



Seasonal olfactory response of *Philaenus spumarius* (Hemiptera: Aphrophoridae) towards traditional Portuguese olive cultivars

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ABSTRACT

In Portugal, the strength of the olive sector relies on the great genetic heritage of traditional olive cultivars. However, this genetic heritage is threatened by the causal agent of olive quick decline syndrome (OQDS), the phytopathogenic bacteria *Xylella fastidiosa*, reported for the first time in 2019 in the country. This vector-borne pathogen is transmitted by xylem-feeding insects such as *Philaenus spumarius* the main European vector. Since there is no cure for this pathogen, the implementation of an integrated approach against the vectors should be considered to prevent and limit the spread of *X. fastidiosa*. In this sense, an in-depth assessment of the host plant preferences of the main European vector of *X. fastidiosa* is crucial to understand their seasonal dynamics towards olive cultivars to determine the most susceptible to vector attack. This work aimed to assess the olfactory response of *P. spumarius*, to five traditional Portuguese olive cultivars: “Cobrançosa”, “Negrinha de Freixo”, “Santulhana”, “Madural”, and “Verdeal Transmontana” in two separate seasons (Spring and Autumn). Our results showed that *P. spumarius* presented significantly different olfactory responses toward the different cultivars under study. In Spring, females and males were significantly attracted to “Negrinha de Freixo”; in Autumn, females were significantly attracted to “Cobrançosa”. In general, the olfactory response toward the five cultivars was sex-dependent and could be related to the volatile composition. In Spring, the cultivar “Negrinha de Freixo” can be more susceptible to *P. spumarius* and that the olfactory response towards the olive cultivars can vary throughout the life cycle of the vector.

1. Introduction

The Olive Quick Decline Syndrome (OQDS) is a severe plant disease that has been spreading through southern Italy, where it is currently responsible for the death of thousands of olive trees, seriously affecting the olive production and economy of the country (Saponari et al., 2019). In 2013, Saponari et al. (2013) determined that the etiological agent responsible for this disease is the plant pathogenic bacterium native to the Americas, *Xylella fastidiosa* Wells et al. (1987) (Xanthomonadales: Xanthomonadaceae) (Wells et al., 1987).

Once this gram-negative bacterium infects the plant, it moves and multiplies within the xylem vessels, creating a biofilm, which leads to the consequent obstruction of the vessels, blocking the passage of water and soluble mineral nutrients (Janse and Obradovic, 2010). Typical symptoms of infection are drying, scorching, wilting of the foliage, and eventually plant death (EFSA, 2013). However, these symptoms can

vary according to the host plants, the bacteria subspecies involved as well as the climatic conditions of the infected region (EFSA, 2013).

Xylella fastidiosa is transmitted to plants exclusively by insects of the infraorder Cicadomorpha that feed on the xylem (Almeida et al., 2005; Hill and Purcell, 1995; Krugner et al., 2019). In Europe, the meadow spittlebug, *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera, Aphrophoridae), was identified as the main vector (Cornara et al. 2017a, 2017b; Saponari et al., 2014). In addition to being widely abundant and distributed throughout Europe (Rodrigues et al., 2014), this insect also has higher bacterial transmission rates than other European vectors (Cavaliere et al., 2019). In olive groves, *P. spumarius* spend most of their life cycle in the herbaceous vegetation cover; however, at the end of the Spring/early Summer, due to mowing or a decrease in the succulence of herbaceous vegetation cover, they move to the olive canopy where they feed and if infected with *X. fastidiosa*, the transmission of the bacteria to the olive tree may then occur (Cornara et al. 2017a, 2019; Dongiovanni

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et al., 2019; Morente et al., 2018; Villa et al., 2020).

In Portugal, the presence of *X. fastidiosa* was first reported in 2019, and since then, new outbreaks have been identified (DGAV, 2021). Although these outbreaks, so far, are confined to ornamental plants (DGAV, 2021), there is a concern that this pathogen may spread to olive groves leading to devastating economic and environmental problems, threatening the olive tree cultivation in Portugal, which holds a remarkable ancient history and tradition, being one of the most emblematic and economically important crops, with a great genetic heritage (Moreira and Veloso, 2009; Rodrigues et al., 2022b).

Since there is no treatment for the OQDS, the only current approaches to control its dissemination are destroying infected trees and managing the vector population (European Union, 2015). Indeed, vector control is identified as the main tool to limit the spread of the disease (Schneider et al., 2020). Understanding the ecology and the seasonal olfactory response of the main European vector for traditional Portuguese olive cultivars is crucial to protect the genetic heritage.

Philaenus spumarius is known to interact socially via substrate-borne vibrations (Avosani et al., 2020), whereas some studies suggest that it can also communicate via pheromones (Sevarika et al., 2022a,b). Furthermore, electrophysiological assays showed that *P. spumarius* can respond to volatile organic compounds (Anastasaki et al., 2021; Germinara et al., 2017). Moreover, behavioral responses showed that different aromatic plants and essential oils could repel or attract insects (Ganassi et al., 2020). Rodrigues et al. (2022a) described that females of *P. spumarius* were significantly attracted to lower concentrations of cis-3-hexenyl acetate and cis-3-hexen-1-ol, two volatile organic compounds commonly found in the olive tree. Cascone et al. (2022) reported that females of *P. spumarius* were attracted by the olive cultivars “Ogliarola”, “Rotondella”, and “Frantoio”, and repelled by “FS-17”, while males didn’t present any olfactory response to different cultivars. The conclusions of these studies support that, although *P. spumarius* has significantly fewer antennal sensilla compared to other species (Ranieri et al., 2016), it can use semiochemical cues to choose and locate host plants.

In this work, we evaluate the olfactory response of *P. spumarius*, to five different Portuguese olive cultivars: “Cobraçosa”, “Negrinha de Freixo”, “Santulhana”, “Madural”, and “Verdeal Transmontana” in Spring (after the emergence of the adults) and Autumn (end of the life cycle of the vector). Understanding cultivar-specific attractiveness can help identify less preferred varieties for planting in high-risk areas, or more attractive varieties for use in monitoring and trap cropping strategies, thereby contributing to integrated management of *P. spumarius* populations and reducing the spread of *X. fastidiosa* in olive groves.

2. Material and methods

2.1. Plants

Three healthy olive trees belonging to five important traditional Portuguese olive cultivars were selected (i.e., “Cobraçosa”, “Negrinha de Freixo”, “Santulhana”, “Madural”, and “Verdeal Transmontana”) and grown in plastic pots (20 cm in height × 11 cm in length). The plants were placed in rearing chambers at 25 ± 1 °C, with 50–60 % relative humidity and a 16:8 h (L:D) photoperiod. All the olive trees were two-year-old and similar in size (approximately 50 cm in height). No pesticide, fungicide, or fertilizer was used in the rearing of the plants.

2.2. Collection of the insects

Adults of *P. spumarius* were collected with an entomological sweep net during Spring and Autumn of 2020 in the herbaceous ground vegetation of an olive grove in Bragança (41°48'10.1"N 6°44'50.9"W). The adults collected were sexed using a binocular stereoscopic microscope and transferred onto different cages (40 cm in height, 30 cm in length, and 43 cm in width) with *Lavandula* sp. plants. The cages were

then placed under controlled conditions (18 ± 1 °C, 65–70 % relative humidity, and a 16:8 h (L:D) photoperiod).

2.3. Olfactometer bioassays

The seasonal olfactory response of *P. spumarius* to the different olive cultivars was assessed using a custom-built eighth chamber olfactometer (Fig. 1). This apparatus was designed and constructed entirely in our laboratory from transparent acrylic sheets (5 mm thickness), following the specifications described below. The outside arena was designed in a square shape (40 cm × 40 cm); in the centre of this arena, a circular arena (18 cm in diameter) was placed. The circular arena was divided into eight chambers, isolated with white PVS tape (Tesaflex® 53988, Tesa SE, Norderstedt, Germany) to eliminate visual interference between chambers. Each chamber included a 1 cm × 1 cm aperture to allow insect entry and was individually connected to a 250 mL gas washing bottle filled with 100 mL of distilled water and activated charcoal (AppliChem, Panreac ITW®). This setup served to purify and humidify the airflow, which was supplied by low volume air pumps at a rate of $12 \text{ cm}^3 \text{ min}^{-1}$ (Sera GmbH, Heinsberg, Germany). The olfactometer apparatus featured an acrylic lid with removable flaps at each corner, allowing for the insertion of insects. All joints and connections were sealed with an odourless transparent adhesive sealant (Poly Max® Crystal Express, 115 g, UHU, Bühl, Germany) to prevent air leakage.

The study was conducted during two seasonal periods: Spring, corresponding to the emergence of adult insects, and Autumn, marking the end of the vector’s life cycle. During each season, the olive cultivars listed in section “2.1 Plants” were tested across two trials. In the first trial, the olfactory responses of *P. spumarius* were assessed using the cultivars ‘Madural’, ‘Cobraçosa’, and ‘Verdeal Transmontana’. In the second trial, the most frequently chosen cultivar from the first trial was tested against two additional cultivars: ‘Negrinha de Freixo’ and ‘Santulhana’.

Overall, ‘Cobraçosa’ was the cultivar most frequently chosen by *P. spumarius*, except in the case of males during Autumn, who also preferred ‘Verdeal Transmontana’. Consequently, for statistical analyses, ‘Cobraçosa’ was selected as the reference cultivar.

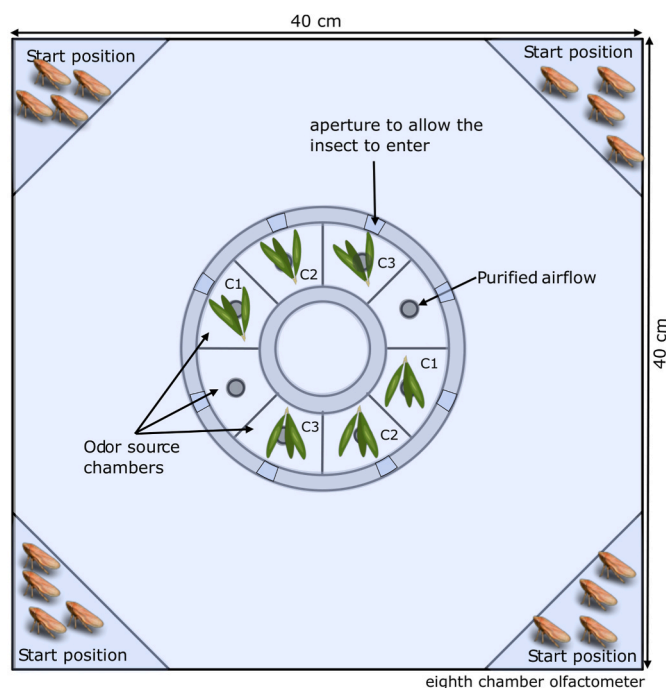


Fig. 1. Schematic drawing of the eight chamber olfactometer built to conduct the olfactometric assays (not drawn to scale).

In each season and trial, three healthy leaves of the same cultivar were placed in two opposite chambers. The petiole of the leaves was protected with aluminium foil to avoid the potential release of volatile compounds related to tissue damage. The remaining two chambers operated as control (purified airflow).

After placing the leaves of the cultivars in the respective chambers, the olfactometer apparatus was closed. Then, sixteen insects, previously kept for 2 h in the absence of food and odors, were equally divided and released into each corner of the olfactometer arena. The olfactory response of the insects was recorded 30 min after their release in the arena. This time point was selected based on a series of preliminary trials conducted prior to the main experiments, which showed that insect responsiveness to plant volatiles was highest around 30 min after release. These pre-tests helped to determine the optimal delay to ensure sufficient accumulation and dispersion of volatiles within the olfactometer chambers, thereby maximizing the behavioural response. Twenty replicates for males and females were performed separately (a total of 320 Males and 320 Females were used). At each repetition, fresh leaves of each cultivar were used. To avoid directional bias, the olfactometer chambers were rotated, and the entire olfactometer was cleaned between trials using neutral soap, rinsed with 70 % ethanol, followed by a final rinse with distilled water.

2.4. Volatile characterization

The volatile profile of five olive cultivars was assessed in spring and autumn using HS-SPME (headspace solid-phase microextraction) coupled with GC/MS (gas chromatography with mass spectrometry) (Shimadzu, Japan). For that, 4 g of healthy leaves from each cultivar were placed in individual 50 mL vials, with the leaf petiole carefully shielded with aluminium foil. Subsequently, the vials were sealed using polypropylene caps equipped with silicon septa. To serve as an internal standard, 5 μ L of 4-methyl-2-pentanol (0.127 mg mL^{-1}) from Sigma Aldrich, USA, was added to each vial with a syringe. The volatiles were released in a water bath at $40 \text{ }^\circ\text{C}$ for 5 min. Following this, a Divinylbenzene/Carboxen/Polydimethylsiloxane-coated fiber (DVB/CAR/PDMS 50/30 μm) from Supelco, Bellefonte, USA, was introduced into the headspace for volatile adsorption. The exposure time was 40 min. Five replicates of HS-SPME analyses were performed for each cultivar. The GC-MS conditions used were the same as those previously described by Rodrigues et al. (2022a).

2.5. Data analysis

Generalized Linear Models (GLMs) with Poisson distribution were used to compare the olfactory response of *P. spumarius* towards the different olive cultivars per trial and season. Sex, cultivar (first trial: “Cobrançosa”, “Madural”, “Verdeal Transmontana”, and Control; second trial: “Cobrançosa”, “Negrinha de Freixo”, “Santulhana”, and Control), and their interaction were used as explanatory variables. The number of individuals that chose the different cultivars was assessed per trial and season and compared using a post hoc pairwise *t*-test ($\alpha = 0.05$). The models were developed in R using the *glm* function (R Core Team, 2022).

To assess differences in volatile profiles among cultivars and seasons, a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using the “adonis2” function from the “vegan” package (Oksanen et al., 2019). Additionally, an Analysis of variance (ANOVA) was performed using the software PAST v.4.03 (Hammer et al., 2001) to compare the volatile profiles among olive cultivars across seasons. A Principal Component Analysis (PCA) was subsequently performed in R software v.3.5.1 (R Core Team, 2022) using the “pca” function from the “FactoMineR” package (Le et al., 2008). The correlation biplot of the first two Principal Components (PCs) was generated using the “fviz_pca_biplot” function from the “factoextra” package (Kassambara and Mundt, 2020).

3. Results

In all seasons and trials, the olfactory response of *P. spumarius* differed significantly among cultivars (Table 1). In Spring, during the first trial, both females and males were significantly more attracted to the cultivar ‘Cobrançosa’ ($p < 0.001$, Fig. 2a). However, this pattern was not observed in the second trial, where the insects were tested against ‘Cobrançosa’, ‘Santulhana’, ‘Negrinha de Freixo’, and a control. In this case, *P. spumarius* showed a significant preference for ‘Negrinha de Freixo’ ($p < 0.001$, Fig. 2a). In Autumn, females were significantly attracted to “Cobrançosa” in both trials ($p < 0.001$, Fig. 3a). Conversely, males were significantly attracted to “Cobrançosa” and “Verdeal Transmontana” in the first trial, but in the second trial, they chose “Negrinha de Freixo” more often than the Control (purified air) ($p < 0.001$, Fig. 3b).

Moreover, the interaction between cultivar and sex significantly affected the behavior of the insects, except in Spring when the insects were exposed to the cultivars “Cobrançosa”, “Santulhana”, and “Negrinha de Freixo” and to the stream of purified air (Table 1).

In total, 78 compounds were identified across two seasons and five cultivars (Table 2).

During spring, 58 volatile organic compounds (VOCs) were identified. The “Santulhana” cultivar exhibited the highest number of VOCs (33), followed by “Negrinha de Freixo” and “Verdeal Transmontana”, each with 31 VOCs, and finally, “Cobrançosa and Madural”, each with 30 volatiles. In Autumn, 77 VOCs were identified. “Negrinha de Freixo” and “Verdeal Transmontana” showed the highest number of VOCs with 24, followed by “Madural”, “Cobrançosa”, and “Santulhana” with 23, 22, and 18 VOCs, respectively.

3-Hexen-1-ol, (Z)- and 3-Hexen-1-ol, acetate, (Z)- were consistently found in all cultivars across both seasons, exhibiting elevated percentages. Similarly, Nonanal was present in every cultivar and season, albeit in relatively lower percentages. D-Limonene, on the other hand, was exclusively identified during Spring season, except for the “Negrinha de Freixo” cultivar.

According to PERMANOVA and PCA analyses, the volatile profile exhibited significant differences between the two seasons and cultivars (Table 3, Fig. 4).

4. Discussion

In Portugal, the strength of the olive sector relies on the high diversity of traditional and autochthonous cultivars (Rodrigues et al., 2020, 2023). However, the introduction of the phytopathogenic bacterium *X. fastidiosa* in the country and its rapid spread threaten this diversity. The scenario observed in southern Italy represents an example of the detrimental impacts associated with this bacterium Saponari et al. (2019).

Several studies started to assess the tolerance and resistance of olive cultivars to *X. fastidiosa*; however, these studies are mainly focused on a few olive cultivars grown in the infected Italian area (e.g., Boscia et al., 2017; Giampetrucci et al., 2016; Baù et al., 2017; Ranieri et al., 2016). So far, only the cultivars FS17 and Leccio showed resistance towards *X. fastidiosa* subspecies *pauc* ST53 (Boscia et al., 2017; EFSA, 2013). The tolerance and resistance of autochthonous cultivars from countries other than Italy are still unknown. An in-depth assessment of the host plant preferences of the main European vector of *X. fastidiosa* is crucial for understanding the seasonal dynamics of a specific plant, identifying the olive cultivars most susceptible to vector attack in our case, and implementing appropriate prevention and control measures against the spread of *X. fastidiosa*.

Although *P. spumarius*, is a polyphagous insect that feeds on the xylem sap of a wide repertoire of host plants (Cornara et al., 2018), several studies showed that polyphagous insects tend to show preferences for specific plant species or growth stages (e.g., Barman et al., 2010; Jackson et al., 2008; Kennedy and Margolies, 1985; Liu et al.,

Table 1

Results of the GLMs developed for the effect of the cultivar and sex, as well as their interaction, on the number of individuals of *Philaenus spumarius* in an eight-chamber olfactometer.

Season	Independent variable	Response variable	First trial			Second trial		
			df	χ^2	P	df	χ^2	P
Spring	Cultivar	Number of individuals	3	95.5	<0.001	3	171	<0.001
	Sex		1	0.1	0.73	1	0.1	0.80
	Cultivar: Sex		3	7.5	0.04	3	0.6	0.89
Autumn	Cultivar	Number of individuals	3	11.3	<0.001	3	13.44	0.003
	Sex		1	1.1	0.29	1	1.63	0.20
	Cultivar: Sex		3	52.8	<0.001	3	21.50	<0.001

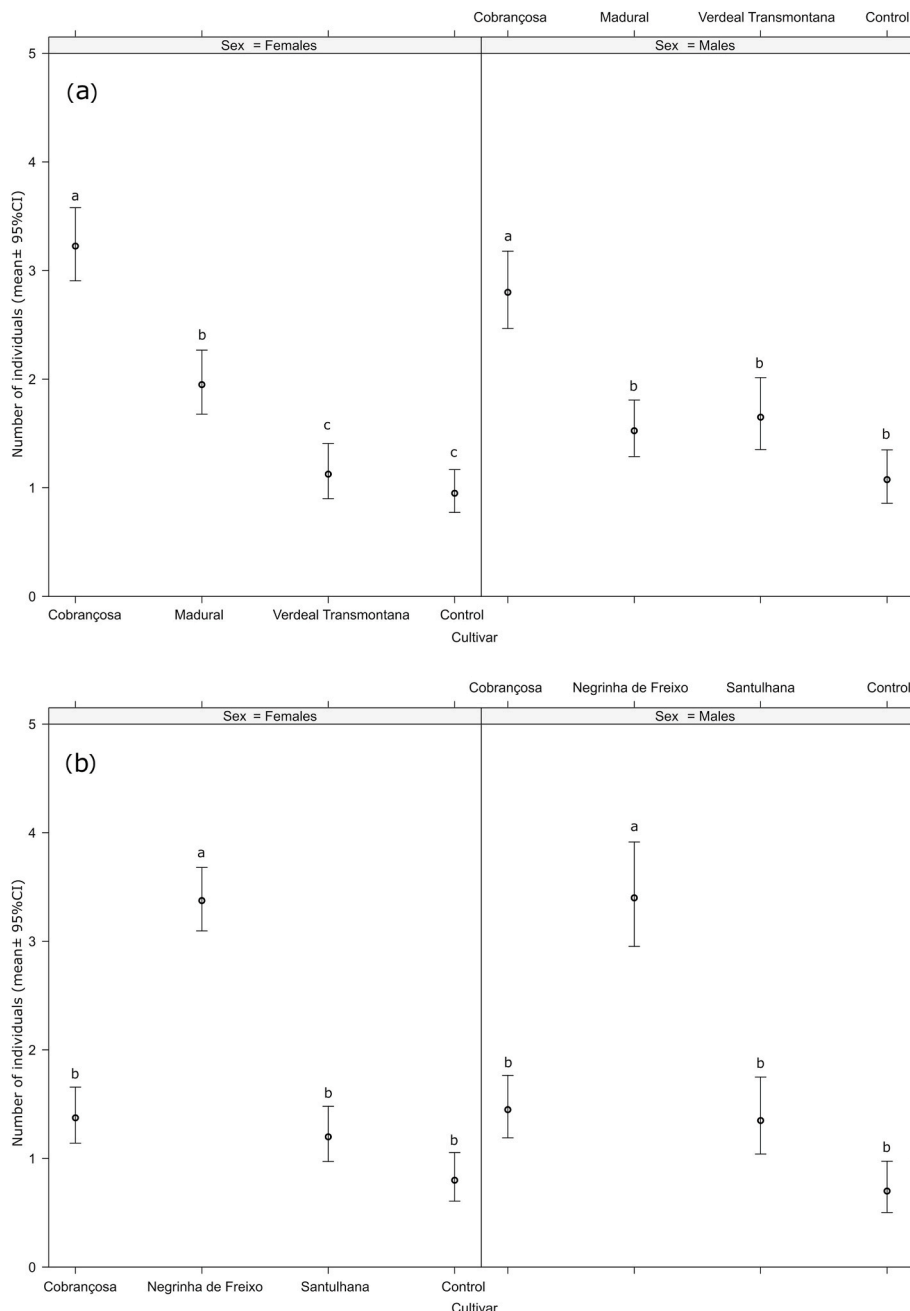


Fig. 2. Olfactory response in Spring of females and males of *Philaenus spumarius* towards traditional Portuguese olive cultivars. (a) First trial (Number of individuals (mean ± 95 %CI) that chose the cultivars “Madural”, “Cobrançosa”, “Verdeal Transmontana” and Control) and (b) Second trial (Number of individuals (mean ± 95 % CI) that chose “Cobrançosa”, “Negrinha de Freixo”, “Santulhana” and Control).

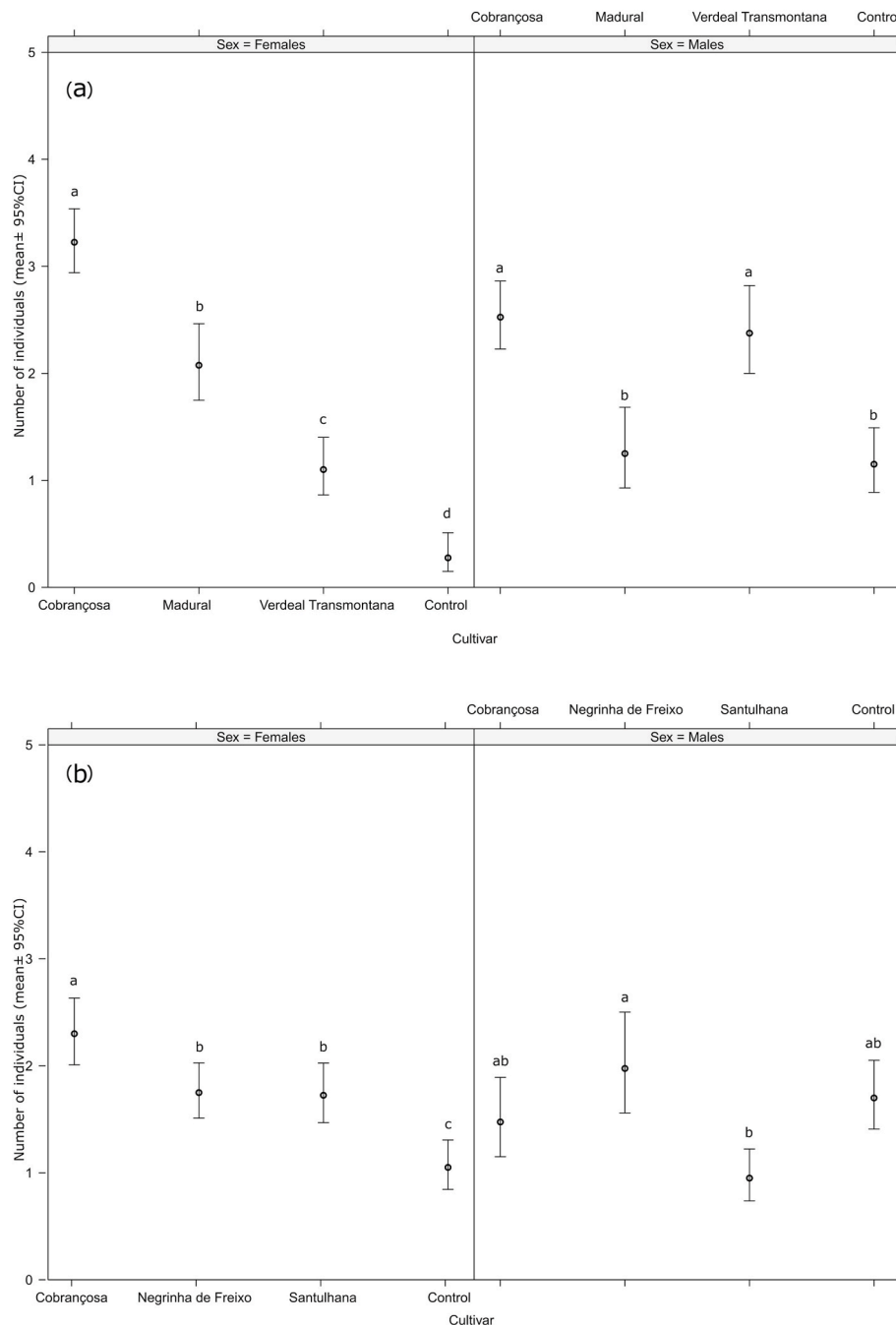


Fig. 3. Olfactory response in Autumn of females and males of *Philaenus spumarius* towards traditional Portuguese olive cultivars. (a) First trial (Number of individuals (mean \pm 95 %CI) that chose the cultivars “Madural”, “Cobrançosa”, “Verdeal Transmontana” and Control) and (b) Second trial (Number of individuals (mean \pm 95 % CI) that chose “Cobrançosa”, “Negrinha de Freixo”, “Santulhana” and Control).

2010; Rwomushana et al., 2008). This vector spends a large part of its life cycle in the herbaceous vegetation, which provides a wide range of host plants where they feed, mate, and lay eggs (Dongiovanni et al., 2019; Moreira and Veloso, 2009; Morente et al., 2018). Nevertheless, several studies reported peaks of abundance in the olive tree canopy in Spring shortly after the emergence of adults (Bodino et al., 2019, 2020; Sanna et al., 2021). In the summer, adults tend to disappear from the olive groves (Antonatos et al., 2020; Ben Moussa et al., 2016; Bodino et al., 2019; Tsagkarakis et al., 2018), returning to the olive groves in Autumn, after the first rains, to proceed to oviposit (Cruaud et al., 2018; Morente et al., 2018; Sanna et al., 2021). Although *P. spumarius* exhibits a lower abundance in the canopy of olive trees in Autumn (Bodino et al., 2019, 2020), Bodino et al. (2021) reported higher acquisition rates of

X. fastidiosa by this vector during this season. For these reasons, we choose to assess the olfactory response of *P. spumarius* adults to the different olive cultivars in two different seasons: Spring and Autumn.

Several studies have shown that *P. spumarius* can respond to olfactory stimuli (e.g., Anastasaki et al., 2021; Cascone et al., 2022; Germinara et al., 2017; Rodrigues et al., 2022a), strongly indicating that these may use chemical cues to choose and locate host plants. In this work, adults of *P. spumarius* presented significantly different olfactory responses toward the different cultivars under study. A previous study conducted by Cascone et al. (2022), where the olfactory response of *P. spumarius* towards the olive cultivars “Leccino”, “FS-17”, “Ogliarola”, “Frantoio”, and “Rotondella” was described, also reported significant differences in olfactory responses to the difference olive cultivars. In that study, male

Table 2

Volatile compound composition (mean percentage \pm SE) of the olive cultivars ‘Cobrançosa’, ‘Madural’, ‘Negrinha de Freixo’ (NF), ‘Santulhana’, and ‘Verdeal Transmontana’ (VT) in Spring and Autumn. For each season, mean values within the same row followed by different letters differ significantly ($P < 0.05$).

Compound	Spring					<i>p</i> -value	Autumn					<i>p</i> -value
	Cobrançosa	Madural	NF	Santulhana	VT		Cobrançosa	Madural	NF	Santulhana	VT	
Alcohol												
1-Dodecanol	0.12 \pm 0.08	nd	nd	nd	0.19 \pm 0.14	0.35	nd	nd	nd	nd	nd	–
1-Dodecanol, 3,7,11-trimethyl	0.55 \pm 0.35	0.68 \pm 0.7	0.16 \pm 0.06	0.05 \pm 0.02	0.38 \pm 0.45	0.11	nd	0.15 \pm 0.15	0.18 \pm 0.06	nd	nd	0.65
1-Heptanol	0.48 \pm 0.40	nd	0.27 \pm 0.17	nd	nd	0.26	nd	nd	nd	nd	nd	–
2-Ethyl-1-hexanol	nd	nd	nd	nd	nd	–	4.21 \pm 4.30	4.33 \pm 4.65	nd	5.4 \pm 3.81	3.04 \pm 1.76	0.8
1-Nonanol	0.53 \pm 0.33	nd	0.1 \pm 0.07	nd	nd	0.02	0.49 \pm 0.30	nd	nd	1.92 \pm 2.86	1.04 \pm 1.30	0.41
1-Octanol	nd	0.71 \pm 0.61	0.28 \pm 0.08	0.18 \pm 0.08	0.92 \pm 1.22	0.3	nd	0.39 \pm 0.48	nd	nd	nd	–
2,7-Dimethyl-1-octanol	nd	nd	nd	nd	nd	–	0.12 \pm 0.10	nd	nd	nd	nd	–
3,7-Dimethyl-1-octanol	nd	nd	nd	nd	nd	–	0.66 \pm 0.01	nd	nd	nd	nd	–
(Z)-3-Hexen-1-ol	9.89 \pm 3.73a	12.16 \pm 6.19 ab	10.46 \pm 2.63 ab	25.41 \pm 10.07b	11.51 \pm 12.81 ab	0.03	15.28 \pm 5.6 ab	7.33 \pm 2.17a	5.83 \pm 1.35a	22.61 \pm 15.17 ab	26.14 \pm 19.48b	0.03
Benzyl alcohol	nd	nd	nd	0.11 \pm 0.06	nd	–	nd	nd	nd	nd	nd	–
2-Phenylethanol	nd	nd	nd	0.31 \pm 0.18	nd	–	nd	nd	nd	nd	nd	–
Aldehyde												
Dodecanal	nd	nd	nd	0.03 \pm 0.01	nd	–	nd	nd	nd	nd	nd	–
Heptanal	nd	nd	nd	0.14 \pm 0.05	nd	–	nd	nd	nd	nd	nd	–
Nonanal	0.79 \pm 0.36	0.95 \pm 0.51	0.6 \pm 0.22	0.62 \pm 0.1	0.85 \pm 0.82	0.73	1.01 \pm 0.90	0.78 \pm 0.9	0.56 \pm 0.29	2.36 \pm 2.16	0.85 \pm 0.67	0.15
Alkane												
Decane	nd	nd	nd	nd	2.25 \pm 2.26	–	3.21 \pm 2.30	nd	nd	nd	nd	–
5-Methyldecane	nd	nd	nd	nd	nd	–	nd	1.39 \pm 1.04	nd	nd	nd	–
Dodecane	0.99 \pm 0.48	1.09 \pm 0.98	0.6 \pm 0.26	0.06 \pm 0.02	0.91 \pm 0.78	0.09	0.74 \pm 0.60	0.41 \pm 0.36	nd	nd	0.55 \pm 0.38	0.56
2,3,5-Trimethylheptane	nd	nd	nd	nd	nd	–	3.01 \pm 1.80	nd	2.49 \pm 0.83	nd	4.8 \pm 3.19	0.45
Hexadecane	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	0.39 \pm 0.32	–
2,2,4-Trimethylhexane	nd	nd	nd	nd	nd	–	nd	nd	4.41 \pm 1.66	nd	nd	–
2,2,5-Trimethylhexane	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	3.93 \pm 2.69	–
Nonane	nd	0.76 \pm 0.47	0.94 \pm 0.43	nd	1.04 \pm 1.07	0.82	nd	nd	nd	nd	nd	–
2,2,4,4,6,8,8-Heptamethylnonane	nd	nd	nd	nd	nd	–	1.35 \pm 1.30	nd	nd	nd	nd	–
3-Methylnonane	nd	nd	nd	nd	nd	–	nd	1.26 \pm 0.79	nd	nd	nd	–
Octane	nd	nd	1.51 \pm 0.61	nd	nd	–	nd	nd	nd	nd	nd	–
2,4,6-Trimethyloctane	nd	nd	0.88 \pm 0.26	nd	nd	–	nd	nd	nd	nd	nd	–
2,7-Dimethyloctane	nd	nd	nd	nd	nd	–	nd	nd	2.12 \pm 1.25	nd	nd	–
Pentadecane	0.77 \pm 0.55	1.28 \pm 1.34	0.33 \pm 0.15	nd	0.85 \pm 0.93	0.41	nd	nd	nd	nd	nd	–
3-Ethyl-2,2-dimethylpentane	nd	nd	nd	nd	nd	–	nd	1.76 \pm 1.37	2.04 \pm 0.46	nd	nd	0.67
Tetradecane	0.85 \pm 0.58	1.17 \pm 1.17	0.51 \pm 0.23	0.07 \pm 0.05	0.89 \pm 0.86	0.17	0.45 \pm 0.40	0.23 \pm 0.2	nd	nd	0.47 \pm 0.38	0.49
Tridecane	0.77 \pm 0.54	0.97 \pm 0.95	0.56 \pm 0.25	nd	0.74 \pm 0.7	0.81	nd	nd	0.4 \pm 0.13	0.67 \pm 0.35	nd	0.15
7-Methylenetridecane	0.2 \pm 0.12	nd	nd	nd	nd	–	nd	nd	nd	nd	nd	–
Undecane	1.25 \pm 0.56	1.41 \pm 0.95	nd	nd	1.73 \pm 1.56	0.58	0.67 \pm 0.30	0.61 \pm 0.24	0.81 \pm 0.53	nd	0.95 \pm 0.58	0.64

(continued on next page)

Table 2 (continued)

Compound	Spring					<i>p</i> -value	Autumn					<i>p</i> -value
	Cobrançosa	Madural	NF	Santulhana	VT		Cobrançosa	Madural	NF	Santulhana	VT	
2,5-Dimethylundecane	0.3 ± 0.19	0.3 ± 0.26	nd	nd	nd	–	nd	nd	nd	nd	nd	–
5,6-Dimethylundecane	nd	nd	nd	nd	nd	–	nd	nd	1.62 ± 0.32	2.68 ± 1.66	2.24 ± 1.68	0.49
1-Dodecene	0.56 ± 0.31	0.54 ± 0.46	nd	nd	0.41 ± 0.37	0.8	0.46 ± 0.50	0.31 ± 0.37	0.26 ± 0.1	0.36 ± 0.32	0.37 ± 0.3	0.91
1-Tridecene	0.7 ± 0.46	0.97 ± 0.96	0.32 ± 0.14	0.05 ± 0.02	0.59 ± 0.6	0.12	0.58 ± 0.50	nd	0.4 ± 0.16	nd	0.83 ± 0.64	0.38
1-Undecene	nd	0.23 ± 0.23	0.18 ± 0.1	nd	0.4 ± 0.46	0.49	nd	nd	nd	nd	nd	–
(E)-2-Dodecene	nd	nd	nd	nd	nd	–	nd	0.79 ± 0.57	nd	1.23 ± 0.44	nd	0.21
(Z)-3-Hexadecene	nd	0.45 ± 0.40	0.06 ± 0.03	nd	0.24 ± 0.24	0.12	nd	nd	nd	nd	nd	–
(Z)-3-Tetradecene	nd	nd	nd	nd	nd	–	nd	0.65 ± 0.77	nd	nd	nd	–
(E)-5-Octadecene	nd	1.16 ± 1.09	nd	0.18 ± 0.07	1 ± 1.11	0.21	1.33 ± 0.90	nd	0.64 ± 0.23	nd	1.58 ± 0.97	0.18
(Z)-7-Hexadecene	0.45 ± 0.23	nd	0.23 ± 0.11	nd	nd	0.1	nd	nd	nd	nd	nd	–
Ester												
(Z)-3-Hexen-1-yl acetate	45.03 ± 23.75	43.98 ± 29.97	71.55 ± 5.18	58.74 ± 9.17	44.1 ± 27.86	0.21	41.33 ± 26.4 ab	59.74 ± 21.96b	60.82 ± 8.63b	25.92 ± 15.28a	26.68 ± 9.39a	0.008
(Z)-3-Hexen-1-yl propanoate	nd	nd	nd	0.57 ± 0.29	nd	–	nd	nd	nd	nd	nd	–
(Z)-Methyl 3-hexenoate	nd	nd	nd	0.37 ± 0.25	nd	–	nd	nd	nd	nd	nd	–
Heptyl acetate	nd	nd	0.19 ± 0.1	nd	nd	–	nd	nd	nd	nd	nd	–
Hexyl acetate	nd	2.89 ± 1.91	nd	2.89 ± 4.04	2.27 ± 1.14	0.9	nd	2.3 ± 0.32	nd	nd	nd	–
Methyl 2-methylbutanoate	4.25 ± 2.65	4.53 ± 2.76	nd	0.28 ± 0.19	4.84 ± 4.56	0.08	5.25 ± 6.60	2.94 ± 2.65	nd	nd	nd	0.48
(E)-3-Hexenyl butanoate	1.45 ± 1.79	nd	0.43 ± 0.19	3.63 ± 1.94	4.9 ± 10	0.52	nd	1.2 ± 1.05	0.87 ± 0.44	0.79 ± 0.38	4.37 ± 7.26	0.38
cis-3-Hexenyl 2-methylbutyrate	0.88 ± 0.94	1.18 ± 0.98	0.33 ± 0.28	1.07 ± 0.89	0.72 ± 0.54	0.46	nd	nd	nd	nd	2.09 ± 3.03	–
Methyl hexanoate	nd	nd	nd	0.27 ± 0.13	nd	–	nd	nd	nd	nd	nd	–
Methyl nonanoate	nd	nd	nd	0.15 ± 0.07	nd	–	nd	nd	nd	nd	nd	–
Methyl octanoate	0.28 ± 0.15	nd	nd	0.23 ± 0.16	0.31 ± 0.21	0.75	nd	nd	nd	nd	nd	–
Bis(2-ethylhexyl) oxalate	nd	nd	nd	nd	nd	–	nd	nd	0.96 ± 0.33	nd	nd	–
Terpene												
β-Ocimene	0.44 ± 0.31	1.19 ± 0.97	nd	0.99 ± 1.29	0.6 ± 0.57	0.51	nd	nd	nd	nd	nd	–
D-Limonene	11.12 ± 12.35	3.89 ± 4.26	nd	1.24 ± 0.44	4.39 ± 5.34	0.19	nd	nd	nd	nd	nd	–
(+)-Cyclosativene	nd	nd	0.19 ± 0.2	0.08 ± 0.03	nd	0.47	nd	nd	0.24 ± 0.07	0.61 ± 0.28	nd	0.55
(+)-Valencene	nd	nd	nd	nd	nd	–	nd	nd	nd	nd	0.7 ± 0.79	–
α-Amorphene	nd	nd	nd	nd	nd	–	nd	nd	nd	0.56 ± 0.28	nd	–
α-Copaene	nd	nd	1.5 ± 0.92	1.36 ± 0.65	nd	0.77	nd	nd	nd	nd	nd	–
α-Selinene	nd	nd	nd	nd	nd	–	nd	nd	nd	0.76 ± 0.48	nd	–
α-Cubebene	nd	0.5 ± 0.41	nd	nd	nd	–	nd	nd	0.46 ± 0.61	nd	nd	–
α-Farnesene	nd	nd	nd	nd	0.1 ± 0.06	–	nd	nd	nd	nd	nd	–
α-Muurolene	nd	nd	0.21 ± 0.17	0.21 ± 0.13	nd	0.96	nd	nd	nd	nd	nd	–
β-Copaene	0.12 ± 0.05	nd	0.12 ± 0.03	0.13 ± 0.09	nd	0.85	nd	nd	nd	nd	nd	–
β-Selinene	nd	nd	nd	0.07 ± 0.04	nd	–	nd	nd	nd	2.58 ± 2.12	nd	–

(continued on next page)

Table 2 (continued)

Compound	Spring					p-value	Autumn					p-value
	Cobrançosa	Madural	NF	Santulhana	VT		Cobrançosa	Madural	NF	Santulhana	VT	
γ -Muuroolene	nd	nd	0.13 \pm 0.07	0.24 \pm 0.41	nd	0.58	nd	nd	nd	nd	nd	–
Caryophyllene	4.43 \pm 3.04	8.04 \pm 8.17	0.45 \pm 0.1	0.97 \pm 0.24	5.18 \pm 5.88	0.11	5.95 \pm 4.20	7.27 \pm 5.37 \pm 1.58	2.32 \pm 2.53	nd	6.3 \pm 4.96	0.31
Copaene	0.61 \pm 0.44	0.97 \pm 0.59	nd	nd	nd	0.31	0.59 \pm 0.40a	0.6 \pm 0.35a	2.53 \pm 0.88a	6.37 \pm 2.76b	0.94 \pm 0.65a	<0.001
Humulene	0.88 \pm 0.61	1.79 \pm 1.75	nd	0.27 \pm 0.09	1.15 \pm 1.33	0.24	0.83 \pm 0.60	1.28 \pm 0.94	0.45 \pm 0.36	1.27 \pm 0.74	1.21 \pm 0.99	0.37
Other												
p-Xylene	nd	nd	4.03 \pm 1.36	nd	nd	–	nd	nd	nd	nd	nd	–
2(3H)-Furanone, dihydro-5-methyl-	nd	nd	nd	0.16 \pm 0.08	nd	–	nd	nd	nd	nd	nd	–
Methyl Isobutyl Ketone	2.98 \pm 1.86	3.15 \pm 2.40	0.72 \pm 0.32	nd	3.24 \pm 2.79	0.1	8.31 \pm 5.10	4.01 \pm 2.61	4.59 \pm 2.03	nd	9.85 \pm 6.52	0.15
o-Cymene	8.09 \pm 12.52	2.35 \pm 1.80	0.91 \pm 0.35	nd	3.03 \pm 2.78	0.35	nd	nd	nd	nd	nd	–
Not identified	0.26 \pm 0.19	0.46 \pm 0.45	nd	nd	0.27 \pm 0.23	0.45	nd	nd	nd	8.35 \pm 4.76	0.29 \pm 0.3	0.001
Oxime-, methoxy- phenyl-	nd	nd	nd	nd	nd	–	4.63 \pm 3.30	nd	4.61 \pm 5.36	15.54 \pm 11.93	nd	0.07
1,6-Cyclodecadiene, 1- methyl-5-methylene- 8-(1-methylethyl)-, [S- (E,E)]-	nd	0.27 \pm 0.21	nd	0.1 \pm 0.08	nd	0.66	nd	0.23 \pm 0.14	0.37 \pm 0.27	nd	0.41 \pm 0.5	0.16

Results are expressed in relative percentage of the total chromatogram area (mean \pm standard deviation, n = 5).

“nd” – detected.

Table 3

Relative importance of the cultivar (“Cobrançosa”, “Negrinha de Freixo”, “Santulhana”, “Madural”, and “Verdeal Transmontana”) and season (Spring and Autumn), on volatile composition as revealed by permutational multivariate analysis of variance (PERMANOVA).

	Df	X ²	F	P
Season	1	0.21	17.51	0.001
cultivar	4	0.16	3.43	0.001
season:cultivar	4	0.16	3.35	0.001
Residual	40	0.47		
Total	49	1		

P. spumarius were unresponsive towards the tested olive varietals, while females were repelled by “FS-17” and attracted to “Ogliarola”, “Roton-della”, and “Frantoio”. In general, in our study, sex also significantly influenced the choice of the cultivars, except in Spring in the second trial. Similar results were also previously reported by Ganassi et al. (2020) and Rodrigues et al. (2022a).

In Spring, *P. spumarius* adults significantly chose the “Negrinha de

Freixo” cultivar. “Negrinha de Freixo” is a variety typically found in Trás-os-Monte’s region, Portugal’s second largest olive-growing region. This cultivar is essentially used to produce table olives since it has a very low oil yield. In fact, this cultivar represents great economic importance in the region since the table olives of this variety are recognized as Protected Denomination Origin (PDO) (European Union, 1996). In Spring this cultivar exhibited a distinct volatile profile compared to the other cultivars. This cultivar was the only one that did not exhibit β -Ocimene and D-Limonene in its volatile profile. β -Ocimene, a secondary metabolite commonly found in plants, plays crucial physiological and ecological roles. It is known for its involvement in attracting pollinators and natural enemies of pests, as well as providing direct protection to plants against herbivores and pathogens (Shimoda et al., 2012; Su et al., 2022). Cascone et al. (2022) described a negative relationship between female attractiveness and the amount of limonene in *P. spumarius*. This cultivar also exhibited one of the lowest percentages of 3-Hexen-1-ol, (Z)-, Rodrigues et al. (2022a) reported that Philaenus are attracted to low concentrations of 3-Hexen-1-ol, (Z)-.

In Autumn, females significantly chose the “Cobrançosa” cultivar.

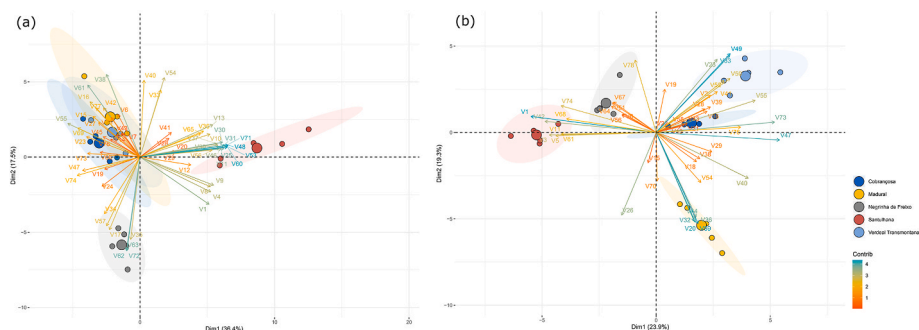


Fig. 4. Principal component analysis (PCA) biplot of volatile organic compounds (VOCs) emitted by “Cobrançosa”, “Negrinha de Freixo”, “Santulhana”, “Madural”, and “Verdeal Transmontana” in the (a) Spring and in the (b) Autumn. The number of each volatile compound can be consulted in Supplementary Table 1.

This dual aptitude cultivar (i.e., it can be used to produce olive oil or table olives) is widely distributed in the main Portuguese olive-producing regions (Reis, 2014). According to the PCA, during Autumn, the profile of this cultivar resembled that of the “Verdeal Transmontana” cultivar. However, females of *P. spumarius* did not exhibit the same level of attraction to this cultivar compared to the “Cobrançosa”. This suggests that there is a specific volatile compound or a combination of volatiles in the “Cobrançosa” profile that is more enticing to *P. spumarius* females. Additionally, “Verdeal Transmontana” cultivar may also possess specific a VOC or combinations of VOC’s that could act as repellents. For example, when comparing the volatile profile of these two cultivars, it is observed that the VOCs: (+)-Valencene, Hexadecane, Hexane, 2,2,5-trimethyl- Undecane, 5,6-dimethyl- were only present in the volatile profile of the “Verdeal Transmontana” cultivar. Valencene is known to have repellent and contact toxic effects against insects (Guo et al., 2019). However, with respect to other compounds, to the best of our knowledge, their effects on insects remain unknown.

On the other hand, in Autumn, males presented a more random olfactory response. In the first trial, they significantly chose the cultivars “Cobrançosa” and “Verdeal Transmontana”, but in the second trial, they showed no statistical differences between the cultivars “Cobrançosa”, “Negrinha de Freixo”, and “Santulhana”. According to Bodino et al. (2019), males have shorter longevity than females because the proportion of males tended to decrease through the season in the olive groves. Therefore, we hypothesized that since Autumn represents the end of the *P. spumarius* cycle, females can resort to olfactory stimuli to find a suitable host plant for oviposition, while males no longer have an active role. It is also important to note that females collected in Spring are unlikely to be mated, whereas those collected in Autumn are presumed to be mated. This distinction is a key aspect of the vector’s biology and may significantly influence their behavior, particularly in relation to host plant selection.

Our results suggested that the *P. spumarius* chose different cultivars depending on seasons, whereas Cascone et al., found no differences in the olfactory response of *P. spumarius* to olive cultivars over time (Cascone et al., 2022). The volatile profile of olive cultivars tends to change according to their morphological state (Malheiro et al., 2015). Additionally, abiotic factors can also lead to a change in the volatile profile (Malheiro et al., 2015; Sofo et al., 2004) so it is expected that the change in the volatile profile can induce different olfactory responses throughout the season.

It is important to acknowledge that the olfactometer bioassays in this study were conducted using excised olive leaves rather than intact plants. While the petiole was wrapped in aluminium foil to minimize the localized release of wound-induced green leaf volatiles (GLVs), it is expected that some GLVs may still have been emitted from the leaf lamina. This could, in theory, influence insect behavior. However, because all cultivars were subjected to the same handling procedure, any potential GLV-related effects would have affected all treatments equally, allowing for valid relative comparisons between cultivars. This methodological choice was made due to practical constraints in using intact plants in olfactometer bioassays, especially when high replication and precise control of odor sources are required. Nonetheless, we recognize that validating these findings with intact plants or extracts derived from intact plants would be an important step to confirm their ecological relevance. Furthermore, although seasonal differences in GLV emissions from excised leaves cannot be completely excluded as a contributing factor to the behavioral changes observed between Spring and Autumn, the standardized methodology across seasons suggests that these differences are more likely linked to natural seasonal variation in the volatile composition of leaves. Therefore, further studies on the olfactory response to volatiles produced by intact olive cultivars in different seasons are needed to validate the results.

5. Conclusions

In the present work, *P. spumarius* showed different olfactory responses to the traditional Portuguese olive cultivars in this study, providing evidence that this insect can use olfactory stimuli to select host plants. In Spring, the cultivar “Negrinha de Freixo” appears to be the most susceptible to vector attack since it was the most chosen by *P. spumarius* adults, whereas in Autumn, the cultivar “Cobrançosa” is favoured, only by the females. Further studies on feeding preference and monitoring the seasonal abundance of *P. spumarius* in mono-cultivar olive groves would be useful for validating of our results. In addition, the characterization of the volatile profile of cultivars and the understanding what differs between them, as well as the olfactory response of *P. spumarius* to volatiles emitted by the cultivars, could help the future implementation of approaches to manipulate the vector behavior.

CRedit authorship contribution statement

Isabel Rodrigues: Writing – original draft, Methodology, Investigation, Data curation. **Jacinto Benhadi-Marín:** Writing – review & editing. **Nuno Rodrigues:** Writing – review & editing, Methodology. **Paula Baptista:** Writing – review & editing, Supervision, Conceptualization. **José Alberto Pereira:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2025.107367>.

Data availability

Data will be made available on request.

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