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Infestation of the gall midge *Dasineura oleae* provides first evidence of induced plant volatiles in olive leaves

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Research Paper

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Abstract

In this study, we present the first characterization of herbivore-induced plant volatiles (HIPVs) released from infested olive leaves. The gall midge *Dasineura oleae* is a specific pest of *Olea europaea* and endemic of the Mediterranean Basin, an area in which severe outbreaks currently occurred. Little is known about the damage caused by the pest and the relationship with its host. Since gall formation and larval feeding activity may lead to the release of specific plant volatile compounds, we investigated the volatile profiles emitted from infested plants compared with healthy plants under both laboratory and field conditions. Additionally, the volatiles emitted from mechanically damaged plants were considered. A blend of 12 volatiles was emitted from olive trees infested by *D. oleae*. Of these, β-copaene, β-ocimene, cosmene, unknown 1 and unknown 3 were found to be exclusively emitted in infested plants. The emission of germacrene-D, (*E*,*E*)-α-farnesene, and (*Z*,*E*)-α-farnesene, α-copaene, (*E*)-4,8-dimethylnona-1,3,7-triene, (*E*)-β-guaiene and heptadecane significantly increased in infested trees. Linalool, β-copaen-4-α-ol, β-bourbonene, β-cubebene, β-elemene, β-copaene and δ-amorphene were found only in the field trial and showed differences depending on the level of infestation and the plant stage. (*Z*)-3-Hexenol, (*E*)-4-oxohen-2-enal, and 2-(2-butoxyethoxy)-ethanol, were exclusively emitted from the leaves after mechanical damage. In a field trial in Italy, we also demonstrated spring synchronization between adults of *D. oleae* and *O. europaea* trees. Analyses of morphoanatomical malformations of gall leaves showed that tissue alterations occur at the spongy parenchyma causing an increase of the leaf blade thickness. We speculate that tissue alterations may lead to HIPV release, in turn potentially attracting *D. oleae* natural enemies.

Introduction

The olive leaf gall midge, *Dasineura oleae* (Angelini, 1831) (Diptera: Cecidomyiidae), is an endemic monophagous species of Mediterranean countries hosted by olive trees (*Olea europaea* L.) (Dogănlar *et al.*, 2011). In recent years, several outbreaks have been reported in the Mediterranean (Simoglou *et al.*, 2012; Caselli *et al.*, 2021; Picchi *et al.*, 2021). In particular, the Tuscany region (Italy) has registered a dramatic increase in infestations throughout its area, with large outbreaks recorded in Grosseto Province (i.e., Gavorrano and Capalbio) and Massa-Carrara Province since 2013 (Tondini and Petacchi, 2019). During 2020, the outbreaks extended farther north, reaching the olive areas around Sarzana on the border between Liguria and Tuscany.

As with other cecidomyiids, *D. oleae* is characterized by a very short adult life span of approximately 12–72 h under laboratory conditions (unpublished data). Adults therefore need to find young olive leaves on which to lay their eggs in a relatively short time. The capacity of the female midge to find a suitable host in the appropriate phenological stage for larval development is fundamental to guarantee the best chances of survival of the progeny (Hall *et al.*, 2012). In gall-forming insects, gall formation is generally caused by the trophic activity of the larva after hatching (Rohfritsch and Shorthouse, 1982). When galls occur on leaves, the photosynthetic capacity of the plant is reduced, and the quantity and quality of the yield could be compromised (Martinez *et al.*, 1992; DeClerck-Floate and Price, 1994; Gonzales *et al.*, 2005; Huang *et al.*, 2014). Studies on the impact of *D. oleae* galls on olive leaf physiology have demonstrated that both net photosynthesis and stomatal conductance are negatively influenced by midge infestation (Caselli *et al.*, 2021).

Several studies have demonstrated that plants under insect attack can trigger direct (e.g., necrosis and neoplasm formation) or indirect defense responses by changing volatile emissions, thus attracting natural enemies (Zong *et al.*, 2012; Reymond, 2013). Therefore, some

predators and parasitoids are attracted by plant-derived volatile organic compounds (VOCs). Other volatiles are released as herbivore-induced plant volatiles (HIPVs) due to the occurrence of the 'crying for help' phenomenon (Whitfield, 2001; Gershenzon, 2007; Bruinsma and Dicke, 2008; Dicke, 2009; Dicke and Baldwin, 2010; Kaplan, 2012; Heil, 2014; Abraham *et al.*, 2015). HIPVs are involved in multitrophic insect–plant interactions, and furthermore, the emission of HIPV signals can be detected by neighboring unaffected plants as a wake-up call (Giacomuzzi *et al.*, 2016). Consequently, neighboring plants are primed to increase their defense as a precautionary response in a phenomenon called 'talking trees' (Abraham *et al.*, 2015; Arimura and Pearse, 2017). From an agronomical point of view, HIPVs could be used very similarly to insect sexual pheromones for pest monitoring, mass trapping, and disruption of host-finding behavior (Regnault-Roger and Philogène, 2008; Rodriguez-Saona and Stelinski, 2009; Preti *et al.*, 2021*a*, 2021*b*).

Studies on VOCs released by olive trees are currently scarce (Baratella, 2011; Malheiro *et al.*, 2016) and none investigated the leaves volatile emissions following herbivore damage. The headspace collection of healthy olive leaf volatiles in Scarpati *et al.* (1993) identified eight compounds. Among these compounds, toluene and α-pinene were the most abundant, having an important role in attracting and repelling *Bactrocera oleae* (Rossi), respectively, the major pest of *O. europaea* (Scarpati *et al.*, 1993; Baratella, 2011). Other compounds such as styrene, xylene, octanal, nonanal, and (*E*,*E*)-α-farnesene were recorded as components of the VOC profile of *O. europaea* leaves (Baratella, 2011). In Malheiro *et al.* (2016), toluene, (*Z*)-3-hexen-1-ol, and (*Z*)-3-hexenyl acetate were the main volatiles collected from the headspace of severed olive leaves. The main compounds identified after hydrodistillation of the leaves were (*E*,*E*)-α-farnesene, kongol, theaspiranes, and (*E*)-β-damascenone in cv Frantoio (Campeol *et al.*, 2001), (*E*)-2-hexenal, (*E*,*E*)-α-farnesene, linalool, and caryophyllene in cv Olivastra Seggianese (Flamini *et al.*, 2003), while the most abundant in cv Chemlali were (*E*)-2-hexenal, nonanal, (*E*)-β-damascenone, 3-ethenylpyridine, and caryophyllene (Abdeljelil Ben *et al.*, 2017). Studies on HIPVs are, to the best of our knowledge, completely absent concerning olive leaf pest infestations. Moreover, it is currently unknown how the VOC profile of *O. europaea* foliage changes after mechanical damage.

In this study, we characterized the headspace volatile compounds released by olive plants of cv Frantoio infested by *D. oleae* under both field and laboratory conditions, comparing these profiles to those of healthy and mechanically damaged plants. For the first time, we described induced volatiles released by infested *O. europaea* leaves. Collecting field data, we examined the synchronization between olive leaf gall midge attack and the phenology of *O. europaea*. Furthermore, we performed a morpho-anatomical analysis of the gall tissue to analyze leaf modifications due to *D. oleae* gall formation.

Materials and methods

Laboratory experiments

One-year-old rooted cuttings of *O. europaea* cv Frantoio were purchased from a plant nursery supplier (SPO, Società Pesciatina Olivicola, Pescia, Pistoia, Italy) in March 2020. Eighteen uniform plants were grown in 1.4-liter plastic pots containing sphagnum peat:pumice (1:1, v:v) and trained on a single shoot. Plants were drip-irrigated every day. The rooted cuttings were singly placed in cylindrical PVC cages (diameter: 25 cm, length: 80 cm) equipped with transparent chiffon fabric (mesh size: 0.05 mm) to allow aeration. On 10 April 2020, nine rooted cuttings were infested by placing five shoots with *D. oleae* galls taken from an infested orchard (length: 8 nodes) into each cage. Before the application, each infested shoot was carefully inspected to avoid any insect pests other than *D. oleae*. Adults of *D. oleae* emerged 24–48 h after the infested shoots were placed into the cages, mating occurred, and females laid eggs on the cutting leaflets. One month after the infestation (May 2020), leaf galls were visible. The other nine rooted cuttings were maintained not infested and treated as a control group. Infested and control plants were kept separated in two different climatic chambers under controlled conditions (24 ± 1°C, 16:8 (L:D) photoperiod) until the end of the experiment to avoid priming effects.

In order to distinguish pest-specific volatiles due to an active damage from the emissions of damaged tissues alone, we also sampled the volatiles emitted by mechanical damage, another 12 *O. europaea* cv Frantoio rooted cuttings (1 year old) were purchased from the nursery and kept under the same environmental conditions of the other laboratory plants. After 2 weeks of adjustment phase, six plants received damage, while the other six plants were considered as a control group. The mechanical damage was performed by using a metal punch (mod. 785, Zenith s.r.l., Manerbio, Italy) to cut 0.55 cm^2 holes on the first ten fully expanded apical leaves (two holes per leaf) of the treatment group. The headspace volatiles were collected from mechanically damaged plants starting immediately after the damage.

Field experiments

Field investigations were done to characterize the volatiles released from *D. oleae* infested trees under field conditions. Three different olive orchards located in Castelnuovo Magra (La Spezia, Italy), an area in which a strong outbreak of *D. oleae* has been recorded, were chosen for these trials. For each orchard, six olive trees of cv Frantoio ranging from 30 to 50 years old were selected. Headspace volatiles were collected from one shoot per tree. The shoots analyzed were ca. 30 cm long with no fruits and were chosen at mid-canopy height by uniformity of leaf number and size through visual observation. Since the whole area surrounding the fields that were sampled registered outbreaks of *D. oleae*, it was not possible to find a field of trees that was not infested as a control group. Therefore, sampling fields were classified as *highly* infested (field H) when the rate of infested leaves was over 20% and gall density was over two galls per leaf or *lightly* infested (fields L1 and L2) when the infestation rate was below 20% and the gall density was below two galls per leaf, according to Tondini and Petacchi (2019).

VOC collection

VOCs were collected using closed loop stripping analysis (CLSA) for 3 h (Kunert *et al.*, 2009). A distal shoot portion of approximately 30 cm was selected and enclosed within a plastic bag (BVOC-bag, Cuki® oven bag, Cofresco, Volpiano, Italy). Six empty plastic bags were sampled as negative controls. Air samples were collected using an adsorbent trap (glass tube, 6.5 × 0.55 × 0.26 cm^3, loaded with 1.5 mg activated charcoal; CLSA filter LR-type; Brechbühler AG, Schlieren, Switzerland). The trap was fitted to a 12-V graphite vacuum pump (Fürgut, Tannheim,

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Germany) using a short Teflon tube. The pump circulated air at a rate of ca. 1 liter min^{-1} within the VOC-bag. After collection, the volatiles were eluted from the CLSA filters with 100 µl of dichloromethane. The volatiles were analyzed by injecting 2 µl of each sample into a gas chromatography–mass spectrometer (GC/MS) (7890, Agilent Technologies, Santa Clara, USA) equipped with a mass selective detector-MS (5975C, Agilent Technologies). A GC/MS nonpolar HP-5 MS column (Agilent Technology, 30 m × 0.25 mm × 0.25 µm film thickness) was used for separation with a constant helium flow of 1.2 ml min^{-1} and at an average velocity of 39.723 cm s^{-1}. The oven temperature program was started at 50°C for 1.8 min and heated to 250°C at a rate of 7.3°C min^{-1}. The total run time was 34.19 min. The mass spectrometric detector was operated in scan mode (*m*/*z* 35–400 amu). Volatiles were initially identified by comparing their mass spectra with those found in two libraries: NIST 14 (National Institute of Standards and Technology, 2008) and Wiley 7 (John Wiley, NY, USA); a mixture of *n*-alkane standards (*n*C8–*n*C20, Sigma-Aldrich, St. Louis, Missouri, USA) was used to calculate linear retention indexes (LRIs) of the detected compounds (Van den Dool and Kratz, 1963). The identification of VOCs was based on the comparison of their retention index *r* with the retention indexes reported in the literature (PubChem, Nist, and Pherobase) (Babushok *et al*., 2011). The compounds were finally confirmed by comparing their retention times with those available from laboratory standards (Sigma-Aldrich, St. Louis, Missouri, USA). Compounds that were also detected in the negative control (VOC-bag) were considered contaminants. The amount of volatiles was reported as the 10^{-4} total ion current (TIC) mean ± standard deviation (SD).

Number of leaves, galls, and measurement of leaf area per sampled shoot

At the end of the collection of volatiles for both field and laboratory experiments, each shoot enclosed within the plastic bags was cut and defoliated to count the number of leaves and galls through visual observation and to measure the area of all the sampled leaves. For the area measurements, an LI-3000C Portable Leaf Area Meter (LI-COR, Lincoln, Nebraska, USA) was used.

Monitoring of the D. oleae flight pattern and phenology of O. europaea

To record the flight curve distribution of *D. oleae*, a field campaign was planned in an unirrigated olive orchard of ca. 1 ha, located at 60 m above sea level (a.s.l.) in Gavorrano (Grosseto, Italy) (42°54′28.30″N, 11°00′10.65″E), where pest infestations have been recorded in the last few years and in which weekly monitoring for the identification of the *D. oleae* developmental stage is ongoing. Standard transparent delta traps (Csalomon®, Budapest, Hungary) with a horizontal sticky base were used for these trials. Traps were nonattractive and odorless to avoid bias due to color and odor preference (Ranamukhaarachchi and Wickramarachchi, 2007). Twenty-five traps, placed 20 m apart from one another, were hung on trees at a height of 0.5 m above the ground, and *D. oleae* males and females captured per trap were recorded weekly during the entire month of April 2020.

O. europaea phenology has been determined by matching the growing degree days (GDDs) of olive trees during the sampling period and phenological observations in the field, with the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale as the reference point for *O. europaea* (Cortés *et al*., 2002). The GDDs were calculated using the formula by Orlandi *et al*. (2005) considering a threshold temperature of 10°C for *O. europaea* (Marchi et al., 2012). The Hydrological Service of Tuscany Region website was used to obtain daily maximum and minimum temperature data (Regione Toscana, 2020). The Braccagni (Grosseto, Italy) meteorological station was chosen because of its proximity to the sampling field.

Characterization of D. oleae attack by gall morphoanatomical observations

For the anatomical observations of the gall structures at the time of the experiment, ten leaves for both the control and infested treatments were chosen. A portion of 0.5 cm^2 was cut from the central region of healthy leaves and from the gall of infested leaves and fixed in an FAA solution (45% ethyl alcohol, 5% glacial acetic acid, 10% formaldehyde 8:1:1, v/v/v). Later, leaf samples, once rinsed in water, were dehydrated in a graded ethanol series and embedded in Histoplast. The tissues were cross-sectioned (10 µm) with a Shandon microtome (Shandon Inc., Pittsburgh, Pennsylvania, USA), mounted on glass slides and stained with 0.01% toluidine blue (Sigma-Aldrich, St. Louis, Missouri, USA) for 12 min. Observations were performed under an optical microscope (Fluophot, Nikon, Shinjuku, Japan). Representative selected sections were photographed with a Leica EC3 digital camera equipped with a microscope.

Statistical analysis

The data were analyzed using R software (R Core Team, 2020). The leaf surface and the number of galls between groups in the laboratory and field trials were analyzed by analysis of variance (ANOVA). In the peak analysis, contaminants found in the solvent alone were excluded from the analysis. Peak area integrations (TIC values) were compared between treatments by ANOVA. The leaf surface and the number of galls were accounted for as controlling variables. Data were log-normalized to fit a normal distribution. In case of a statistically significant treatment or field effect, a Tukey post-hoc test was performed. Although the potted plants had no (control) or some (infested) galls, the field plants had different degrees of infestation. Thus, it was not possible to consider a control field, and the amount of galls was considered a controlling variable. In the laboratory trial, since no gall was present on the control plants, only the number of leaves and the leaf surfaces were controlled for. Principal component analysis (PCA) of the laboratory data was performed using the R package 'factoextra' (Kassambara and Mundt, 2020). The PCA calculated the combination of the VOC area data by extracting eigenvalues and eigenvectors of a correlation matrix and then highlighting principal components. A two-dimensional score plot was created to compare the volatile profiles from control and infested plants. All the data are reported as the mean ± SD. The figures were created by using the R packages 'ggplot2' and 'cowplot' (Wickham, 2016; Wilke, 2020).

Results

VOC characterization of laboratory experimental plants

Thirty-one compounds were detected in infested and uninfested olive-potted plants, and among them, a variety of green leaf volatiles, aromatics, and terpenes were found (table 1). Sixteen were

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identified using authentic standard compounds, and others were identified based on their mass spectra and available LRIs. For four compounds, it was not possible to assign any chemical structures due to the low similarity to any compounds in the databases. Among the collected VOCs, the main peaks were 2-ethylhexanol (584.3 ± 310.8), (*Z*)-3-hexenyl acetate (333.3 ± 263), and nonanal (145.4 ± 33.8) for the control group, while in the infested plants, (*E*,*E*)-α-farnesene (545.8 ± 256.1) and β-ocimene (393.9 ± 116.6) became the main volatiles, while 2-ethylhexanol remained one of the main volatiles (259.1 ± 256.9). Four VOCs were present only in the infested group: β-ocimene, cosmene, β-copaene, and unknown 1. Others increased their concentration in infested plants: (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), α-copaene, germacrene D, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, and heptadecane. Within the same group, the leaf surface in the shoot influenced the amount of germacrene D ($F_{1,5} = 7.4$, $P < 0.05$), (*Z*,*E*)-α-farnesene ($F_{1,4} = 20.68$, $P < 0.05$), and heptadecane ($F_{1,5} = 15.47$, $P < 0.05$). Three compounds were higher in the control group: (*Z*)-3-hexenyl acetate, 2-ethylhexanol, and methyl salicylate. Comparing laboratory and field experiments, three compounds were found only in the laboratory collection, regardless of the infestation status: 3-hexenyl butyrate, tetradecane, and β-oplopenone.

PCA allowed us to determine the clustering of the volatile profiles collected from infested and healthy-potted saplings. The score plot (fig. 1) reports the PCA results and shows the difference between infestation conditions. Although healthy plants are clustered together, infestation by *D. oleae* caused a remarkable deviation along the principal components. Since PC1 explains almost half of the total variability (45.4%) the power left to PC2 (12.9%) is only a few points away from PC3 (9.89%) and PC4 (7.12%), resulting in dimension 2 contributing a relatively low explanatory power.

VOC characterization of mechanically damaged plants

Five compounds were identified as released by mechanically damaged leaves: 3-hexen-1-ol, (*E*)-4-oxohen-2-enal, (*Z*)-3-hexenyl-acetate, 2-(2-butoxyethoxy)ethanol, and (*E*,*E*)-α-farnesene (table 2). Among these, 3-hexenyl-acetate was the most abundant (1603.3 ± 1023), while 2-(2-butoxyethoxy)ethanol had the lowest amount (44.4 ± 29.8).

VOC characterization of infested field plants

A total of 37 compounds were collected from the field trees. Among them, caryophyllene and germacrene D were the most abundant, without differences between the olive orchards and the relative infestation level, while β-copaene and unknown 1 were remarkably more abundant in the more highly infested field (field H) (table 1). Some VOCs such as linalool, β-bourbonene, β-cubebene, β-elemene, aromadendrene, α-muurolene, β-copaen-4 α-ol, α-bisabolol, and unknown 4 (table 1) were found only in the field trials, regardless of the infestation level. Many VOCs were present only in highly infested plants: linalool, DMNT, cosmene, α-copaene, (*E*,*E*)-α-farnesene, unknown 1, and heptadecane, thus confirming what was observed under laboratory conditions. Additionally, (*Z*)-3-hexenyl acetate, higher in the control-potted plants, was more abundant in the low infested fields (fields L1 and L2). Benzaldehyde, which was not different between the potted plants, was instead not detected in field H. However, some compounds such as methyl salicylate showed a reversed pattern that increased in the highly infested field. The compounds β-bourbonene, β-cubebene, β-elemene, β-copaene, (*Z*,*E*)-α-farnesene, and δ-amorphene differed between the fields regardless of the infestation level (table 1).

The number of galls positively affected the amount of linalool ($F_{2,10} = 5.23$, $P < 0.05$), β-copaen-4 α-ol ($F_{2,12} = 4.48$, $P < 0.05$) and heptadecane ($F_{2,12} = 8.36$, $P < 0.05$). The germacrene D amount was positively influenced by the leaf surface ($F_{2,12} = 9.44$, $P < 0.05$).

Number of leaves, galls, and measurement of leaf area

Laboratory conditions

Leaf areas ranged between 179.59 ± 52.89 and 198.09 ± 58.13 cm^2 per plant in the control and infested groups, respectively. Statistical analysis showed no significant differences between the two groups ($F_{1,16} = 0.499$, $P = 0.4363$) (fig. 2a). The number of leaves per plant was not significantly different ($F_{1,16} = 0.674$, $P = 0.3296$) between control (60.78 ± 10.83) and infested (71.56 ± 24.85) plants (fig. 2a). The number of galls per shoot in the infested plants was 134.89 ± 111.82, while the number of galls per leaf was 1.73 ± 1.06. In the cuttings used for the volatiles released by mechanical damage, the amount of leaves per shoot was 50.60 ± 16.77 and 50.33 ± 12.81 ($F_{1,10} = 0.582$, $P = 0.658$) for control and damaged plants, respectively, while the leaf surface was 156.24 ± 52.89 and 163.16 ± 45.83 cm^2 ($F_{1,10} = 0.637$, $P = 0.547$), thus showing no significant difference between the groups.

Field conditions

Measurements of the leaf area and the total number of leaves per shoot were performed for the three different olive orchards. The leaf area ranged between 227.81 ± 103.51 cm^2 in field L1, 226.72 ± 53.31 cm^2 in field L2, and 213.83 ± 100.44 cm^2 in field H. Statistical analysis showed no significant difference between fields ($F_{2,15} = 0.049$, $P = 0.5873$) (fig. 2b). The number of leaves per shoot was not significantly different ($F_{2,15} = 0.715$, $P = 0.5191$ (fig. 2b). The number of galls per shoot was significantly higher in field H (594.33 ± 250.06) compared to that in fields L1 and L2 (44.16 ± 33 and 14.5 ± 7.12, respectively) ($F_{2,15} = 49.5$, $P = 0.0017$) (fig. 2b). The number of galls per leaf was 6.68 ± 3.05 in field H, 0.49 ± 0.24 in field L1, and 0.22 ± 0.13 in field L2, confirming a significant difference between fields ($F_{2,15} = 24.9$, $P < 0.001$).

Flight curve of D. oleae and O. europaea phenology

The flight curves of *D. oleae* males and females showed the same trend, with a greater presence of males than females during the whole period of sampling (fig. 3). On 8 April, a peak of adult flight activity was evident for both sexes, as the traps recorded a total of 2066 males and 1004 females. On this date, several swarms of *D. oleae* were noticed around young olive tree shoots and females during oviposition. The minimum presence of *D. oleae* was observed on 22 April, the last date of sampling, with a total catch of 512 males and 190 females.

Through visual observation and referring to the BBCH scale for *O. europaea*, phenological stage number 9, corresponding to external leaflets opening with their extremity intercrossing, was reached on 8 April, at 55.9 GDDs. On 15 April, at 75.7 GDDs, phenological stage number 33 was identified (fig. 3).

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Table 1. Summary table of the volatile compounds identified by GC/MS analysis of the headspace of *O. europaea* cv Frantoio shoots and their amounts (10^{-4} TIC ± SD) under laboratory (n = 9 + 9) and field conditions (n = 6 + 5 + 6)

				Laboratory plants		Field plants		
Compound		LRI[a]	LRI[b]	Control (9)	Infested (9)	L1 (6)	L2 (5)	H (6)
Aldehydes								
1	Benzaldehyde	965	964†	(9) 97.1 ± 23.6	(9) 77.1 ± 24.3	(6) 111.5 ± 25.4	(5) 109 ± 15.4	–
2	Nonanal	1107	1104†	(8) 145.4 ± 33.8	(9) 112.6 ± 73.2	(6) 274.6 ± 91.7	(5) 210.1 ± 88.3	(6) 228.1 ± 112
Alcohols								
3	2-Ethylhexanol	1032	1031†	(9) 584.3 ± 310.8*	(9) 259.1 ± 256.9	(6) 534.9 ± 296.4	(4) 272 ± 128.3	(6) 316 ± 219.2
4	Linalool	1105	1101†	–	–	(5) 32.1 ± 14.9	(3) 41.2 ± 12	(6) 301.5 ± 192.2***
5	β-Copaen-4 α-ol	1598	1580[4]	–	–	(6) 34.4 ± 8.3	(5) 29.6 ± 6.9	(5) 54.8 ± 26.1*
Alkanes								
6	Tetradecane	1400	1400†	(5) 30.7 ± 8.7	(5) 35.3 ± 15.4	–	–	–
7	Heptadecane	1700	1700†	(9) 32 ± 14.2	(7) 50.3 ± 23.3**	(5) 43.7 ± 12.4	(3) 39.6 ± 15.4	(4) 274.3 ± 148.6***
Esters								
8	Hexyl acetate	1007	1006[3]	(9) 56.2 ± 6.5	(9) 67.5 ± 16.8	(6) 99.1 ± 23.6	(5) 104.3 ± 46.4	(6) 156.9 ± 50.6
9	(*Z*)-3-Hexenyl acetate	1012	1010†	(9) 333.3 ± 263***	(7) 83.9 ± 50.7	(6) 353.4 ± 176.6**	(5) 155.6 ± 122	(6) 75.2 ± 34.4
10	3-Hexenyl butyrate	1191	1171[3]	(6) 59.7 ± 29	(3) 57 ± 33.6	–	–	–
11	Methyl salicylate	1196	1198†	(9) 65 ± 33.7*	(8) 35 ± 11.2	(6) 62 ± 27.8	(5) 46.9 ± 26.1	(6) 285.4 ± 86.7***
Ketones								
12	Sulcatone	984	974[1]	(7) 42.9 ± 14.1	(3) 24.2 ± 15.3	(3) 103.8 ± 106.3	(3) 42.9 ± 23.2	(3) 80.6 ± 40.6
Terpenes								
13	Myrcene	995	992[3]	(8) 43.6 ± 20.1	(7) 49.2 ± 15.9	(5) 92.4 ± 44	(4) 99.7 ± 12.1	(6) 147.3 ± 41.5
14	**β-Ocimene**	1053	1052†	–	(5) 393.9 ± 116.6	(5) 150.1 ± 170.5*	(3) 20.4 ± 8.1	(6) 179.1 ± 125.2*
15	DMNT	1122	1118†	(6) 44.7 ± 16.2	(5) 154 ± 115.1**	(6) 82.8 ± 46.9	(5) 139.7 ± 68.3	(6) 482.5 ± 328.6**
16	**Cosmene**	1135	1133†	–	(5) 170.3 ± 61.7	(4) 64 ± 49.5	(5) 139.7 ± 68.3	(6) 265 ± 125.2**
17	Carveol	1209	1209[2]	(9) 78.4 ± 21.6	(9) 94 ± 34.9	(6) 246.3 ± 121.1	(5) 164.5 ± 111.2	(6) 385.7 ± 138
Sesquiterpenes								
18	α-Copaene	1380	1380[3]	(7) 32.4 ± 6	(6) 65.1 ± 21.3***	(6) 129.4 ± 64.4	(4) 195.5 ± 97.9	(6) 506.6 ± 247.5**
19	β-Bourbonene	1381	1385[3]	–	–	(6) 115.2 ± 57.1	(4) 705.9 ± 430.1***	(6) 716.5 ± 250.9***
20	β-Cubebene	1388	1386[4]	–	–	(5) 40.5 ± 21.3	(4) 168.1 ± 137.4**	(6) 111.3 ± 83.8**
21	β-Elemene	1395	1390[4]	–	–	(5) 46.1 ± 26.4	(1) 133.9*	(4) 207.2 ± 110.7*
22	Caryophyllene	1424	1427†	(9) 82.5 ± 56.3	(9) 169.3 ± 172.7	(6) 1894 ± 2383.4	(5) 5067.6 ± 4496.2	(6) 4182.8 ± 2139.7
23	**β-Copaene**	1433	1433[4]	–	(5) 38.4 ± 38.6	(6) 107.1 ± 75.6	(4) 374.4 ± 239.7**	(6) 336.7 ± 88.8**

(*Continued*)

Table 1. (*Continued.*)

Compound		LRI[a]	LRI[b]	Laboratory plants		Field plants		
				Control (9)	Infested (9)	L1 (6)	L2 (5)	H (6)
24	Humulene	1458	1463†	(8) 37.9 ± 12.7	(6) 57.3 ± 36.2	(6) 331.4 ± 339.1	(4) 805.4 ± 478.4*	(6) 793.6 ± 390.4*
25	Aromadendrene	1468	1460[4]	–	–	(5) 76.5 ± 39.1	(4) 161.3 ± 161	(6) 180.3 ± 71.9
26	Bicyclosesquiphellandrene	1470	1463[4]	(7) 34.4 ± 14.4	(3) 44.1 ± 3.9	(2) 36.7 ± 14.4	(3) 68 ± 22.3	(3) 116.5 ± 35.2
27	Germacrene D	1485	1489†	(7) 36.5 ± 14.4	(7) 200 ± 285.4*	(6) 609.6 ± 395.3	(4) 1437.3 ± 1109*	(6) 1502.5 ± 416.5*
28	(*E*)-β-Guaiene	1500	1499[4]	(9) 29.5 ± 12.3	(9) 63.8 ± 35.4*	(6) 139.3 ± 76	(1) 145.9	(6) 716.5 ± 430.5**
29	(*Z*,*E*)-α-Farnesene	1506	1498†	(9) 22.8 ± 11.3	(6) 210.7 ± 204.2***	(5) 61.1 ± 24.2	(4) 134.2 ± 104**	(6) 271.2 ± 105.5**
30	(*E*,*E*)-α-Farnesene	1513	1511†	(7) 24.2 ± 9.5	(6) 545.8 ± 256.1***	(5) 263.7 ± 235.8	(4) 250.6 ± 244.3	(6) 1614.2 ± 1293.7*
31	γ-Cadinene	1519	1519[3]	(7) 108.4 ± 81.3	–	(5) 95.1 ± 44.6	(4) 116.5 ± 83.9	(6) 182.9 ± 66.2
32	δ-Amorphene	1529	1529[3]	(2) 20.1	(6) 31.7 ± 23.9	(6) 68.3 ± 37.3	(4) 154.2 ± 90.7**	(6) 177.2 ± 56.3**
33	Liguloxide	1534	1532[3]	(5) 23.6 ± 8.5	(6) 43.1 ± 17.1*	(6) 55 ± 22	(4) 31.4 ± 6.8	(6) 238.9 ± 125.7***
34	α-Muurolene	1543	1541[2]	–	–	(4) 29.1 ± 10.1	(4) 58.3 ± 35.7	(5) 66.5 ± 21.1
35	β-Oplopenone	1601	1600[1]	(3) 30.1 ± 12	(3) 16.1 ± 3.8	–	–	–
36	α-Bisabolol	1664		–	–	(4) 43 ± 6	(3) 120.5 ± 84.4	(5) 88.5 ± 54.9
Unknowns								
37	**Unknown 1**	1592		–	(5) 37.9 ± 17.5	(2) 27.2 ± 22.5	(3) 66.2 ± 47.9	(6) 327 ± 269.8*
38	Unknown 2	1605		(8) 63.6 ± 18.7	(8) 53.9 ± 19.9	(6) 39.6 ± 19.9	(5) 65.4 ± 37.3	(6) 234.3 ± 142.1**
39	**Unknown 3**	1659		–	(5) 35.4 ± 13.2	–	(2) 39.6 ± 17.6	(6) 170.3 ± 103.3*
40	Unknown 4	1695		–		(2) 50.7 ± 1.1	(3) 55.4 ± 33.3	(6) 243.3 ± 174.6

[a]LRI = linear retention index calculated in relation to *n*-alkanes.
[b]LRI = linear retention index already published in peer-reviewed journals and listed in Pherobase (1), NIST WebBook (2), or PubChem (3). The review of Babushok *et al.* (2011) has also been considered (4). When possible, the LRI was verified by a standard compound (†).
Sampling fields were classified as highly infested (H) or lightly infested (L1 and L2). The volatile compounds were collected by the CLSA technique for 3 h. The compounds were identified by mass spectrometry and confirmed by LRIs available from the literature or laboratory standards when available. The table also reports the *P*-value of the ANOVA test of the TIC amount. The number of samples where the compound was found is reported between brackets. Compounds in bold are present only in infested plants, while the gray background identifies the compounds varying between infestation conditions. Asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

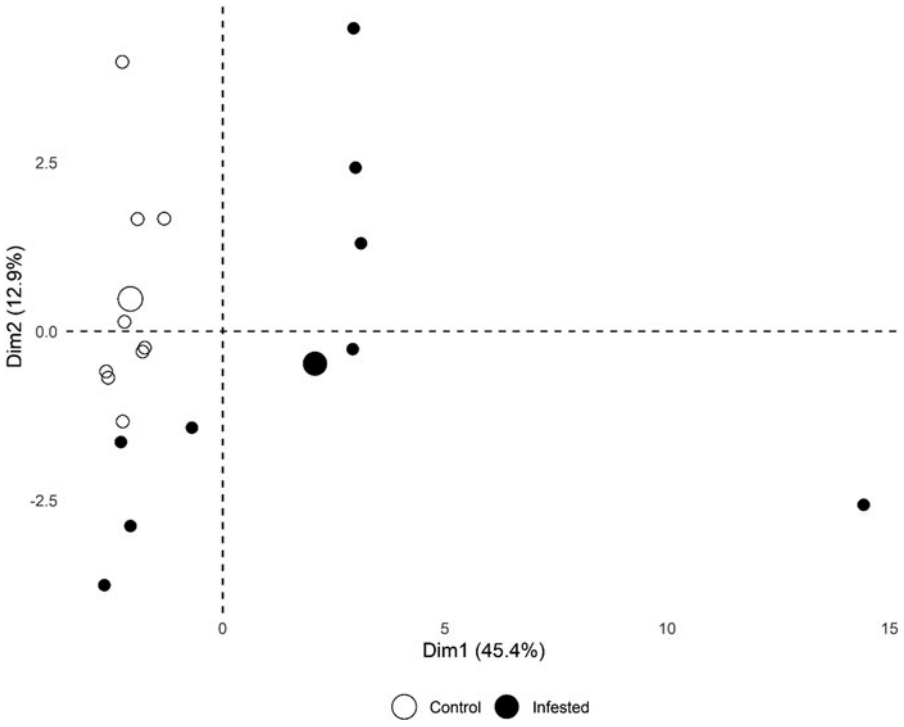

Figure 1. PCA score plot of the volatile profiles from healthy (1–9) olive tree saplings and saplings infested with *D. oleae* (Diptera: Cecidomyiidae) (10–18). Clusters of volatiles attributed to infestation status are indicated by the circles.

Table 2. VOCs released by *O. europaea* cv Frantoio cuttings after mechanical damage and their amounts (10^{-4} TIC ± SD)

Compound	LRI[a]	LRI[b]	Amount
(*Z*)-3-Hexen-1-ol	856	859†	658.8 ± 333.9
(*E*)-4-Oxohex-2-enal	963	956	259.8 ± 88.3
(*Z*)-3-Hexenyl-acetate	1012	1010†	1603.3 ± 1023
2-(2-Butoxyethoxy)ethanol	1192	1192	44.4 ± 2.9.8
(*E*,*E*)-α-Farnesene	1513	1511†	216.3 ± 163.4

Volatiles were collected from six potted plants after 20 holes of 3 mm were made on ten leaves. Volatile emissions were compared with those of control plants collected on the same day. The volatiles were collected by the CLSA technique for 3 h.
[a]LRI = Linear retention index calculated in relation to n-alkanes.
[b]LRI = Linear retention index already published in peer-reviewed journals and listed in PubChem. When possible, the LRI was verified by a standard compound (†).

Morphoanatomical observations

The healthy leaves of *O. europaea* cv Frantoio have a typical elongate-elliptical shape, with a smooth dark green adaxial surface and a lighter and less smooth abaxial surface (fig. 4a). A longitudinal healthy leaf section is presented in fig. 4b, in which palisade parenchyma I and II (PPI and PPII), spongy parenchyma (SP), and peltate trichomes (PT) are clearly recognizable. *D. oleae* galls are roundish, a few millimeters long (3–6 mm), and they are irregularly present just on the adaxial leaf surface (fig. 4a). The section in fig. 4c shows a belt of cell proliferation just near the larval chamber at the SP. The larval chamber is clearly recognized in fig. 4d. The thickness of this leaf section (2 mm) is threefold greater than the thickness of the healthy leaf (0.35 mm) (fig. 4b).

Discussion

Studies on olive leaf volatile profiles are currently scarce and based on hydrodistillate fractions, which are different from headspace emissions (Campeol *et al.*, 2001; Flamini *et al.*, 2003; Brahmi *et al.*, 2012; Malheiro *et al.*, 2016). In the present study, headspace collections of olive volatiles were recorded under both laboratory and field conditions. Control-potted plants emitted a blend of 26 VOCs, including the aldehydes nonanal and benzaldehyde, the esters methyl salicylate, 3-hexenyl butyrate and hexyl acetate, the ketone sulcatone, and the sesquiterpenes caryophyllene, (*E*,*E*)-α-farnesene and germacrene D, previously identified by Malheiro *et al.* (2015, 2016). Other VOCs are reported here for the first time, such as myrcene, (*Z*)-3-hexenyl acetate, 2-ethylhexanol, DMNT, carveol, α-copaene, tetradecane, humulene, bicyclosesquiphellandrene, (*E*)-β-guaiene, (*Z*,*E*)-α-farnesene, δ-amorphene, liguloxide, β-oplopenone, heptadecane, γ-cadinene, and unknown 2.

This study, for the first time, provides evidence about HIPVs for olive trees due to insect attack to the leaves. Previous studies have only investigated fruit HIPV profile due to *B. oleae* larvae infestation (Alagna *et al.*, 2016) and the behavioral responses of its parasitoid *Psyttalia concolor* to olive drupe HIPVs (Giunti *et al.*, 2016*a*). Moreover, although gallers usually manage to suppress VOCs (Borges, 2018), *D. oleae* induces a change of the volatile profiles of attacked plants in the amount of emissions and in the released compounds. A blend of 12 VOCs is distinctive of olive leaves infested by *D. oleae*: DMNT, β-ocimene, α-copaene, β-copaene, cosmene, germacrene D, (*E*)-β-guaiene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, unknown 1, unknown 3, and heptadecane. Some were found to be specific only to infested plants in laboratory trials (β-ocimene, cosmene, β-copaene, unknown 3, and unknown 4), while others showed increased emission in infested plants both in the laboratory and in field collection (DMNT, α-copaene, (*E*)-β-guaiene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, liguloxide, and heptadecane). This herbivore-induced emission observed in the study is in agreement with other studies that reported some of the same compounds in infested plants of other species (Suckling *et al.*, 2012). DMNT and α-copaene were released in *Medicago truncatula* Gaertn.

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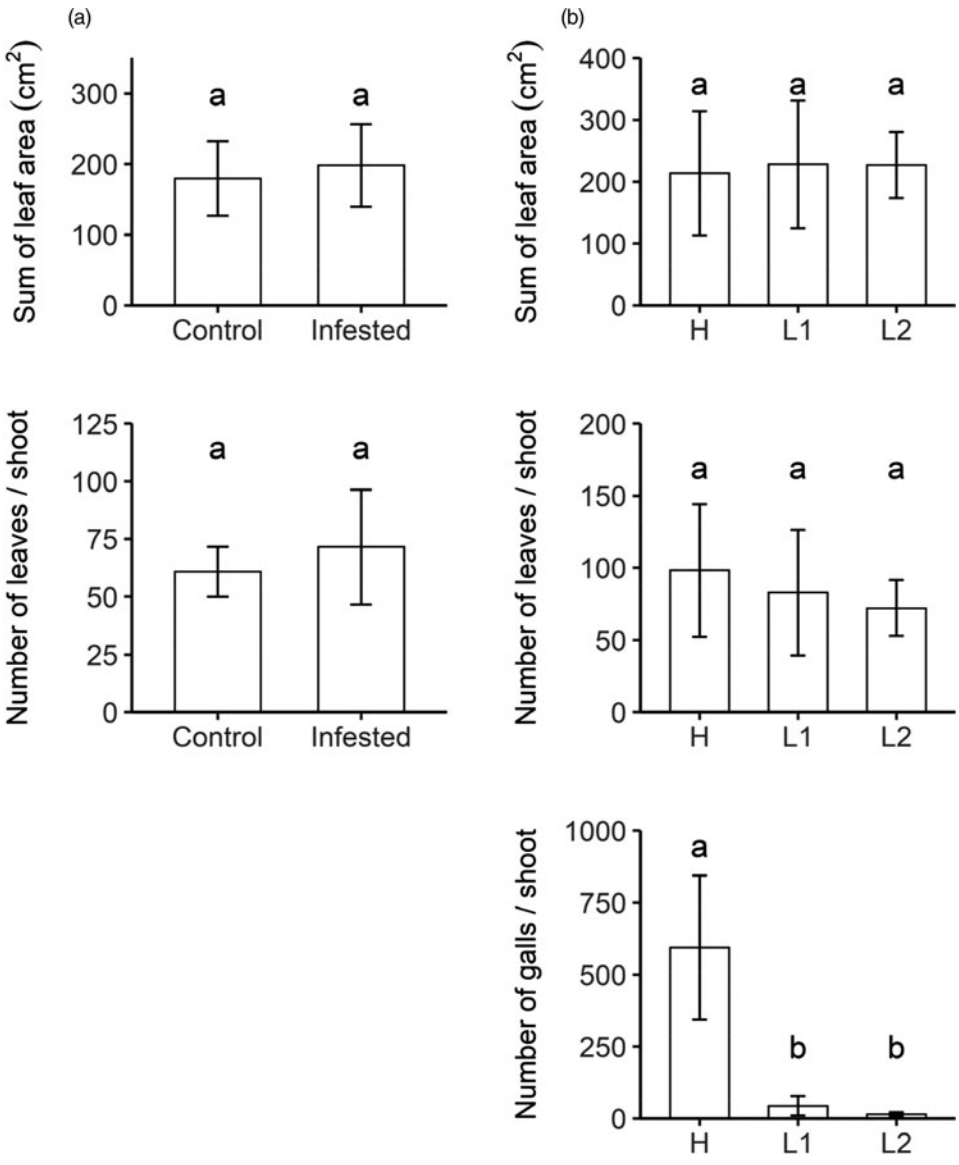

Figure 2. (a) Sum of leaf area and number of leaves per shoot in *O. europaea* (cv Frantoio) rooted cuttings treated as control ($n = 9$) and infested by *D. oleae* (Diptera: Cecidomyiidae) ($n = 9$). (b) Sum of leaf area, number of leaves per shoot, and number of galls per shoot in three different olive orchards (cv Frantoio) located in Castelnuovo Magra (La Spezia, Italy) having different levels of *D. oleae* infestation: field H ($n = 6$) is highly infested, while fields L1 ($n = 6$) and L2 ($n = 5$) are infested at a lower level. Letters report statistical significance.

following the feeding activity of *Spodoptera exigua* (Hübner) (Arimura *et al.*, 2008). In the study of Zeng *et al.* (2017), α-farnesene and ocimene were released after tea leaf (*Camellia sinensis* (L.) Kuntze) stimulation by jasmonic acid combined with mechanical damage, simulating aphid damage. (*E*, *E*)-α-Farnesene and β-ocimene were also emitted by cotton plants treated with methyl jasmonate (Rodriguez-Saona *et al.*, 2001). The sesquiterpene (*E*,*E*)-α-farnesene and the monoterpene β-ocimene are already known as constituents of olive oils, and when infested by *B. oleae*, olive fruits increase the emission of these two compounds (Giunti *et al.*, 2016*b*). In the present study, (*E*, *E*)-α-farnesene and β-ocimene were the most abundant molecules in the infested potted plants, thus confirming a key role of these molecules in the olive tree–insect interactions. (*E*)-β-Ocimene is reported as an HIPV from *B. oleae* exploited by virgin males of the braconid parasitoid *P. concolor* to boost their mate searching activity (Giunti *et al.*, 2018).

The monoterpene cosmene, identified in the present study, was also found as an HIPV in apple leaves infested by *Pandemis heparana* Denis & Schiffermüller (Giacomuzzi *et al.*, 2016). Most of the other compounds are in agreement with other studies, as they were identified as VOCs of olive trees (Flamini *et al.*, 2003; Baratella, 2011). The main VOCs collected from the field trials were caryophyllene and germacrene D, regardless of the infestation level between fields. Although caryophyllene is one of the main constituents of the olive leaf profile (Flamini *et al.*, 2003), germacrene D might instead act as an HIPV since its emission increased in response to midge infestation in the laboratory collection. Moreover, statistical analysis revealed that germacrene D is also affected by the leaf surface of the sampled shoots both in laboratory and field trials. Two other VOCs, (*Z*,*E*)-α-farnesene and heptadecane, are commonly emitted in small amounts by apple leaves and olive leaves (Bengtsson *et al.*, 2001; Dursun *et al.*, 2017). In the present study, they were directly related to the leaf surface of the shoots sampled in the laboratory trials, and their emission increased in infested plants.

A substantial number of VOCs are usually emitted due to oxidation processes after plant tissue injury. Considering the volatile emission due to mechanical damage allowed us to notice the

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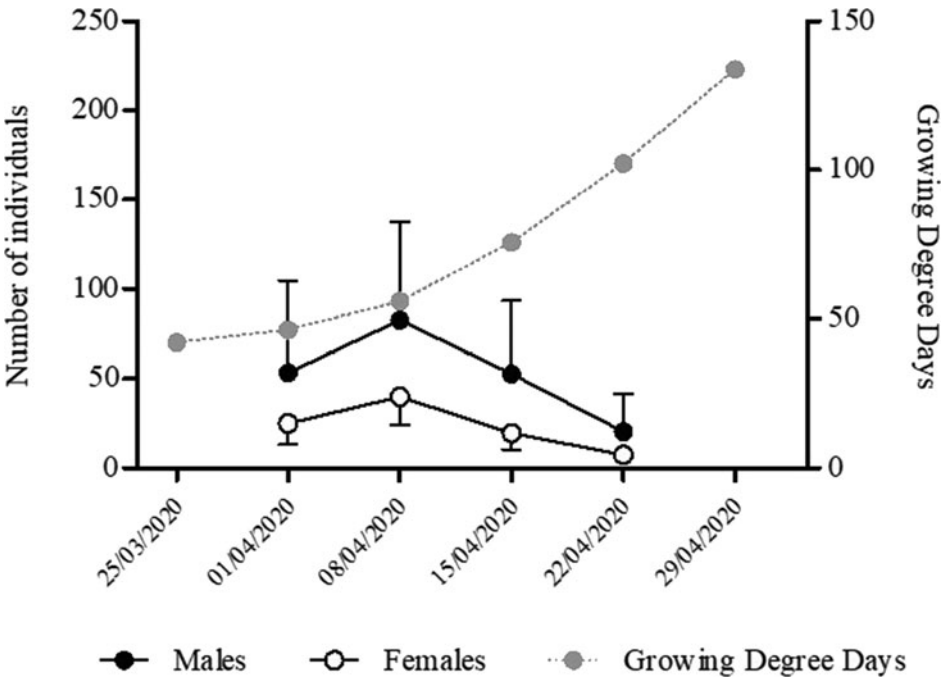

Figure 3. Flight curves of *D. oleae* (Diptera: Cecidomyiidae) males and females (mean ± SD) and phenology of *O. europaea* expressed as the sum of GDDs and BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) stages (9 and 33). Data were collected from 1 April 2020 using a standard transparent delta trap (Csalomon®) in an olive orchard placed in Gavorrano (Grosseto, Italy). On 8 April, a peak of *D. oleae* adults, both males and females, was recorded. On the same date, researchers observed that the external leaflets of *O. europaea* started to open and their tips intercrossed (9th stage on the BBCH scale) at GDD values of 55.9. On 15 April, olive shoots reached 30% of the final length (33rd stage of the BBCH scale), at 75.7 GDD.

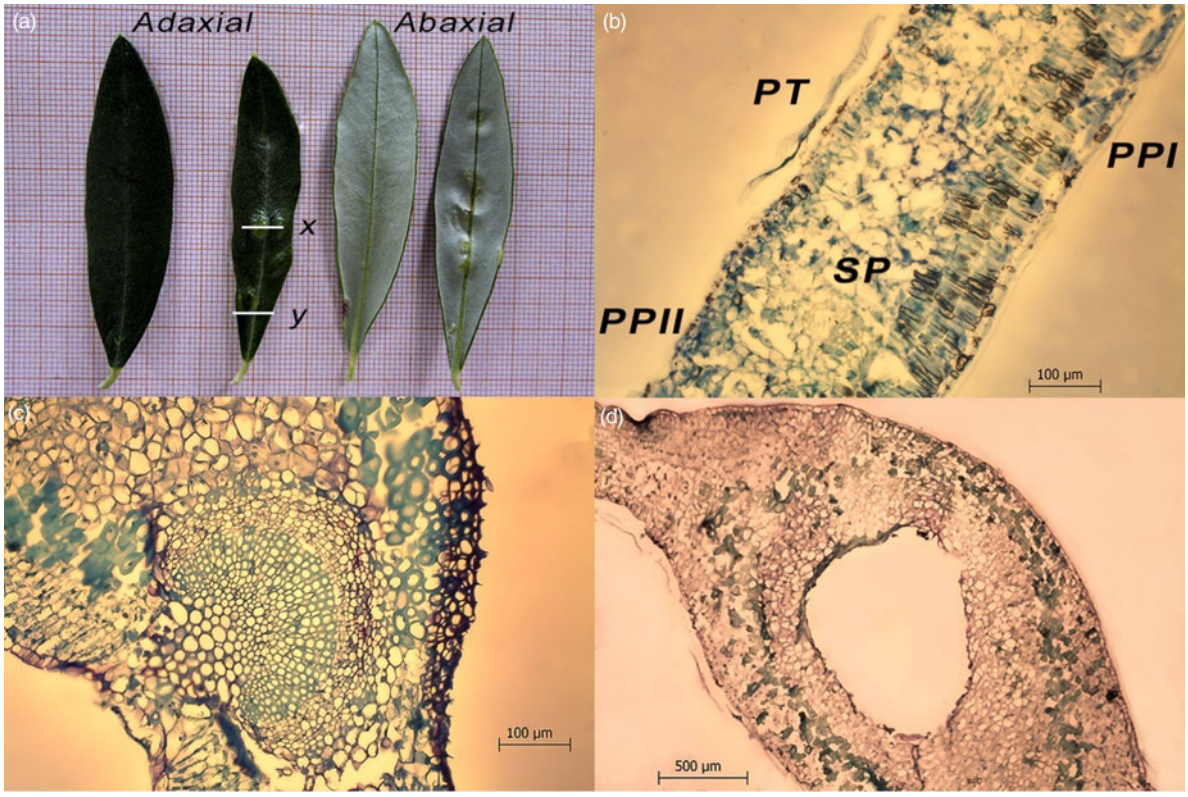

Figure 4. Adaxial and abaxial *O. europaea* (cv Frantoio) leaf surface in control and infested treatments (a) and cross sections of olive leaves stained with 0.01% toluidine blue (Sigma-Aldrich, St. Louis, Missouri, USA) and observed under an optical microscope (Fluophot, Nikon, Shinjuku, Japan) (b–d). (b) Section of a control leaf (10×): PPI and PPII, SP, and PT. (c) Leaves infested by *D. oleae* (Diptera: Cecidomyiidae): cell proliferation near the larval chamber (10×). The cut point for the cross section is represented in (a) with the letter 'y'. (d) Leaves infested by *D. oleae* (4×) showing the larval chamber. The cut point for the cross section is represented in (a) with the letter 'x'.

difference between mechanical wounding and insect activity, thus identifying the set of specific herbivore-induced volatiles. The analysis revealed that (*Z*)-3-hexenyl acetate was the main released compound after mechanical wounding. Indeed, this ester is known to be released from damaged leaves (Oluwafemi *et al.*, 2011), usually together with its original alcohol (*Z*)-3-hexen-

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1-ol (Kikuta *et al.*, 2011; Sufang *et al.*, 2013), which was also found in our collected VOCs. (*Z*)-3-Hexen-1-ol is found in a typical green leaf formed by lipoxygenase of linolenic acid of the cell membranes. Both 3-hexen-1-ol and (*Z*)-3-hexenyl acetate were indeed reported as the main VOCs collected by severed olive leaves in Malheiro *et al.* (2016) confirming their emission after cell membrane breakup. Among the 12 compounds previously reported as HIPVs by *D. oleae*, (*E*,*E*)-α-farnesene was also released following mechanical damage in our study, but the 20-fold rise of emission in the infested plants and the consistency of the data under both laboratory and field conditions confirmed the role of this sesquiterpene as HIPV (Giacomuzzi *et al.*, 2016).

The laboratory trials allowed for the use of uninfested plants, thus revealing four molecules (β-ocimene, cosmene, β-copaene, and unknown 1) released only in the plants that were attacked and not detected in the control plants. However, although cosmene, β-copaene, and unknown 1 were also consistently higher in the plants of the highly infested field, β-ocimene was higher in the highly infested field and in one less-infested field. Some compounds were reduced in the infested plants in the laboratory trials. γ-Cadinene, although completely absent in infested potted plants, was present in the mature field plants but without a difference between infestation levels. (*Z*)-3-Hexenyl acetate and 2-ethylhexanol, which are more abundant in control-potted plant collections, were always present in field trials. However, the lack of uninfested field trees prevented comparison with the volatile profiles of controlled conditions and understanding whether the emission of these VOCs was actually reduced due to infestation. For this reason, the number of galls in the sampled branch was used to predict the amount of volatiles. Linalool, β-copaen-4 α-ol, and heptadecane were found to be dependent on the number of galls. Although linalool and heptadecane are known as common HIPVs (Moayeri *et al.*, 2007; War *et al.*, 2011), β-copaen-4 α-ol has so far been reported only as a sesquiterpene characteristic of many plant species (Dória *et al.*, 2010; Magierowicz *et al.*, 2020), but not as an herbivory-induced compound. Moreover, β-copaen-4 α-ol was detected only in the field collection and not in the laboratory collection, thus suggesting an emission more related to mature plants or other parameters. Linalool, which was also not observed in the laboratory trial, is characteristic of highly infested plants in the field, together with DMNT, cosmene, α-copaene, (*E*,*E*)-α-farnesene, unknown 1, and heptadecane, already observed in potted plants as HIPVs. The reason linalool appeared only in the field collections might be dependent on the age or the phenological stage of the plant, since the plants used in the laboratory trials were young saplings. For instance, in Rao *et al.* (2010) older leaves of palmarosa (*Cymbopogon martinii* (Roxburgh) Watson) contained more linalool than young leaves. Also in hop (*Humulus lupulus* L.), the linalool content increased with the age of the leaves (Matsui *et al.*, 2012). Hence, β-copaen-4 α-ol and linalool might act as HIPVs only in mature plants. A possible effect due to plant age might also explain the difference in the compounds β-bourbonene, β-cubebene, β-elemene, β-copaene, and δ-amorphene between field L1 and fields L2 and H. These sesquiterpenoids were not present in the young potted saplings, thus appearing only in field plants, regardless of the infestation level. Sesquiterpenoids are often involved in communication between species since they can travel long distances via advective transport in gas (Huang and Osbourn, 2019). Other VOCs were detected only in the field collection, with no difference between fields (aromadendrene, α-muurolene, α-bisabolol, and unknown 4). Although certain studies pointed out differences in volatile emissions between young and old leaves (Takabayashi *et al.*, 1994; Rao *et al.*, 2010; Matsui *et al.*, 2012), only a few studies are available about these differences when comparing young (few-week-old saplings) and mature trees (30-year-old olive trees). In the study of Street *et al.* (1997), the emissions of *Pinus pinea* L. seemed to vary significantly from mature forest to young plantation, and age-related changes were found in the volatiles released by wounded phloem of *Picea abies* (L.) Karsten seedlings (Kännaste *et al.*, 2013). Whether similar changes can occur in broad-leaf species such as *O. europaea* has not been reported, but it might provide an explanation for the emissions observed in the present study. A genotype effect cannot be excluded, but it seems to be unlikely, since olive trees are reproduced mainly by cutting, and the cultivar chosen for the study was the same for all the trials.

The relationship between gall-inducing species and host plants is a complex association in which insects redirect plant physiology and, consequentially, the growth of the attacked organs for the purpose of gaining advantages (e.g., nourishment and shelter) (Shorthouse *et al.*, 2005). For this reason, the synchronization of gall-inducing insects with their host is fundamental, particularly for short-lived insects, such as cecidomyiids (Yukawa, 2000; Tondini and Petacchi, 2019). Furthermore, in a tritrophic interaction (host plant–pest–natural enemies) each component has its biological rhythm concerning the behavior and the physiology of all the members (Allemand *et al.*, 1994; Fantinou *et al.*, 1998; Turlings *et al.*, 1998; Zhang *et al.*, 2010). The efficiency of the tritrophic system is maintained by synchronizing the rhythms across the trophic levels that is influenced by biotic and abiotic factors (Zhang *et al.*, 2010). For instance, the emission of HIPVs by plants to recruit natural enemies of herbivore is a typical phenomenon involved in a synchronized tritrophic system (Zhang *et al.*, 2010). However, studies about rhythm synchronization in tritrophic interactions are currently scarce. This paper gives the first demonstration of the synchronization between *D. oleae* adults (both females and males) and the phenology of *O. europaea*. A greater number of *D. oleae* adults during the 2020 sampling campaign was observed on 8 April. This is in accordance with our previous data that recorded the highest amount of *D. oleae* exactly in the same period also during the previous years, although in those cases only females were recorded (Tondini and Petacchi, 2019). In the same flying period, the phenology of *O. europaea* reached the 9th stage of the BBCH scale, in which the external leaflets opened and their tips intercrossed (Cortés *et al.*, 2002). The GDD on 8 April was 55.9. However, the GDD recorded in conjunction with the 9th stage of the BBCH scale usually ranged between 70 and −100 (Marchi *et al.*, 2012). This phenomenon can be explained by the climatic conditions verified during the period between January and April 2020. The temperature during these months has never been too frigid, with a mean temperature of 9.9°C and a quite scarce rainfall, particularly in February (4.6 mm) (Regione Toscana, 2020). During March, rainfall was more abundant (57 mm) and concurrently with mild weather, abundant rainfall can be the cause of a vegetative restart of *O. europaea*, even if the GDD was not greater than 56 (Regione Toscana, 2020). During the third sampling date, on 15 April, researchers observed that the olive shoots reached 30% of the final length, classifying this phenological stage as the 33rd stage of the BBCH scale (Cortés *et al.*, 2002). During the whole period of sampling, *D. oleae* eggs were observed on olive leaflets, confirming the

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preference of this cecidomyiid for fresh leaves, unlike other gall midges colonizing hard leaves or other tissues, such as the yew gall midge, *Taxomyia taxi* (Inchbald) and the blueberry gall midge, *Dasineura oxycoccana* Johnson (Dernisky *et al*., 2005; Miller and Raman, 2019).

The hypothesis that the *D. oleae* sex ratio presents a bias in favor of females in the proportion of 2:1 by Hallett and Heal (2001) is confuted in the current study, having observed exactly the opposite relationship. However, this phenomenon may result in a false estimation, since during direct sampling from the field several secondary factors are considered, such as the differential mortality of sexes due to food quality and/or overwintering diapause (Tabadkani *et al*., 2012).

Leaf galls induced by cecidomyiid trophic activity generally show alterations in cells and tissues, with a consequent modification of the leaf blade shape (Albert *et al*., 2013; de Alcantara Guimarães *et al*., 2013). A basic study on the leaf tissue modifications as a consequence of *D. oleae* feeding action has been recently reported by our group (Caselli *et al*., 2021). We observed that the first-stage larva forms an entrance hole on the adaxial olive leaf surface and then it develops in a gallery within the SP of the leaves. Spongy parenchymatic tissue is often modified after gall agent attack, similar to *Clusia lanceolata* Cambessèdes following *Clusiamyia nitida* Maia trophic action (de Alcantara Guimarães *et al*., 2013) and *Copaifera langsdorffii* Desfontaines following midrib gall development (Oliveira and Isaias, 2010). Additionally, the feeding activity of *Dasineura mali* on the upper apple leaves surface, causes characteristic distortions followed by a change in the foliage color (Lo *et al*., 2015). Near the larval chamber of *D. oleae*, the olive SP cells divide and become spherical with a small intercellular space, such as in *Guarea macrophylla* Vahl subsp. *tuberculata* Vellozo (Kraus *et al*., 1996). Here, it is demonstrated that the formation of the larval chamber by *D. oleae* induces the development of new cell layers in the SP, as described for other cecidomyiids by Albert *et al*. (2013) and de Alcantara Guimarães *et al*. (2013). We observed a spongy hyperplastic parenchyma, as described for *Aspidosperma spruceanum* Benth. ex Müll. Arg. by Formiga *et al*. (2011) and consequentially, the galled leaf portion is thicker than the unaffected leaf portion. Further studies may focus on the metabolic compounds formed in the parenchyma tissues during the *D. oleae* gall development. Furthermore, parenchymal alteration may have a role in inducing a plant defense response (Sousa *et al*., 2020). Among plant defense strategies, the emission of HIPVs plays an important role in the recruitment of natural enemies that may control the attack of herbivore insect pests (Gebreziher, 2020).

Conclusions

The analysis of the volatiles emitted by plants infested by galls of *D. oleae* provided a clear set of 12 insect-induced compounds, and the data were consistent between the laboratory and the field trials. Following the insect attack, the emission amount of some VOCs increased significantly. Other HIPVs were not present in control plants and appeared only after the infestation. The sampling technique did not cause any mechanical damage that might interfere with the plant volatile emissions. Some volatiles seemed to be related to the age of the plant rather than to the infestation status.

This study confirms a close relationship between *D. oleae* and its exclusive host, *O. europaea*, highlighting a spring synchronization of midge flight activity and the vegetative restart of the olive tree. As a consequence of the larval feeding action, the olive leaves show modifications of the leaf shape blade due to the structural alteration of the spongy parenchymatic tissue. Further studies are needed to investigate the relationship between leaf structure modifications and VOCs emitted by *O. europaea*, shedding light on the ecological role of volatiles. Forthcoming behavioral tests might investigate whether the change in the volatile profile of the plant elicits a response in adults of *D. oleae* and its natural enemies.

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References

Abdeljelil Ben Z, Tekaya M, Mechri B, Flamini G and Hammami M (2017) Changes in volatiles of olive tree *Olea europaea* according to season and foliar fertilization. *International Journal of Agriculture and Biology* **19**, 1633–1639.

Abraham J, Giacomuzzi V and Angeli S (2015) Root damage to apple plants by cockchafer larvae induces a change in volatile signals below and aboveground. *Entomologia Experimentalis et Applicata* **156**, 279–289.

Alagna F, Kallenbach M, Pompa A, De Marchis F, Rao R, Baldwin IT, Bonaventure G and Baldoni L (2016) Olive fruit infested with olive fly larvae respond with an ethylene burst and the emission of specific volatiles. *Journal of Integrative Plant Biology* **58**, 413–425.

Albert S, Rana S and Gandhi D (2013) Anatomy and ontogenesis of foliar galls induced by *Odinadiplosis odinae* (Diptera: Cecidomyiidae) on *Lannea coramandelica* (Anacardiacee). *Acta Entomologica Serbica* **18**, 161–175.

Allemand R, Pompanon F, Fleury F, Fouillet P and Bouletreau M (1994) Behavioral circadian rhythms measured in real-time by automatic image analysis: applications in parasitoid insects. *Physiological Entomology* **19**, 1–8.

Arimura G and Pearse IS (2017) From the lab bench to the forest. How plants communicate with their biotic environment. *Advances in Botanical Research* **82**, 3–17.

Arimura GI, Garms S, Maffei M, Bossi S, Schulze B, Leitner M, Mithöfer A and Boland W (2008) Herbivore-induced terpenoid emission in *Medicago truncatula*: concerted action of jasmonate, ethylene and calcium signaling. *Planta* **227**, 453–464.

Babushok VI, Linstrom PJ and Zenkevich IG (2011) Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data* **40**, 043101.

https://doi.org/10.1017/S0007485321001000 Published online by Cambridge University Press

Baratella V (2011) *Development of a Continuous Small-Rearing technique on Natural Host for the Olive Fruit fly Bactrocera oleae (Rossi) and Study on Volatile Organic Compounds (VOCs) Emitted by Olea europaea L. as Potential host allelochemics* (PhD thesis). University of Tuscia, Viterbo, Italy.

Bengtsson M, Bäckman AC, Liblikas I, Ramirez MI, Borg-Karlson AK, Ansebo L, Anderson P, Löfqvist J and Witzgall P (2001) Plant odor analysis of apple: antennal response of codling moth females to apple volatiles during phenological development. *Journal of Agricultural and Food Chemistry* **49**, 3736–3741.

Borges RM (2018) The galling truth: limited knowledge of gall-associated volatiles in multitrophic interactions. *Frontiers in Plant Science* **9**, 1139.

Brahmi F, Flamini G, Issaoui M, Dhibi M, Dabbou S, Mastouri M and Hammami M (2012) Chemical composition and biological activities of volatile fractions from three Tunisian cultivars of olive leaves. *Medicinal Chemistry Research* **21**, 2863–2872.

Bruinsma M and Dicke M (2008) Herbivore-induces indirect defence: from induction mechanisms to community ecology. In Schaller A (ed.), *Induced Plant Resistance to Herbivory*. Berlin, Germany: Springer, pp. 31–60.

Campeol E, Flamini G, Chericoni S and Catalano S (2001) Volatile compounds from three cultivars of *Olea europaea* from Italy. *Journal of Agricultural and Food Chemistry* **49**, 5409–5411.

Caselli A, Francini A, Minnocci A and Petacchi R (2021) *Dasineura oleae*: morphological and physiological characterization following the midge attack on olive leaves. *Journal of Plant Disease and Protection* **128**, 173–182.

Cortés FS, Martínez-Calvo J, Badenes ML, Bleiholder H, Hack H, Llácer G and Meier U (2002) Phenological growth stages of olive trees (*Olea europaea*). *Annals of Applied Biology* **140**, 151–157.

de Alcantara Guimarães AL, Brasil Bizarri CH, Silva Barbosa L, Nakamura MJ, Freiman de Souza Ramos M and de Macêdo Vieira AC (2013) Characterisation of the effects of leaf galls of *Clusiamyia nitida* (Cecidomyiidae) on *Clusia lanceolate* Cambess. (Clusiaceae): anatomical aspects and chemical analysis of essential oil. *Flora* **208**, 165–173.

DeClerck-Floate R and Price PW (1994) Impact of a bud-galling midge on bud populations of *Salix exigua*. *Oikos* **70**, 253–260.

Dernisky AK, Evans RC, Liburd OE and Mackenzie K (2005) Characterization of early floral damage by cranberry tipworm (*Dasineura oxycoccana* Johnson) as a precursor to reduced fruit set in rabbiteye blueberry (*Vaccinium ashei* Reade). *International Journal of Pest Management* **51**, 143–148.

Dicke M (2009) Behavioural and community ecology of plants that cry for help. *Plant, Cell and Environment* **32**, 654–665.

Dicke M and Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science* **15**, 167–175.

Dogănlar M, Sertkaya E and Skuhrava M (2011) Pest status of olive leaf gall midge *Dasineura oleae* (Angelini, 1831), description of *Lasioptera oleicola* Skuhravá sp. New (Diptera: Cecidomyiidae) and effectiveness of parasitoids on their populations in Hatay Turkey. *Turkiye Entomolojii Dergisi-Turkish Journal of Entomology* **35**, 265–284.

Dória GA, Silva WJ, Carvalho GA, Alves PB and Cavalcanti SC (2010) A study of the larvicidal activity of two *Croton* species from northeastern Brazil against *Aedes aegypti*. *Pharmaceutical Biology* **48**, 615–620.

Dursun A, Güler Z, Özkan D and Konuşkan DB (2017) Identification of volatile compounds (VCs) in the leaves collected from 'Gemlik', 'Halhalı' and 'Sarı Hasebi' olive tree varieties. *International Journal of Secondary Metabolite* **4**, 195–204.

Fantinou AA, Alexandri MP and Tsitsipis JA (1998) Adult emergence rhythm of the egg-parasitoid *Telenomus busseolae*. *BioControl* **43**, 141–151.

Flamini G, Cioni PL and Morelli I (2003) Volatiles from leaves, fruits, and virgin oil from *Olea europaea* cv. Olivastra Seggianese from Italy. *Journal of Agricultural and Food Chemistry* **51**, 1382–1386.

Formiga AT, Gonçalves Soares GL and dos Santos Isaias RM (2011) Responses of the host plant tissues to gall induction in *Aspidosperma spruceanum* Müell. Arg. (Apocynaceae). *American Journal of Plant Sciences* **2**, 823–834.

Gebreziher HG (2020) Advances in herbivore-induced plant volatiles (HIPVs) as plant defense and application potential for crop protection. *International Journal of Botany Studies* **5**, 29–36.

Gershenzon J (2007) Plant volatiles carry both public and private messages. *Proceedings of the National Academy of Science of the USA* **104**, 5257–5258.

Giacomuzzi V, Cappellin L, Khomenko I, Biasioli F, Schütz S, Tasin M, Knight AL and Angeli S (2016) Emission of volatile compounds from apple plants infested with *Pandemis heparana* larvae, antennal response of conspecific adults, and preliminary field trial. *Journal of Chemical Ecology* **42**, 1265–1280.

Giunti G, Benelli G, Flamini G, Michaud JP and Canale A (2016a) Innate and learned responses of the tephritid parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae) to olive volatiles induced by *Bactrocera oleae* (Diptera: Tephritidae) infestation. *Journal of Economic Entomology* **109**, 2272–2280.

Giunti G, Benelli G, Conte G, Mele M, Caruso G, Gucci R, Flamini G and Canale A (2016b) VOCs-mediated location of olive fly larvae by the braconid parasitoid *Psyttalia concolor*: a multivariate comparison among VOC bouquets from three olive cultivars. *BioMed Research International* **2016**, 7827615.

Giunti G, Benelli G, Palmeri V and Canale A (2018) *Bactrocera oleae*-induced olive VOCs routing mate searching in *Psyttalia concolor* males: impact of associative learning. *Bulletin of Entomological Research* **108**, 40–47.

Gonzales WL, Caballero PP and Medel R (2005) Galler-induced reduction of shoot growth and fruit production in the shrub *Colliguaja integerrima* (Euphorbiaceae). *Revista Chilena de Historia Natural* **78**, 393–399.

Hall DR, Amarawardana L, Cross JV, Francke W, Boddum T and Hillbur Y (2012) The chemical ecology of cecidomyiid midge. *Journal of Chemical Ecology* **38**, 2–22.

Hallett RH and Heal J (2001) First Nearctic record of the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), a pest of cruciferous crops in Europe. *The Canadian Entomologist* **133**, 713–715.

Heil M (2014) Herbivore-induced plant volatiles: targets, perception and unanswered questions. *New Physiologist* **204**, 297–306.

Huang A and Osbourn A (2019) Plant terpenes that mediate below-ground interactions: prospects for bioengineering terpenoids for plant protection. *Pest Management Science* **75**, 2368–2377.

Huang MY, Chou HM, Chang YT and Yang CM (2014) The number of cecidomyiid insect galls affects the photosynthesis of *Machilius thunbergii* host leaves. *Journal of Asia-Pacific Entomology* **17**, 151–154.

Kännaste A, Zhao T, Lindström A, Stattin E, Långström B and Borg-Karlson AK (2013) Odors of Norway spruce (*Picea abies* L.) seedlings: differences due to age and chemotype. *Trees* **27**, 149–159.

Kaplan I (2012) Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? *Biological Control* **60**, 77–89.

Kassambara A and Mundt F (2020) Factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. Available at https://CRAN.R-project.org/package=factoextra.

Kikuta Y, Ueda H, Nakayama K, Katsuda Y, Ozawa R, Takabayashi J, Hatanak A and Matsuda K (2011) Specific regulation of pyrethrin biosynthesis in *Chrysanthemum cinerariaefolium* by a blend of volatiles emitted from artificially damaged conspecific plants. *Plant & Cell Physiology* **52**, 588–596.

Kraus JE, Sugiara HC and Cutrupi S (1996) Morfologia e ontogenia em galhas entomogenas de *Gurea macrophylla* subsp. of *tuberculata* (Meliaceae). *Fitopatologia Brasileira* **21**, 349–356.

Kunert M, David A, Becher J and Boland W (2009) Volatile sampling from biological sources by the closed-loop-stripping technique. *Cold Spring Harbor Protocols* **2009**, pdb.prot5233.

Lo PL, Walker JTS and Suckling DM (2015) Prospects for the control of apple leaf midge *Dasineura mali* (Diptera: Cecidomyiidae) by mass trapping with pheromone lures. *Pest Management Science* **71**, 907–913.

Magierowicz K, Górska-Drabik E and Sempruch C (2020) The effect of *Tanacetum vulgare* essential oil and its main components on some ecological and physiological parameters of *Acrobasis advenella* (Zinck.) (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology* **162**, 105–112.

https://doi.org/10.1017/S0007485321001000 Published online by Cambridge University Press

Malheiro R, Ortiz A, Casal S, Baptista P and Pereira JA (2015) Electrophysiological response of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) adults to olive leaves essential oils from different cultivars and olive tree volatiles. *Industrial Crops and Products* **77**, 81–88.

Malheiro R, Casal S, Cunha SC, Baptista P and Pereira JA (2016) Identification of leaf volatiles from olive (*Olea europaea*) and their possible role in the ovipositional preferences of olive fly, *Bactrocea oleae* (Rossi) (Diptera: Tephritidae). *Phytochemistry* **121**, 11–19.

Marchi S, Giudotti D, Ricciolini M and Sebastiani L (2012) Un esempio di supporto on line alle decisioni per gli olivicoltori. *L'Informatore Agrario* **4**, 60.

Martinez E, Montenegro G and Elgueta M (1992) Distribution and abundance of two gall makers on the euphorbiaceous shrub *Colliguaia odorifera*. *Revista Chilena de Historia Natural* **65**, 75–82.

Matsui H, Inui T, Ishimaru M, Hida Y and Oka K (2012) The influence of the age of a hop plant on the quality of hop aromas in beer. In *III International Humulus Symposium 1010*, pp. 171–182.

Miller DG and Raman A (2019) Host-plant relations of gall-inducing insects. *Annals of the Entomological Society of America* **112**, 1–19.

Moayeri HRS, Ashouri A, Poll L and Enkegaard A (2007) Olfactory response of a predatory mirid to herbivore induced plant volatiles: multiple herbivory vs. single herbivory. *Journal of Applied Entomology* **131**, 326–332.

Oliveira DC and Isaias RMS (2010) Redifferentiation of leaflet tissue during midrib gall development in *Copaifera langsdorffii* (Fabaceae). *South African Journal of Botany* **76**, 239–248.

Oluwafemi S, Bruce TJA, Pickett JA, Ton J and Birkett MA (2011) Behavioral responses of the leafhopper, *Cicadulina storeyi* China, a major vector of maize streak virus, to volatile cues from intact and leafhopper-damaged maize. *Journal of Chemical Ecology* **37**, 40–48.

Orlandi F, Ruga L, Romano B and Fornaciari M (2005) Olive flowering as an indicator of local climatic changes. *Theoretical and Applied Climatology* **81**, 169–176.

Picchi MS, Tondini E, Albertarelli N, Monteforti G and Petacchi R (2021) Following the outbreak: preliminary findings on the landscape effect on *Dasineura oleae* and its parasitoids in Central Italy. *Phytoparasitica*. doi: 10.21203/rs.3.rs-174210/v

Preti M, Knight AL, Mujica VM, Basoalto E, Larsson Herrera S, Tasin M and Angeli S (2021*a*) Development of multi-component non-sex pheromone blends to monitor both sexes of *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Applied Entomology* **145**, 822–830.

Preti M, Favaro R, Knight AL and Angeli S (2021*b*) Remote monitoring of *Cydia pomonella* adults among an assemblage of nontargets in sex pheromone-kairomone-baited smart traps. *Pest Management Science* **77**, 4084–4090.

Ranamukhaarachchi SL and Wickramarachchi KS (2007) Color preference and sticky traps for field management of thrips *Ceratothripodes claratris* (Thysanoptera: Thripidae) in tomato in central Thailand. *International Journal of Agriculture and Biology* **9**, 392–397.

Rao BRR, Rajput DK, Patel RP and Purnanand S (2010) Essential oil yield and chemical composition changes during leaf ontogeny of palmarosa (*Cymbopogon martinii* var. Motia). *Natural Product Communications* **5**, 1947–19450.

R Core Team (2020) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at https://www.R-project.org/ (accessed February, 2021).

Regione Toscana (2020) Settore Idrogeologico Regionale. Available at http://www.sir.toscana.it/ (accessed February 2021).

Regnault-Roger C and Philogène BJR (2008) Past and current prospects for the use of botanicals and plant allelochemicals in integrated pest management. *Pharmaceutical Biology* **46**, 41–52.

Reymond P (2013) Perception, signaling and molecular basis of oviposition-mediated plant responses. *Planta* **238**, 247–258.

Rodriguez-Saona CR and Stelinski LL (2009) Behavior-modifying strategies in IPM: theory and practice. In Peshin R and Dhawan AK (eds), *Integrated Pest Management: Innovation-Development Process*. Dordrech: Springer, pp. 263–315.

Rodriguez-Saona C, Crafts-Brandner SJ, Paré PW and Henneberry TJ (2001) Exogenous methyl jasmonate induces volatile emissions in cotton plants. *Journal of Chemical Ecology* **27**, 679–695.

Rohfritsch O and Shorthouse JD (1982) Insect gall. In Kahl G and Schell J (eds), *Molecular Biology of Plant Tumor*. New York, USA: Academic Press, pp. 131–152.

Scarpati MS, Lo Scalzo R and Vita G (1993) Volatiles attractive and repellent to the olive fruit fly (*Dacus oleae*, Gmelin). *Journal of Chemical Ecology* **19**, 881–891.

Shorthouse JD, Wool D and Raman A (2005) Gall-inducing insect-nature's most sophisticated herbivores. *Basic and Applied Ecology* **6**, 407–411.

Simoglou KB, Karataraki A, Roditakis NE and Roditakis E (2012) *Euzophera bigella* (Zeller) (Lepidoptera: Pyralidae) and *Dasineura oleae* (F. Low) (Diptera: Cecidomyiidae): emerging olive crop pests in the Mediterranean? *Journal of Pest Science* **85**, 169–177.

Sousa ALV, Silva DB, Silva GG, Bento JMS, Penãflor MFGV and Souza B (2020) Behavioral response of the generalist predator *Orius insidiosus* to single and multiple herbivory by two cell content-feeding herbivores on rose plants. *Arthropod–Plant Interactions* **14**, 227–236.

Street RA, Owen S, Duckham SC, Boissard C and Hewitt CN (1997) Effect of habitat and age on variations in volatile organic compound (VOC) emissions from *Quercus ilex* and *Pinus pinea*. *Atmospheric Environment* **31**, 89–100.

Suckling DM, Twidle AM, Gibb AR, Manning LM, Mitchell VJ, Sullivan TES, Wee SL and El-Sayed AM (2012) Volatiles from apple trees infested with light brown apple moth larvae attract the parasitoid *Dolichogenidia tasmanica*. *Journal of Agricultural and Food Chemistry* **60**, 9562–9566.

Sufang Z, Jianing W, Zhen Z and Le K (2013) Rhythms of volatiles release from healthy and insect-damaged *Phaseolus vulgaris*. *Plant Signaling & Behavior* **8**, 10.

Tabadkani SM, Ashouri A, Rahimi-Alangi V and Fathi-Moghaddam M (2012) When to estimate sex ratio in natural population of insects? A study on sex ratio variation of gall midges within a generation. *Entomological Science* **16**, 54–59.

Takabayashi J, Dicke M, Takahashi S, Posthumus MA and Van Beek TA (1994) Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. *Journal of Chemical Ecology* **20**, 373–386.

Tondini E and Petacchi R (2019) First observations on the parasitoids complex and on the biology of *Dasineura oleae* during an outbreak in Tuscany, Italy. *Bulletin of Insectology* **72**, 93–102.

Turlings TCJ, Lengwiler UB, Bernasconi ML and Wechsler D (1998) Timing of induced volatile emission in maize seedlings. *Planta* **207**, 146–152.

Van den Dool H and Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography A* **11**, 463–471.

War AR, Sharma HC, Paulraj MG, War MY and Ignacimuthu S (2011) Herbivore induced plant volatiles: their role in plant defense for pest management. *Plant Signaling & Behavior* **6**, 1973–1978.

Whitfield J (2001) Marking crops cry for help. *Nature* **410**, 736–737.

Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Wilke CO (2020) cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.0. Available at https://CRAN.R-project.org/package=cowplot (accessed February 2021).

Yukawa J (2000) Synchronization of gallers with host plant phenology. *Population Ecology* **42**, 105–113.

Zeng L, Liao Y, Li J, Zhou Y, Tang J, Dong F and Yang Z (2017) α-Farnesene and ocimene induce metabolite changes by volatile signaling in neighboring tea (*Camellia sinensis*) plants. *Plant Science* **264**, 29–36.

Zhang S, Wei J, Guo X, Liu TX and Kang L (2010) Functional synchronization of biological rhythms in a tritrophic system. *PLoS ONE* **5**, e11064.

Zong S, Zhang H and Kampungu G (2012) Mechanism of herbivore-induced plant volatiles in host plant selection by herbivorous insects. *Entomologia Generalis* **33**, 251–261.