plant:Water content (%) | 3 | 3.3 | 0.34 |
| Plant:Hour | 3 | 26.3 | <0.001 |
| Water content (%):Hour | 1 | 0 | 0.82 |
| Plant:Water content (%):Hour | 3 | 1.2 | 0.75 |

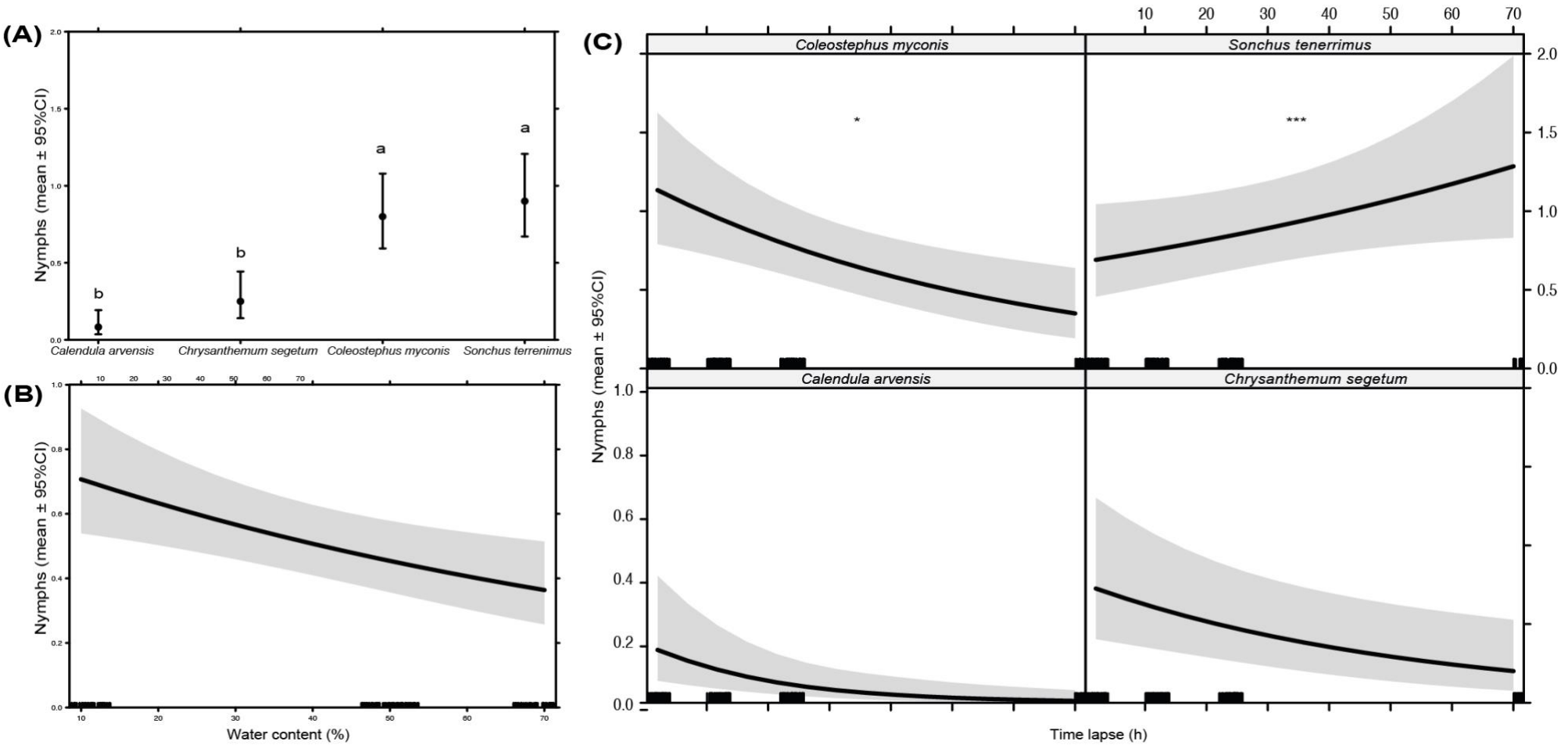



**Figure 13.** Water content assay: (A)- Number of plants chosen by nymphs of *Philaenus spumarius* (mean ± 95%CI), (B)- The effect of the water content on the choice of nymphs *Philaenus spumarius* (mean ± 95%CI), (C)- The choice by nymphs of *Philaenus spumarius* of each plant species during time at different humidity levels.

## 4.3 The olfactory preference of *P. spumarius* adults

The results of the olfactory preference assay for *P. spumarius* revealed that the sex of the insects did not have a significant influence on their choice of plants (Table 3). However, the choice of plants was significantly affected by the plant species itself.

These findings regarding the plant preference by adult *P. spumarius* align with the observations made by Villa et al. (2020) in the field. The previous study also reported that adult *P. spumarius* showed a preference for *S. tenerrimus* and *C. myconis* over other plant species.

The olfactory preference assay indicated that the choice of plants by adult *P. spumarius* was influenced by the plant species, with *S. tenerrimus* being preferred by females and the olive plant being more frequently chosen by males.

**Table 3.** The results of the GEE developed for the effect of plant species and sex of the insects on the choice of adults of *Philaenus spumarius.*

| | Df | $X^2$ | P-value |
|---|---|---|---|
| Plant | 5 | 62.2 | <0.001 |
| Sex | 1 | 1.3 | 0.25 |
| Plant:Sex | 5 | 3.6 | 0.60 |

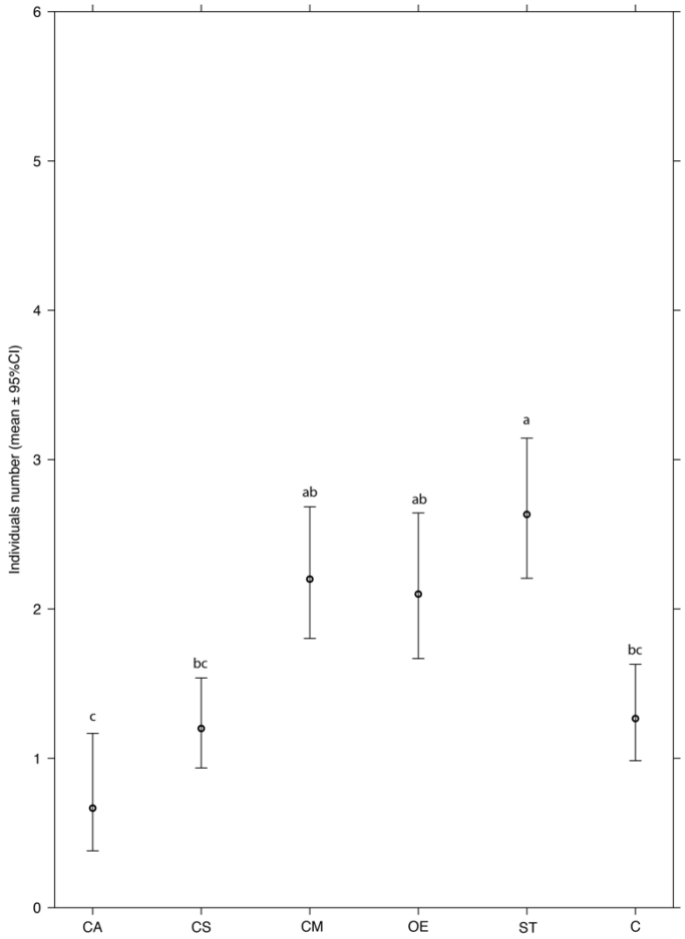



**Figure 14.** The olfactory response of *Philaenus spumarius* (Mean±95% CI) towards: CA: *Calendula arvensis*, CS: *Chrysanthemum segetum* , CM: *Coleostephus myconis*, OE: *Olea europea*, ST: *Sonchus tenerrimus* and C: Control.

## 4.4 Volatile organic compounds characterization

In total, 110 organic compounds were identified from the four plant species investigated (Table 4). The plant species and their respective number of volatile organic compounds (VOCs) are as follows: *C. arvensis* had the highest number with 51 VOCs, followed by *C. segetum* with 39 VOCs, *C. myconis* with 11 VOCs, and *S. tenerrimus* with 9 VOCs (Table 4).

The main constituents of *C. arvensis* were. alpha.-Pinene, accounting for 31% of the VOCs, followed by Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (13.9%), and 3-Hexen-1-ol, (Z)- (13.4%).

For *C. segetum*, the most abundant components were Butanoic acid, 3-hexenyl ester, (E)- (22.8%), cis-.beta.-Farnesene (18.5%), and 3-Hexen-1-ol, acetate, (Z)- (10.3%).

*C. myconis* was dominated by Benzene, 1,2,3-trimethyl- (35.6%), followed by Benzene, 1-methyl-3-propyl- (17.3%) and Indane (15.7%).

In *S. tenerrimus*, the major component was 3-Hexen-1-ol, (Z)-, constituting 51.2% of the VOCs.

Based on the results of Principal Component Analysis (PCA) and ANOVA analyses, the volatile profiles of *C. myconis* and *S. tenerrimus* exhibited a similar profile, as shown in Figure 15. Additionally, the volatile profiles of these two plants were significantly different from those of *C. segetum* and *C. arvensis*. The first and second principal components (PC1 and PC2) explained 59.3% of the variation observed in the volatile profiles (Figure 15).

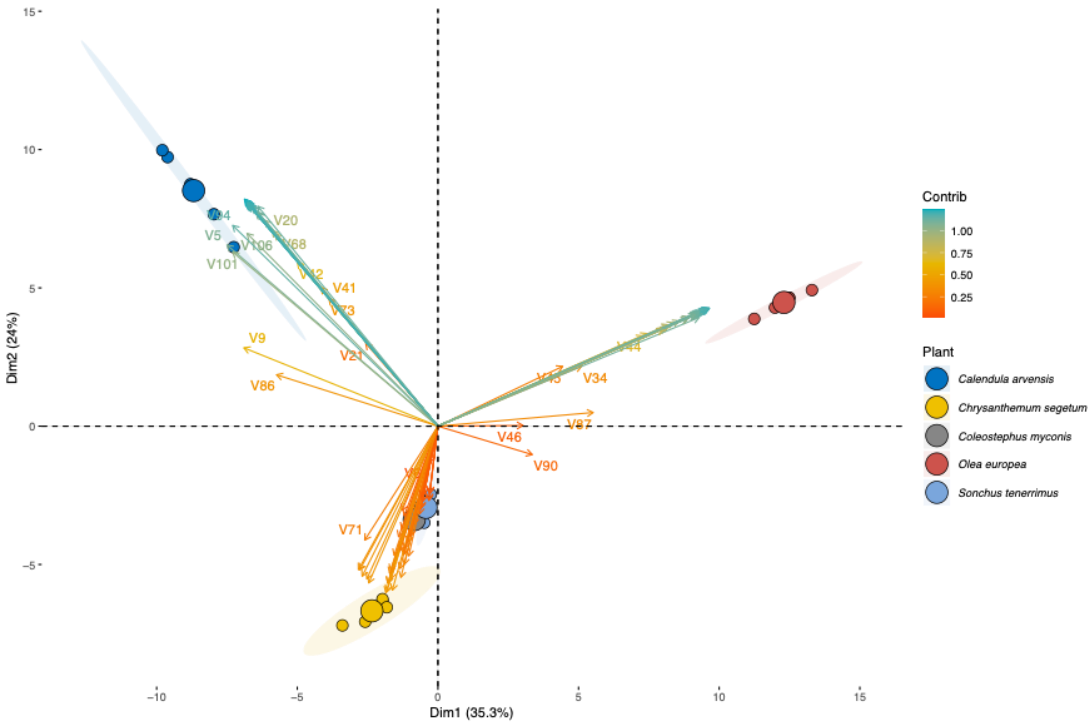



**Figure 15.** Principal component analysis score plot obtained from the volatile profile of *Calendula arvensis, Chrysanthemum segetum, Coleostephus myconis*, *Olea europea* and *Sonchus tenerrimus*, "the numbers within the panels correspond to the numbers of the families in Table 4".

**Table 4.** Volatile organic compounds (VOCs) (Mean±SE) of *Calendula arvensis*, *Chrysanthemum segetum*, *Coleostephus myconis*, *Olea europea* and *Sonchus tenerrimus* obtained by gas chromatography coupled with Mass spectrophotometry (GC-MS).

| Code | Compound | *Calendula arvensis* | *Chrysanthemum segetum* | *Sonchus tenerrimus* | *Coleostephus myconis* | *Olea europaea* | P-value |
|---|---|---|---|---|---|---|---|
| | | Mean ± S. E | Mean ± S. E | Mean ± S. E | Mean ± S. E | Mean ± S. E | |
| V1 | .alpha.-Calacorene | 0.07 ± 0.01 | - | - | - | - | - |
| V2 | .alpha.-Cubebene | 0.06 ± 0.01 | - | - | - | - | - |
| V3 | .alpha.-Farnesene | | 1.42 ± 0.41 | - | - | - | - |
| V4 | .alpha.-Guaiene | 0.04 ± 0.00 | - | - | - | - | - |
| V5 | .alpha.-Pinene | 30.96 ± 1.46 | - | - | - | - | - |
| V6 | .alpha.-Terpineol | 0.06 ± 0.00 | - | - | - | - | - |
| V7 | .beta.-Myrcene | 0.84 ± 0.08 | - | - | - | - | - |
| V8 | .beta.-Phellandrene | 0.60 ± 0.04 | - | - | - | - | - |
| V9 | .gamma.-Muurolene | 0.49 ± 0.06 | - | - | - | - | - |
| V10 | .gamma.-Terpinene | 3.43 ± 0.23 | - | - | - | - | - |
| V11 | .tau.-Cadinol | 0.29 ± 0.08 | - | - | - | - | - |
| V12 | (-)-.alpha.-Panasinsen | 0.01 ± 0.00 | - | - | - | - | - |
| V13 | (-)-.beta.-Bourbonene | 1.26 ± 0.12 | - | - | - | - | - |
| V14 | (.+/-.)-Lavandulol, chlorodifluoroacetate | - | - | - | - | 0.07 ± 0.00 | - |
| V15 | (+)-4-Carene | 1.20 ± 0.08 | - | - | - | - | - |
| V16 | (E)-.beta.-Famesene | - | 9.15 ± 5.85 | - | - | - | - |
| V17 | 1-Heptadecene | 0.02 ± 0.00 | - | - | - | - | - |
| V18 | 1-Heptanol | 0.04 ± 0.01 b | 0.26 ± 0.04 a | - | - | - | <0.001 |
| V19 | 1-Hexanol | - | - | - | - | 3.26 ± 0.25 | - |
| V20 | 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]- | 0.02 ± 0.00 | - | - | - | - | - |
| V21 | 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1S-(1.alpha.,4.alpha.,4a.beta.,8a.beta.)]- | 0.00 ± 0.00 | - | - | - | - | - |
| V22 | 1-Nonanol | - | - | - | - | 0.09 ± 0.01 | - |
| V23 | 1-Octanol | 0.05 ± 0.01 b | 0.43 ± 0.05 a | - | - | - | <0.001 |
| V24 | 1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta | 0.02 ± 0.00 | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V25 | 1,3-Pentanediol, 2,2,4-trimethyl- | | - | | | - | | | - | | | - | | | 0.49 | ± 0.04 | | - |
| V26 | 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- | 0.07 | ± 0.01 | | | - | | | - | | | - | | | | - | | - |
| V27 | 1,5-Hexadien-3-ol | | - | | | - | | | - | | | - | | | 0.37 | ± 0.03 | | - |
| V28 | 1,7-Dioxaspiro[5.5]undecane | | - | | 1.35 | ± 0.80 | | | - | | | - | | | | - | | - |
| V29 | 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- | 3.40 | ± 0.11 | | | - | | | - | | | - | | | | - | | - |
| V30 | 1H-Indene, 2,3-dihydro-4-methyl- | | - | | 0.22 | ± 0.05 | | | - | | | - | | | | - | | - |
| V31 | 1H-Indene, 2,3-dihydro-5-methyl- | | - | | 0.28 | ± 0.07 | | | - | | | - | | | | - | | - |
| V32 | 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)- | | - | | 0.32 | ± 0.15 | | | - | | | - | | | | - | | - |
| V33 | 2-Hexenal, (E)- | | - | | | - | | | - | | | - | | | 0.42 | ± 0.12 | | - |
| V34 | 2-Pentanol, 4-methyl- | | - | | | - | | | - | | | - | | | 1.04 | ± 0.68 | | - |
| V35 | 2-Propanol, 1-(2-methoxy-1-methylethoxy)- | | - | | | - | 1.66 | ± 0.52 | | | | - | | | | - | | - |
| V36 | 2-Tolyloxirane | | - | | | - | | | - | 11.44 | ± 2.21 | | | | | - | | - |
| V37 | 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate | | - | | | - | | | - | | | - | | | 0.25 | ± 0.04 | | - |
| V38 | 2,4-Dodecadienal, (E,E)- | | - | | | - | | | - | | | - | | | 0.12 | ± 0.02 | | - |
| V39 | 2,4-Hexadienal, (E,E)- | | - | | | - | | | - | | | - | | | 0.45 | ± 0.07 | | - |
| V40 | 2,4,4-Trimethyl-1-hexene | | - | | | - | | | - | | | - | | | 0.08 | ± 0.01 | | - |
| V41 | 2,4,6-Octatriene, 2,6-dimethyl- | 0.01 | ± 0.01 | | | - | | | - | | | - | | | | - | | - |
| V42 | 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- | 0.02 | ± 0.01 | | | - | | | - | | | - | | | | - | | - |
| V43 | 2(3H)-Furanone, 5-ethyldihydro- | | - | | | - | | | - | | | - | | | 0.16 | ± 0.02 | | - |
| V44 | 2(5H)-Furanone, 5-ethyl- | | - | | | - | | | - | | | - | | | 0.12 | ± 0.05 | | - |
| V45 | 3-Hexen-1-ol, (Z)- | 13.42 | ± 3.13 | c | | - | 51.23 | ± 7.41 | a | 1.51 | ± 0.37 | d | 35.80 | ± 3.16 | b | <0.001 | | |
| V46 | 3-Hexen-1-ol, acetate, (Z)- | 3.87 | ± 2.24 | | 10.30 | ± 3.03 | | | - | | | - | | | 9.21 | ± 2.05 | | - |
| V47 | 3-Hexen-1-ol, formate, (Z)- | | - | | | - | | | - | | | - | | | 0.07 | ± 0.01 | | - |
| V48 | 3-Hexenoic acid, methyl ester, (Z)- | | - | | | - | | | - | | | - | | | 0.84 | ± 0.25 | | - |
| V49 | 3-Octanone | | - | | | - | | | - | 1.91 | ± 0.77 | | | | | - | | - |
| V50 | 3-Pentanone | | - | | | - | | | - | | | - | | | 0.70 | ± 0.66 | | - |
| V51 | 4-Pentenal, 2-methyl- | | - | | | - | | | - | | | - | | | 2.17 | ± 0.89 | | - |
| V52 | 4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]- | 0.02 | ± 0.00 | | | - | | | - | | | - | | | | - | | - |
| V53 | 5-Hepten-2-one, 6-methyl- | | - | | | - | | | - | | | - | | | 0.05 | ± 0.01 | | - |
| V54 | 5-Octadecene, (E)- | | - | | | - | | | - | | | - | | | 0.09 | ± 0.01 | | - |
| V55 | Acetic acid, hexyl ester | | - | | | - | | | - | | | - | | | 0.25 | ± 0.03 | | - |
| V56 | Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- | 0.15 | ± 0.03 | | | - | | | - | | | - | | | | - | | - |
| V57 | Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]- | | - | | | - | | | - | | | - | | | 0.03 | ± 0.00 | | - |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V58 | Benzene, 1-ethyl-2,4-dimethyl- | - | | | - | | | - | | | 1.39 | ± | 0.39 | | - | | | - | |
| V59 | Benzene, 1-ethyl-3-methyl- | - | | | 1.42 | ± | 0.16 | b | - | | | 4.44 | ± | 0.82 | a | - | | | 0.01 |
| V60 | Benzene, 1-methyl-2-propyl- | - | | | 0.24 | ± | 0.05 | b | - | | | 4.30 | ± | 1.34 | a | - | | | 0.02 |
| V61 | Benzene, 1-methyl-3-propyl- | - | | | - | | | | - | | | 17.34 | ± | 3.63 | | - | | | - |
| V62 | Benzene, 1-methyl-4-propyl- | - | | | - | | | | - | | | 2.79 | ± | 2.79 | | - | | | - |
| V63 | Benzene, 1,2,3-trimethyl- | - | | | - | | | | - | | | 38.30 | ± | 3.40 | | - | | | - |
| V64 | Benzene, 1,2,3,4-tetramethyl- | - | | | 0.14 | ± | 0.09 | | - | | | - | | | | - | | | - |
| V65 | Benzene, 1,2,3,5-tetramethyl- | - | | | 0.09 | ± | 0.04 | | - | | | - | | | | - | | | - |
| V66 | Benzocyclobutene | - | | | - | | | | - | | | - | | | 0.34 | ± | 0.05 | | - |
| V67 | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- | 13.89 | ± | 0.89 | - | | | | - | | | - | | | | - | | | - |
| V68 | Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)- | 0.41 | ± | 0.13 | - | | | | - | | | - | | | | - | | | - |
| V69 | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- | 3.25 | ± | 0.17 | - | | | | - | | | - | | | | - | | | - |
| V70 | Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl- | - | | | 0.00 | ± | 0.00 | | - | | | - | | | | - | | | - |
| V71 | Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- | 0.02 | ± | 0.00 | 0.10 | ± | 0.04 | | - | | | - | | | | - | | | - |
| V72 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- | 0.01 | ± | 0.00 | b | 0.11 | ± | 0.01 | a | - | | | - | | | - | | | <0.001 |
| V73 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- | 0.01 | ± | 0.00 | | - | | | | - | | | - | | | - | | | - |
| V74 | Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene)-, [1S-(1R*,9S*)]- | 0.06 | ± | 0.01 | b | 0.43 | ± | 0.06 | a | - | | | - | | | - | | | <0.001 |
| V75 | Butanoic acid, 3-hexenyl ester, (E)- | - | | | 22.79 | ± | 5.47 | a | - | | | - | | | 0.42 | ± | 0.06 | b | 0.0035 |
| V76 | Butanoic acid, 3-hexenyl ester, (Z)- | - | | | - | | | | - | | | - | | | 0.07 | ± | 0.01 | | - |
| V77 | Butanoic acid, heptyl ester | - | | | - | | | | - | | | - | | | 0.24 | ± | 0.02 | | - |
| V78 | Butanoic acid, hexyl ester | - | | | 2.72 | ± | 1.02 | a | - | | | - | | | 0.07 | ± | 0.02 | b | 0.0307 |
| V79 | Butylated Hydroxytoluene | - | | | - | | | | 0.64 | ± | 0.22 | - | | | - | | | | - |
| V80 | Camphene | 0.17 | ± | 0.01 | - | | | | - | | | - | | | - | | | | - |
| V81 | Caryophyllene | - | | | - | | | | - | | | - | | | 0.79 | ± | 0.11 | | - |
| V82 | cis-.beta.-Farnesene | - | | | 9.40 | ± | 4.30 | | - | | | - | | | - | | | | - |
| V83 | Copaene | - | | | 0.55 | ± | 0.10 | | - | | | - | | | - | | | | - |
| V84 | Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis- | - | | | 4.02 | ± | 3.31 | | - | | | - | | | - | | | | - |
| V85 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 0.62 | ± | 0.04 | - | | | | - | | | - | | | - | | | | - |
| V86 | D-Limonene | 5.60 | ± | 0.50 | a | 5.10 | ± | 1.91 | b | - | | | - | | | 0.51 | ± | 0.10 | c | 0.0153 |
| V87 | Decanal | 0.01 | ± | 0.00 | b | 0.07 | ± | 0.02 | ab | - | | | - | | | 0.08 | ± | 0.02 | a | 0.0301 |
| V88 | Decane | - | | | 0.55 | ± | 0.09 | | - | | | - | | | | - | | | - |
| V89 | Decanoic acid, methyl ester | - | | | - | | | | 0.16 | ± | 0.03 | - | | | - | | | | - |
| V90 | Dodecane | - | | | - | | | | 0.08 | ± | 0.02 | a | - | | 0.03 | ± | 0.01 | b | 0.0314 |
| V91 | Heptanal | - | | | - | | | | - | | | - | | | 0.12 | ± | 0.02 | | - |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V92 | Hexanoic acid, 2-ethyl-, methyl ester | 0.05 | ± 0.00 | | - | | | - | | | - | | | - | | | - | | |
| V93 | Hexanoic acid, 3-hexenyl ester, (Z)- | | - | | 0.32 | ± 0.10 | | | - | | | - | | | - | | | - | |
| V94 | Humulene | 4.02 | ± 0.28 | a | 0.80 | ± 0.11 | b | | - | | | - | | | 0.08 | ± 0.01 | c | <0.001 | |
| V95 | Indane | | - | | | - | | | - | | | 15.67 | ± 4.46 | | | - | | | - | |
| V96 | Methyl isovalerate | | - | | 7.54 | ± 3.24 | | | - | | | - | | | | - | | | - |
| V97 | n-Valeric acid cis-3-hexenyl ester | | - | | 1.02 | ± 0.43 | | | - | | | - | | | | - | | | - |
| V98 | Naphthalene | | - | | 0.71 | ± 0.13 | | | - | | | - | | | | - | | | - |
| V99 | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- | 2.83 | ± 0.49 | | | - | | | - | | | - | | | | - | | | - |
| V100 | Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | | - | | | - | | | - | | | - | | | 0.04 | ± 0.01 | | | - |
| V101 | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- | 2.86 | ± 0.43 | a | 0.81 | ± 0.28 | b | | - | | | - | | | | - | | | 0.0038 |
| V102 | Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- | 0.02 | ± 0.00 | | | - | | | - | | | - | | | | - | | | - |
| V103 | Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]- | | - | | 0.17 | ± 0.06 | | | - | | | - | | | | - | | | - |
| V104 | Nonanal | 0.04 | ± 0.00 | | | - | | 0.22 | ± 0.06 | | | - | | | 0.65 | ± 0.06 | | | <0.001 |
| V105 | Nonane, 5-(1-methylpropyl)- | | - | | | - | | | - | | | - | | | 0.08 | ± 0.01 | | | - |
| V106 | o-Cymene | 2.26 | ± 0.18 | | 0.54 | ± 0.23 | | | - | | | - | | | 0.15 | ± 0.02 | | | - |
| V107 | Octanal | | - | | | - | | | - | | | - | | | 0.39 | ± 0.06 | | | - |
| V108 | Octanoic acid, methyl ester | 0.06 | ± 0.00 | b | 0.28 | ± 0.04 | | 1.09 | ± 0.16 | a | | - | | | 0.02 | ± 0.01 | b | | <0.001 |
| V109 | Oxime-, methoxy-phenyl-_ | 0.47 | ± 0.10 | | 0.88 | ± 0.09 | | 3.50 | ± 1.85 | | 0.90 | ± 0.16 | | | | - | | | - |
| V110 | p-Cymene | | - | | 0.09 | ± 0.09 | | | - | | | - | | | | - | | | - |
| V111 | Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester | | - | | | - | | | - | | | - | | | 0.14 | ± 0.02 | | | - |
| V112 | Phenylethyl Alcohol | | - | | | - | | | - | | | - | | | 0.11 | ± 0.03 | | | - |
| V113 | Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester | | - | | | - | | | - | | | - | | | 0.40 | ± 0.05 | | | - |
| V114 | Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester | | - | | | - | | | - | | | - | | | 17.17 | ± 1.30 | | | - |
| V115 | Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester | | - | | | - | | | - | | | - | | | 20.30 | ± 1.49 | | | - |
| V116 | Propanoic acid, 2-methyl-, anhydride | | - | | | - | | | - | | | - | | | 0.07 | ± 0.02 | | | - |
| V117 | Propanoic acid, 5-hexen-1-yl ester | | - | | | - | | | - | | | - | | | 0.20 | ± 0.01 | | | - |
| V118 | Sulfurous acid, cyclohexylmethyl hexadecyl ester | | - | | | - | | | - | | | - | | | 0.67 | ± 0.05 | | | - |
| V119 | Sulfurous acid, dicyclohexyl ester | | - | | | - | | | - | | | - | | | 0.10 | ± 0.01 | | | - |
| V120 | Thujone | 0.03 | ± 0.01 | | | - | | | - | | | - | | | | - | | | - |
| V121 | trans-.beta.-Ocimene | 0.14 | ± 0.02 | | | - | | | - | | | - | | | | - | | | - |
| V122 | Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans- | | - | | 0.20 | ± 0.10 | | | - | | | - | | | | - | | | - |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V123 | Tricyclo[3.2.1.02,7]oct-3-ene, 2,3,4,5-tetramethyl- | 0.63 | ± 0.04 | | - | | - | | - | | - | |
| V124 | Undecane | | - | | - | 0.22 | ± 0.04 | | - | | - | |
| V125 | α-Muurolene | 1.58 | ± 0.24 a | 0.16 | ± 0.05 | | - | | - | 0.12 | ± 0.02 b | <0.001 |
| V126 | β-copaene | | - | | - | | - | | - | 0.08 | ± 0.02 | - |
| V127 | β-Cubebene | | - | | - | | - | | - | 0.05 | ± 0.01 | - |
| V128 | β-Dihydroagarofurane | | - | | - | | - | | - | 0.07 | ± 0.01 | - |
| V129 | β-Ocimene | | - | | - | | - | | - | 0.10 | ± 0.02 | - |
| V130 | γ-Muurolene | | - | | - | | - | | - | 0.04 | ± 0.01 | - |
| V131 | δ-Cadinene | | - | | - | | - | | - | 0.17 | ± 0.02 | - |
| V132 | Caryophyllene | 0.53 | ± 0.05 | 6.30 | ± 0.70 | | - | | - | | - | - |
| V133 | Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | | - | 0.05 | ± 0.02 | | - | | - | | - | - |

"-"—not detected.

## 4.5 The potential effect of the VOCs on the behavior of nymphs and adults of *P. spumarius*

The statistical analysis conducted on the potential effect of Volatile Organic Compounds (VOCs) on the behavior of *P. spumarius* nymphs and adults revealed interesting findings. The results are summarized in Table 5 for nymphs and Table 6 for adults.

Regarding *P. spumarius* nymphs, three VOCs were found to have a noticeable effect (Table 5). Specifically, 1-Heptanol and D-Limonene had a significant repellent effect, indicating that they deterred the nymphs from the tested environment. On the other hand, 1,7-Dioxaspiro[5.5]undecane had an attractive effect, implying that it attracted the nymphs towards the tested environment.

For *P. spumarius* adults, the VOCs emitted by plants also had a significant impact on their behavior (Table 6). Three volatile organic compounds were found to repel the adults: Butanoic acid, 3-hexenyl ester (E)-($P=0.0102$), D-Limonene ($P<0.001$), and β-Cubebene ($P=0.0397$). This suggests that these compounds acted as deterrents, causing the adults to avoid the tested environment. Conversely, 1,7-Dioxaspiro[5.5]undecane ($P<0.001$) was found to have an attractive effect on the adults, indicating that it lured them towards the tested environment.

These results highlight the influence of specific VOCs on the behavior of *P. spumarius* nymphs and adults. 1-Heptanol and D-Limonene acted as repellents for nymphs and adults, while 1,7-Dioxaspiro[5.5]undecane had an attractive effect on both life stages. For adults, in addition to 1,7-Dioxaspiro[5.5]undecane, three other VOCs (Butanoic acid, 3-hexenyl ester (E)-, D-Limonene, and β-Cubebene) were found to repel them.

**Table 5.** The results of the potential effects of the VOCs on the behavior of nymphs of *Philaenus spumarius*.

| | Estimate | S.E | Z value | P -value |
|---|---|---|---|---|
| (Intercept) | -0.16549 | 0.09901 | -1.671 | 0.09465 |
| V18 | -5.38078 | 1.98515 | -2.711 | 0.00672 |
| V28 | 1.43229 | 0.25192 | 5.685 | <0,001 |
| V86 | -0.38161 | 0.08339 | -4.576 | <0,001 |

**Table 6.** The results of the potential effects of the VOCs on the behavior of adults of *Philaenus spumarius*.

| | Estimate | S.E | Z value | P-value |
|---|---|---|---|---|
| (Intercept) | 1.841185 | 0.460006 | 4.003 | <0,001 |
| V28 | 0.703492 | 0.162822 | 4.321 | <0,001 |
| V36 | -0.051325 | 0.026464 | -1.939 | 0.0525 |
| V45 | -0.013223 | 0.007733 | -1.710 | 0.0873 |
| V75 | -0.031653 | 0.012328 | -2.568 | 0.0102 |
| V86 | -0.366415 | 0.077158 | -4.749 | <0,001 |
| V95 | -0.025287 | 0.014303 | -1.768 | 0.0771 |
| V109 | -0.059058 | 0.036763 | -1.606 | 0.1082 |
| V127 | -8.915954 | 4.334513 | -2.057 | 0.0397 |







# CHAPTER5

Discussion

The development of *P. spumarius* control strategies are critical for limiting *X. fastidiosa* spread. The majority of *P. summaries'* life cycle is spent in the spontaneous ground cover vegetation. Nonetheless, during the dry season, they seek refuge in major crop plants such as olive trees where the transmission of *X. fastidiosa* to insects through spontaneous ground cover vegetation and then to the crop plants once the bacteria are acquired, and the insects are infected (Cornara et al., 2017). That's why understanding *P. spumarius'* mechanisms for locating and selecting host plants is critical to develop control and management strategies.

In this study, we chose to evaluate the host plant preferences of *P. spumarius* nymphs and adults, as well as investigate the effect of these plants on the insects' development. However, the study of the impact of the four selected ground cover plant species on the morphological parameters of *P. spumarius* revealed that the plant species appear to have a significant impact on certain aspects of the morphology of these insects, while other aspects are unaffected. This could potentially indicate some form of adaptive evolution or could reflect differences in the physical or nutritional environments provided by the different plants. The findings from our study aligns with the findings from a study conducted by Wood & Jones, (2020) on Kent Island, a boreal island in the Bay of Fundy, who studied the influence of plants on the growth of insects, plant species altered the length of the insects' bodies. Indeed, the emerged insects from *Solidago rugosa* and *Anaphalis margaritacea* had larger body sizes than the other insects, also discovered that using fertilizers in a section of the field accelerated the development of the nymphs to the adult phase when compared to the other unfertilized section of the field.

Furthermore, our study observed a decrease in the choice of plants by *P. spumarius* nymphs as the water level increased, this can be explained by the fact that high water levels in xylem vessels lead to extremely diluted nutrients, requiring a great deal of work to extract, particularly organic nitrogen. The nitrogen concentration in a plant is one of plant properties that herbivores value the most. Nitrogen, on the other hand, is a key element in the growth of all living organisms due to its prominent involvement in all metabolisms, as well as cellular structure and genetic coding. Many creatures benefit from more Nitrogen in terms of health, growth, reproduction, and survival (Mattson, 1980).

*P. spumarius* are often associated with legumes plants, however, this is the first record of an insect-host interaction specific to nitrogen-fixing plants. Spittlebugs are likely drawn to nitrogen-fixing plants because they serve as a reasonably abundant, consistent source of organic nitrogen compounds for xylem-feeder insects (Thompson,

1994), which logically explains the decrease in plant choice when nutrients are diluted at high water levels.

Additionally, a study conducted by Huberty & Denno, (2004) revealed that chronical water stress, also, had a significant detrimental impact on the performance of sap-feeding insects. Overall, their findings call into question the long-held belief that herbivorous insects demonstrate enhanced performance and outbreak dynamics on water-stressed plants. Conversely, there is significant evidence that prolonged water stress also can affect many phytophagous insects, particularly sap-feeders (Huberty & Denno, 2004).

Despite what various scientists have earlier documented (Cascone et al., 2022; Rodrigues et al., 2022; Germinara et al., 2017), the olfactory response of *P. spumarius* adults to different plants is not greatly impacted by their sex. The olfactometer experiment revealed that the sex of *P. spumarius* has no effect on plant selection by adults of *P. spumarius*, however, the choice was affected mainly by the species of plants where, *S. tenerrimus* being the most preferred plant of *P. spumarius* when compared to *C. segetum*, *C. arvensis*, and the control, and *O. europea*.

According to Ranieri et al, (2016), spittlebugs have a wide range of responses to VOCs despite having a small number of antennal sensory structures. This demonstrates that spittlebugs use such cues to choose and locate their host plants. The profiles of the volatile organic compounds from the four chosen plants were identified and characterized. 110 VOCs were found after this identification, where *C. arvensis* had the highest number of VOCs and *S. tenerrimus* had the lowest number.

A study of the potential impact of VOCs on the behavior of nymphs and adults of *P. spumarius* was conducted after the identification of VOCs, and the results showed that there is in real sense, an interaction between VOCs and insects. The type of interactions between insects and the VOCs can have an attractive or repellent effect. However, in our work, we found that for nymphs, there was two repellent VOCs and one attractant, and for adults, three repellent VOCs and only one attractant.

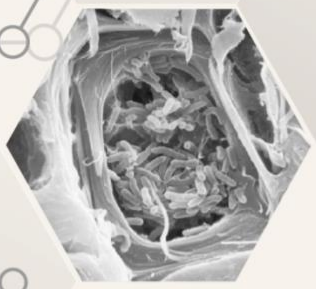
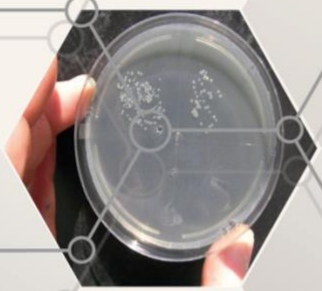
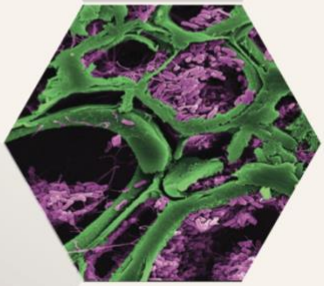
Understanding how *P. spumarius* interacts with its environment is critical for developing successful control measures. By adopting control measures for dry and rainy seasons, our results can aid refine present strategies in the fight against this insect's damage. Considering that plant selection diminishes naturally by the plant at high soil humidity levels, with outliers such as *S. tenerrimus*, where selection increases at high soil humidity levels, the tillage of the ground cover vegetation and killing the insects can be adjusted with the seasons and the water levels in the soil. The interaction of *Philaenus*

*spumarius* with VOCs, whether positive or negative, is also relevant for developing and implementing successful control approaches, such as the push-pull strategy, in which VOCs could be chosen and deployed as repellents that make the protected resource repulsive or unusable to the insect (push) and as trap-attractant to draw the insects toward an attractive source (pull), where the pests are then removed or killed. Which consists essentially of combining stimuli and (Cook et al, 2007).

It is also crucial to investigate what allows one plant to be preferred over another at a certain humidity level, as well as the various stimuli that influence insect choice, such as colors, xylem flow vibrations, and a variety of other factors.





# CHAPTER6

*Conclusion & future perspectives*

In this study, we investigated the host plant preferences of *P. spumarius* (meadow spittlebug) and their impact on the morphological parameters of the insect. Our findings verified that *S. tenerrimus* and *C. myconis* were the most preferred ground cover plants for *P. spumarius*. Interestingly, we observed that plant species did not significantly influence the morphological characteristics of *P. spumarius*.

Moreover, we established that the selection of host plants by *P. spumarius* is influenced by soil humidity levels over time. As water levels increase, the choice of plants by nymphs and adults decreases, likely due to the higher water content in the xylem vessels, which dilutes the nutrients and makes them more challenging to extract.

Furthermore, our research demonstrated that *P. spumarius* interacts with the volatile organic compounds (VOCs) emitted by the four plant species, both negatively and positively. This interaction between the insects and VOCs opens possibilities for developing innovative pest control strategies. Further investigation, such as studying the sensilla and electro-antennographic responses of *P. spumarius*, can contribute to a better understanding of the role of VOCs in host plant selection and the insect's interactions with its surroundings and neighboring plants.

By expanding our knowledge of the sensory mechanisms and responses of *P. spumarius*, we can develop more targeted and effective approaches to limit the population of this pest. Additionally, a deeper understanding of the role of VOCs in the choice of host plants can provide insights into the ecological dynamics between *P. spumarius* and its environment.

Future research should focus on investigating the specific mechanisms underlying the insect's responses to VOCs and exploring the potential for using VOCs as tools in integrated pest management strategies. This could lead to the development of novel control methods that are environmentally friendly and sustainable while helping to minimize the impact of *P. spumarius* on crops and vegetation.

# References


Abbott, A. ( 2016 ). Olive tree gridlock eases. News in Focus, 533 ,299-300.

Abbott, A. (2017). Italy rebuked in olive fiasco. News in Focus, 546, 193-194.

Abbott, A. (2018). Deadly olive-tree disease spreads. News in Focus, 563, 306-307.

Almeida, R. (2016). Can Apulia's olive trees be saved? Science, 353, 346- 348.

Almeida, R., Blua, M. J., Lopes, J. R. , & Purcell, A. H. (2005). Vector transmission of *Xylella fastidiosa*: applying fundamental knowledge to generate disease management strategies. Annals of the Entomological Society of America, 98(6), 775-786.

Almeida, R., & Nunney, L. (2015). How do plant diseases caused by *Xylella fastidiosa* emerge? Plant disease, 99(11), 1457–1467.

Anse, J. D., & Obradovic, A. (2010). Xylella fastidiosa: Its biology, diagnosis, control and risks. Journal of Plant Pathology, 92, 35-48.

Asteggiano, A., Franceschi, P., Zorzi, M., Aigotti, R., Bello, F. D., Baldassarre, F., Ciccarella, G. (2021). Hplc-hrms global metabolomics approach for the diagnosis of “olive quick decline syndrome” markers in olive trees leaves. Metabolites, 11(1), 1-15.

Avosani, S., Daher, E., Franceschi, P., Ciolli, M., Verrastro, V., & Mazzoni, V. (2020). Vibrational communication and mating behavior of the meadow spittlebug *Philaenus spumarius*. Entomologia Generalis, 40(3), 307-321.

Bodino, N., Cavalieri, V., Dongiovanni, C., Simonetto, A., Saladini, M. A., Plazio, E., Bosco, D. (2017). Dispersal of *Philaenus spumarius* (Hemiptera: Aphrophoridae), a Vector of *Xylella fastidiosa*, in olive grove and meadow agroecosystems. Environmental Entomology, 50(2), 267-279.

Bruce, J.A. , & Pickett, J.A. . (2011). Perception of plant volatile blends by herbivorous insects – finding the right mix. Phytochemistry, 72, 1605–1611.

Bruce, T., & John, A.P. (2011). Perception of plant volatile blends by herbivorous insects – finding the right mix. Phytochemistry, 72, 1605-1611.

Cascone, P., Quarto, R., Iodice, L., Cencetti, G., Formisano, G., Spiezia, G., Guerrieri, E. (2022). Behavioural response of the main vector of *Xylella fastidiosa* towards olive VOCs. Entomologia Generalis, 42, 35–44.

Cavalieri, V., Altamura, G., Fumarola, G., di Carolo, M. , Saponari, M. , Cornara, D. , & Dongiovanni, C. (2019). Transmission of Xylella fastidiosa subspecies pauca sequence type 53 by different insect species. Insects, 10, 324.

Colella, C., Carradore, R., & Cerroni, A. (2019). Problem setting and problem solving in the case of olive quick decline syndrome in Apulia, Italy: A sociological approach. Phytopathology, 09(2), 187-199.

Cook, S.M., Khan, Z.R., & Pickett, J.A. (2007). The use of push-pull strategies in integrated pest management. Annual Revews Entomology, 52(375-400).

Cornara, D. , Bosco, D. , & Fereres, A. (2018). *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. Journal of Pest Science, 91, 957-972.

Cornara, D., Marra, M., Morente, M., Garzo, E., Moreno, A., Saponari, M., & Fereres, A. (2020). Feeding behavior in relation to spittlebug transmission of *Xylella fastidiosa*. Journal of Pest Science, 93(4), 1197–1213.

Cornara, D., Saponari, M., Zeilinger, A.R., de Stradis, A., Boscia, D., Loconsole, G., . . . Porcelli, F. (2017). Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. Journal of Pest Science, 90, 521-530.

Craig, T.P. , & Ohgushi, T. . (2002). Preference and performance are correlated in the spittlebug aphrophora pectoralis on four species of willow. Ecological Entomology, 27, 529–540.

Cruz, A.C., Luvisi, A. , De Bellis, L., & Ampatzidis, Y. (2017). X-FIDO: an effective application for detecting olive quick decline syndrome with deep learning and data fusion. Frontiers in Plant Science, 8, 01741.

De Lima, J. E. O., Miranda, V. S., Hartung, J. S., Brlansky, R. H. , Coutinho, A., Roberto, S. R., & Carlos, E. F. (1998). Coffee leaf scorch bacterium: axenic culture, pathogenicity, and comparison with *Xylella fastidiosa* of citrus. Plant disease, 82(1), 94-97.

Delong, D.M., & Severin, H.H.P. (1950). Spittle-insect vectors of Pierce's disease virus. I. characters, distribution, and food plants. Journal of Agricultural Science, 19, 339-357.

Denancé, N., Legendre, B. , Briand, M. , Olivier, V., & Jacques, M.A. (2017). Several subspecies and sequence types are associated with the emergence of *Xylella fastidiosa* in natural settings in France. Plant Pathology, 66(7), 1054–1064.

Di Serio, F., Bodino, N., Cavalieri, V., Demichelis, S., Di Carlo, M., Dongiovanni, C., Bosco. (2019). Collection of data and information on biology and control of vectors of *Xylella fastidiosa*. EFSA Journal, 16(5), 1628.

Dongiovanni, C., Cavalieri, V., Bodino, N., Tauro, D., Di Carolo, M., Fumarola, G., & Bosco, D. (2019). Plant selection and population trend of spittlebug immatures (Hemiptera: Aphrophoridae) in olive groves of the Apulia region of Italy. Journal of Economic Entomology, 112(1) , 67-74.

Dormann, C.F, Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Lautenbach, S. (2013). Collinearity: a review of methods to deal with it and simulation study evaluating their performance. Ecography, 36, 027-046.

EFSA (European Food Safety Authority). (2013). Statement of EFSA on host plants, entry and spread pathways and risk reduction options for *Xylella fastidiosa* Wells et al. EFSA Journal, 11(11), 3468.

EFSA (European Food Safety Authority). (2015). Scientific opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal, 13(1), 3989.

EFSA (European Food Safety Authority). (2018). Scientific report on the update of the *Xylella* spp. host plant database. EFSA Journal, 21(1), 7726.

EPPO. (2022). Récupéré sur https://gd.eppo.int/taxon/XYLEFA/distribution/ES

European commission. (2020). Producing 69% of the world's production, the EU is the largest producer of olive oil. Agriculture and rural development.

Frem, M., Champan, D., Fucilli, V., Choueiri, E., El Moujabber, M., La Notte, A., & Nigro, F. (2020). *Xylella fastidiosa* invasion of new countries in Europe, the middle east and north africa: ranking the potential exposure scenarios. NeoBiota, 59, 77–97.

Ganassi, S., Cascone, P., Di Domenico, C., Pistillo, M., Formisano, G., Giorgini, M., Guerrieri, E. (2020). Electrophysiological and behavioural response of *Philaenus spumarius* to essential oils and aromatic plants. scientific Reports, 10, 3114.

Germinara, G.S., Ganassi, S., Postillo, M., Di Domenico, C., DE Cristifaro, A., & DI Palma, A.M. (2017). Antennal olfactory responses of adult meadow spittlebug, *Philaenus spumarius*, to volatile organic compounds (VOCs) Giacinto. Plos one, 12(12), e0190454.

Goheen, A.C., Nyland, G., & Lowe, S.K. (1973). Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology, 63, 341-45.

Guzmán, G. , Cabezas, J. M. , Sánchez-Cuesta, R., Lora., Bauer, T., Strauss, P., Gómez, J. A. (2019). A field evaluation of the impact of temporary cover crops on soil properties and vegetation communities in southern Spain vineyards. Agriculture, Ecosystems and Environment, 272, 135–145.

Halkka, 0. (1964). Geographical, spatial and temporal variability in the balanced polymorphism of *Philænus spumarius*, 19, 383-401.

Halkka, O. (1962). Equilibrium populations of *Philænus spumarius* L. . Nature International Journal of Science, 193(4810), 93-94.

Hamilton, K.G.A. . (1982). The spittlebugs of Canada, Homoptera: Cercopidae. Insects and arachnids of Canada. Agriculture Canada publication, 10 , 102.

Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). Paleontological statestics software package for education and data analysis. Palaeontologia Electronica, 4(1), 4-9.

Hopkins, D. (1989). Xylem-limited bacterial pathogen of plants. Annual Review of Pathology, 27, 271-290.

Hopkins, D.L. (1982). Relation of Pierce's disease bacterium to a wilt-type disease in citrus in the greenhouse. Phytopathology, 72, 1090-92.

Horsfield, D. (1978). Evidence for xylem feeding by *Philaenus spumarius*(L.) (Homoptera: Cercopidae). Entomologia Experimentalis et Aplicata, 24, 95-99.

Huberty, A.F., & Denno, R.F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. The Ecological Society of America, 85(5), 1383-1398.

Huberty, A.F., & Denno, R.F. (2004). Plant water stress and its consequences for herbivorous insects: a new sythesis. Ecology, 35(5), 1383-1398.

Janse, J. D., & Obradovic, A. . (2010). *Xylella fastidiosa*: its biology, diagnosis, control and risks. Journal of Plant Pathology, S35-S48.

Janse, J., & Obradovic, A. (2015). Società Italiana di patologia Vegetale ( SIPaV ). Journal of Plant Pathology, 80(2), 85–103.

Leu, L., & Su, C.C. (1993). Isolation, cultivation, and pathogenicity of *Xylella fastidiosa*, the causal bacterium of pear leaf scorch disease in Taiwan. Plant Disease, 77(6) , 642.

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. Journal of Statestical Software, 25(1), 1-18.

Liccardo, A., Fierro, A., Garganese, F., Picciotti, U., & Porcelli, F. (2020). A biological control model to manage the vector and the infection of *Xylella fastidiosa* on olive trees. Plos One, 15(4).

Luvisi, A., Nicolì, F. , & De Bellis, L. (2017). Sustainable management of plant quarantine pests: The case of olive quick decline syndrome. Sustainability (Switzerland), 9(4), 659.

Martelli, G.P., Boscia, D., & Porcelli, F. (2016). The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency. European Journal of Plant Pathology, 144, 235–243.

Mattson, W.J. (1980). Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics, 11(1), 119-161.

McGavin, G. (2001). Essential entomology: An order-by-order introduction. Oxford University Press, 20.

Nuti, M., Giovannetti, G., Scortichini, M., Pergolese, G., Sarcino,M., & Doveri, G. (2021). The olive quick decline syndrome: A syndemic outbreak in the Apulia region, southern Italy. Journal of Agronomy Research, 3(3):1, 3-25.

Ranieri, E., Ruschioni, S., Riolo, P., Isidoro, N., & Romani, R. (2016). Fine structure of antennal sensilla of the spittlebug Philaenus spumarius L. (Insecta: Hemiptera: Aphrophoridae). I. Chemoreceptors and thermo-/hygroreceptors. Arthropod Structure & Development, 45(5), 432-439.

Retchless, A.C., Labroussaa, F., Shapiro, L., Stenger, D.C., Lindow, S.E., & ALmeilda, R.P.P. (2014). Genomic insights onto Xylella fastidiosa interactions with plant and insect hosts. Genomics of Plant-Associated Bacteria, 177-202.

Rodrigues, I., Benhadi- Marín, J., Rodrigues, N., Baptista, P., & Pereira, J.A. (2022). Olfactory responses to volatile organic compounds and movement parameters of Philaenus spumarius and Cicadella viridis. Journal of Applied Entomology, 1–12.

Rodrigues, I., Benhadi- Marín, J., Rodrigues, N., Baptista, P., & Pereira, J.A. (2022). Olfactory responses to volatile organic compounds and movement parameters of Philaenus spumarius and Cicadella viridis . Journal of Applied Entomology, 1-12.

Sabella, E., Aprile, A., Genga, A., Siciliano, T., Nutricati, E., Nicolì, F., & Luvisi, A. . (2019). Xylem cavitation susceptibility and refilling mechanisms in olive trees infected by Xylella fastidiosa. Scientific Reports, 9(9602).

Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca,S., D'attoma,G., . . . Martelli, G.P. (2017). Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. Scientific Reports, 7, 1-13.

Saponari, M., Boscia, D., Nigro, F., & Martelli, G.P. (2013). Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). Journal of Plant Pathology, 95, 668.

Saponari, M., Loconsole, G., Cornara, D., Yolomi, R.K., DE Stradis, A., Boscia, D., & Porcelli. F. (2014). Infectivity and transmission of *Xylella fastidiosa* by *Philænus spumarius* (hemiptera: Aphrophoridae) in Apaulia, Italy. Journal of Economic Entomology, 107(4), 1316-1319.

Scortichini, M. (2004). *Xylella fastidiosa*. European And Mediterranean Plant Protection Organization Bulletin, 34(2), 187-192.

Seabra, S. G. , Pina-Martins, F. , Marabuto, E., Yurtsever, S. , Halkka, O. , Quartau, J. A. , & Paulo, O. S. (2020). Corrigendum to "Molecular phylogeny and DNA barcoding in the meadow-spittlebug *Philaenus spumarius* (Hemiptera, Cercopidae) and its related species". Molecular Phylogenetics and Evolution, 56, 462-467.

Serio, F. D. , Bodino, N., Cavalieri, V., Demichelis, S. , Carolo, M. D. , Dongiovanni, C., & Bosco, D. (2019). Collection of data and information on biology and control of vectors of *Xylella fastidiosa*. EFSA Supporting Publications, 16(5), 1628.

Sevarika, M., Rondoni, G., Ganassi, S., Pistillo, O.M., Germinara, G.S., De Cristofaro, A., Conti, E. (2022). Behavioural and electrophysiological responses of *Philaenus spumarius* to odours from conspecifics. Scientific Reports, 12, 8402.

Sicard, A., Zeilinger, A.R., Vanhove, M., Schartel, T.E., Beal, D.J., Daugherty, M.P., & Almeida, R.P. (2018). *Xylella fastidiosa*: insights into an emerging plant pathogen. Annual Review Phytopathology, 56, 181–202.

Stancanelli, G., Almeida, R., Bosco, D., Caffier, D., Czwienczek, E. , Gregoire, J. C. , & Bragard, C. (2015). Assessing the risk posed to plant health by *Xylella fastidiosa* in the European Union. CIHEAM International Center for Advanced Mediterranean Agronomic Studies, Watch Letter, 33, 1-8.

Thompson, V. (1994). Spittlebug indicators of nitrogen-fixing plants. Ecological entomology, 19(4), 391-398.

Tumlinson, J. (2014). The Importance of volatile organic compounds in ecosystem functioning. Journal of Chemestry Ecolology, 40, 212–213.

Villa, M., Rodrigues, I., Baptista, P., Fereres, A., & Pereira, J.A. (2020). Populations and host/non-host plants of spittlebugs nymphs in olive orchards from northeastern Portugal. Insects, 11.

Vitousek, P., D'antonio, C., Loope, L., & Westbrooks, R. (2014). Biological invasions as global environnementale change. American Scientist, 468-478.

Wells, J.M. , Raju, B.C. , Hung, H.Y., Weisburg, W.G., Mandelco-Paul, L. , & Brenner, D.J. . (1987). *Xylella fastidiosa* gen. nov., sp. nov. Gram-negative, xylem-limited, fastidious plant bacteria related to Xanthomonas spp. International Journal of Systematic Bacteriology, 37, 136–143.

Wood, Z.M., & Jones, P.L. (2020). The effects of host plant species and plant quality on growth and development in the meadow spittlebug (*Philaenus spumarius*) on kent island in the bay of fundy. Northeastern Naturalist, 27(1), 168-185.

Yurtsever, S. (2000). On the polymorphic meadow spittlebug, *Philaenus spumarius* (L.) (Homoptera: Cercopidae). Turkish Journal of Zoology, 24, 447-459.

# Supplementary documents

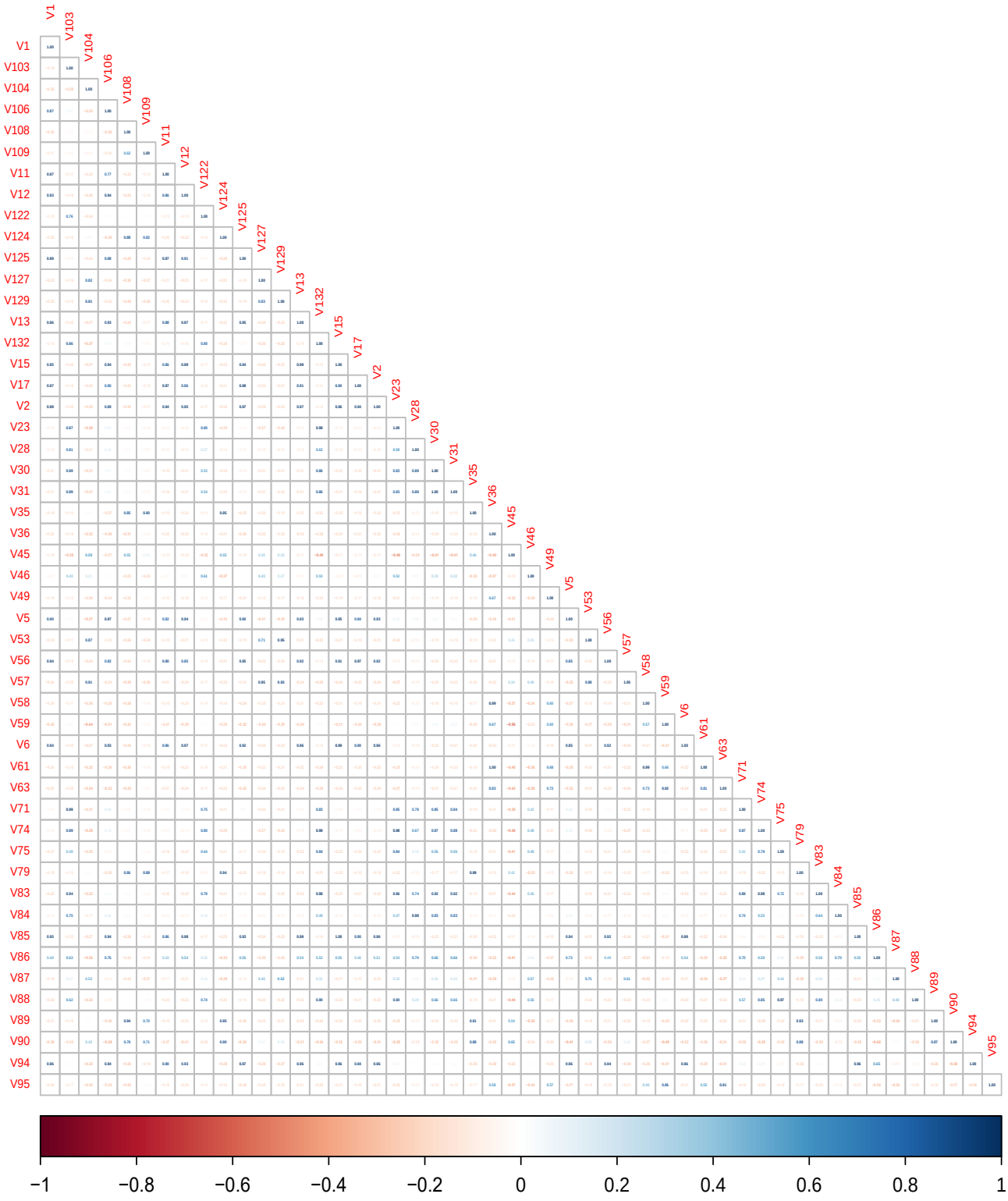



**Figure S 1.** The corrplot from the Pearson correlations used to study the relationship between the different volatile organic compounds emitted by *Calendula arvensis*, *Chrysanthemum segetum* , *Coleostephus myconis* and *Sonchus tenerrimus* to study their potential effect on the *Philaenus spumarius* nymphs.


**Figure S 4.** The corrplot from the Pearson correlations used to study the relationship between the different volatile organic compounds emitted by *Calendula arvensis*, *Chrysanthemum segetum* , *Coleostephus myconis*, *Olea europea* and *Sonchus tenerrimus* to study their potential effect on the *Philaenus spumarius* adults.