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RECEIVED 10 December 2025
REVISED 08 May 2026
ACCEPTED 01 June 2026
PUBLISHED 22 June 2026

CITATION

Cao L, Vrecko V, Huguenin J,
Buatois B, Ormeño E, Bagnères A-G
and Proffit M (2026) Emission
of volatile organic compounds in
Mediterranean fig trees bearing
receptive flowers: dynamics under
diurnal temperature light conditions.
Front. Ecol. Evol. 14:1764436.
doi: 10.3389/fevo.2026.1764436

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Emission of volatile organic compounds in Mediterranean fig trees bearing receptive flowers: dynamics under diurnal temperature light conditions

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Floral scents are formed by a complex mixture of Volatile Organic Compounds (VOCs) which play major roles in mediating encounter with partners in plant-pollinator interactions. Hence, VOC temporal patterns may be regulated to respond to both pollinator's activity and pollination status. In the nursery pollination mutualism between the dioecious fig tree, *Ficus carica*, and its exclusive pollinator the fig wasp, *Blastophaga psenes*, it has been reported that VOCs emitted by receptive figs mediate species-specific interaction and that a combination of four VOCs - benzyl alcohol, (S)-linalool, (Z)-linalool oxide (furanoid) and (E)-linalool oxide (furanoid) - are responsible for pollinator attraction. However, the temporal pattern of their emissions during the day has never been studied. Previous studies on floral scents have mostly relied on offline collection methods that provide only discrete snapshots of emission rates. To bridge this gap and capture the continuous 'pulse' of floral signaling, we employed real-time Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOF-MS). This high-resolution approach allows us to characterize, for the first time, the fine-scale diurnal kinetics of VOC emissions in the *F. carica* nursery pollination system and to assess how these signals withstand natural environmental fluctuations. In addition, we estimated the influence of light intensity and temperature on these emissions under semi-natural conditions, based on simultaneous measurements and statistical modeling. Emissions of VOCs from flowering trees followed a diurnal pattern with a peak at midday, corresponding to the period when the pollinator is most active, with very conserved proportions of VOCs attractive to pollinators around this peak. Our results indicate that VOC emissions exhibited a diurnal rhythm closely associated with the natural fluctuation of temperature and

light. While the conditions used in our studies did not allow us to disentangle the effects of these two factors, our analyses suggest nonlinear relationships between these factors and the emissions of compounds attractive to pollinators. Further experimental studies are needed on *F. carica*, as well as on other species, to better assess how light intensity and temperature drive floral VOC emissions.

KEYWORDS

abiotic factors, figs, pollinator attraction, PTR-ToF-MS, rhythmic emission, volatile organic compounds

1 Introduction

Floral Volatile Organic Compounds (VOCs) play major roles in the interactions between plants and their biotic environment by attracting pollinators and natural enemies of herbivores, while deterring herbivores and pathogens (Agrawal, 2011; Becerra et al., 2009; Dötterl and Gershenzon, 2023; Raguso, 2008; Junker and Parachnowitsch, 2015). The production and emission of floral VOCs can be very variable over time. Floral VOCs emission is often developmentally and rhythmically regulated (Picazo-Aragónés et al., 2020) and influenced by the endogenous biological clock, i. e. circadian clock (Zeng et al., 2017). For example, in *Hoya carnososa*, a circadian rhythm of floral VOCs emission has been reported using 3-hour sampling intervals, and this rhythm was maintained even during exposure to continuous dark (Matile and Altenburger, 1988). Also, temporal variation between day and night in floral VOC emissions has been demonstrated in snapdragons and petunias based on measurements taken every 4 hours (Kolosova et al., 2001; Fenske et al., 2015). Nevertheless, a very limited number of studies (Marotz-Clausen et al., 2018; Powers et al., 2020) have examined the temporal variation in VOC emissions on a very fine time scale and the factors that may influence these emission patterns.

As floral odors are crucial for plant reproduction (Dötterl and Gershenzon, 2023; Raguso, 2008; Hossaert et al., 2010), the timing of pollinator activities and attraction are major factors driving the temporal pattern of floral VOC emission. Many flowers exhibit daily patterns of scent emission (Delle-Vedove et al., 2017; Powers et al., 2020), some of which can be linked to pollinator activity (Balao et al., 2011; Friberg et al., 2014; Majetic et al., 2015; Chapurlat et al., 2018). For instance, diacetin emission peaks during the day, allowing the oil bee to locate their oil secreting host plants (Schäffler et al., 2015). Flowers of *Petunia axillaris* (Hoballah et al., 2005; Oyama-Okubo et al., 2005), *Nicotiana attenuata* (Kessler et al., 2008; Yon et al., 2016) and *Myrtaceae* species (Cordeiro et al., 2019) emit a greater quantity of VOCs at night compared to the day to attract night-active pollinators. The timing of scent emission in blueberry flowers aligns with the activity period of their pollinator (Rodríguez-Saona et al., 2011). Furthermore, the composition of these floral scents corresponds to the antennal sensitivity of these pollinators (Hoballah et al., 2005).

In addition, abiotic factors can modulate the emission of floral VOCs in long and short-term (Borghi et al., 2019; Farré-Armengol et al., 2020). For example, environmental conditions occurring during the plant development such as temperature, precipitation or carbon dioxide (CO₂) concentration can affect floral VOCs emission (Cordeiro

and Dötterl, 2023). In addition to long-term effects of abiotic factors, ambient environmental conditions can also affect VOCs emission by flowers. In particular, VOC emissions by plants are strongly associated with temperature and light intensity (Borghi et al., 2019; Hu et al., 2013) via different mechanisms: (1) through their direct effects on VOCs biosynthesis (Cna'ani et al., 2015); (2) indirectly by affecting various other plant physiological traits such as photosynthesis and therefore carbon availability for VOCs production (Loreto et al., 1996; Niinemets et al., 2002; Rasulov et al., 2009); (3) through their effects on the physicochemical properties of VOCs such as volatility (Niinemets et al., 2004). These multiple effects result in complex emission patterns of floral VOCs following increases in temperature and/or light intensity. Floral emission of various Mediterranean plants has been reported to increase with temperature up to an optimum, that coincided to the maximum temperatures that plant species can experience during flowering, and thereafter decreased (Farré-Armengol et al., 2014). Moreover, distinct classes of VOCs, or even of compounds, often differentially respond to temperature and light intensity (Niinemets and Reichstein, 2002; Llusà and Peñuelas, 2000; Farré-Armengol et al., 2020). These differential effects of temperature and light intensity on the emission rates of certain volatile compounds could result in a change in the relative proportions of VOCs in the floral odor. This variation can pose a significant ecological challenge, particularly for specialized nursery pollination mutualisms where signal fidelity is critical for both partner reproduction (Hossaert et al., 2010).

The Mediterranean fig tree, *Ficus carica* L. (Moraceae), represents a perfect model to investigate the timing of floral VOC emission and the factors affecting this dynamic. *Ficus* trees possess figs characterized by an enclosed wall with a unique entrance (i.e., the ostiole) with the hundreds of tiny florets lining the inside of a central cavity, the inflorescences. Figs are exclusively pollinated by agaonid wasps, *Blastophaga psenes* L. (Agaonidae), that develop exclusively in the figs of their host species. Once emerging from its natal fig, pollinators live less than 24 hours. Pollinators are active exclusively during the day. It has been reported that receptive figs of *F. carica* emitted 36 VOCs (mainly terpenoid compounds) at the receptive stage (i.e., in the developmental stage when they are ready to be pollinated) (Soler et al., 2012) although this wasp only uses four of them (benzyl alcohol, (S)-linalool, (Z)-linalool oxide and (E)-linalool oxide) within a particular proportion to locate their host (Proffit et al., 2020). Previous studies have shown that receptive periods of *F. carica* can last for more than two weeks, and that after the introduction of at least three wasps into a receptive fig, receptivity stops in less than 72h (Hossaert-McKey et al., 1994; Khadari et al., 1995). While strong selection pressure likely favors signal stability, it remains unknown whether and how the strict

chemical specificity required for attraction is maintained under the substantial diurnal fluctuations of temperature and light intensity that occur during the receptive period.

To date, very few studies have performed real-time measurements of floral VOCs to understand their high-resolution temporal dynamics (but see Powers et al., 2020; Losch et al., 2023). This is partly because Proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS), the most appropriate technique for this type of study, while offering high time-resolution, cannot separate compounds with the same molecular weight. However, the *F. carica* system presents a unique opportunity to overcome this limitation. Since the specific VOCs responsible for attracting its pollinator have been fully characterized (Proffit et al., 2020) and verified with synthetic standards (Dubuisson et al., 2024), we can utilize PTR-ToF-MS in a targeted manner to accurately characterize the fine-scale emission dynamics of these specific compounds and describe the effect of key abiotic factors on their emission.

The aim of this study was to characterize: (1) the daily emission patterns of VOCs in fig trees bearing receptive figs of *F. carica*; (2) the effects of light intensity and temperature on receptive fig emissions. We hypothesize that the emission of attractive VOCs is synchronized with the diurnal activity of the pollinator. Specifically, we predict that VOC emissions will track the natural diurnal rise in temperature and light intensity, peaking at mid-day when *B. psenes* is most active. Following the patterns observed in other Mediterranean plants (Farré-Armengol et al., 2014), we also expect a non-linear response, where emissions may stabilize or decrease if afternoon temperatures exceed physiological optima.

To test our hypothesis, we performed a study in a greenhouse under semi-natural conditions using fig trees of *F. carica* bearing inflorescence that were maintained receptive during 72 hours. Released floral VOCs were studied both using real-time analysis by PTR-ToF-MS and headspace collection and Gas chromatography - Mass Spectrometry (GC-MS) analyses during two different moments of the day. We focused particularly on the VOCs responsible for attracting pollinators, as well as sesquiterpenes, which are also emitted by receptive figs but do not play a role in attracting pollinators. During the 72 hours, we measured light intensity and temperature to test their possible association with VOCs emission. Unlike studies performed in climate chambers under strictly controlled constant environmental factors, this study was conducted in a greenhouse under semi-natural conditions. This approach allows us to monitor VOC emission dynamics in a realistic scenario where temperature and light fluctuate concomitantly, aiming to reflect as closely as possible the actual environmental variability encountered by the fig trees and their pollinators.

2 Methods and materials

2.1 Study system and site

Ficus carica is a dioecious fig species with the male tree bearing figs where its specific pollinator, fig-wasp, oviposits and develops, and female tree bearing figs that produce seeds where pollinators

cannot either oviposit or develop (Kjellberg et al., 1987). Male fig tree bear figs twice a year (in spring and summer) and female only once (in summer) (for more details about the reproductive biology see Kjellberg and Lesne, 2020; Proffit et al., 2020).

The experiment took place in spring (April-May) 2022, when males of the fig tree bear receptive figs for the first time of the year, in the greenhouse of the Terrain d'Experience (TE) technical platform of the Laboratoire d'Excellence Centre Méditerranéen Environnement et Biodiversité (LabEx CeMEB) in the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE, CNRS Montpellier, France). In the greenhouse, the temperature was controlled and fixed at 25 °C in the day and 15 °C during the night under natural light conditions (approximately 15 h/9 h, day/night periods, consistent with the spring season). We used 6-year-old trees grown in 7L pots, obtained by cuttings of male wild fig trees provided by nurserymen (Pépinières Baud[®], <http://www.fig-baud.com>). These potted trees were maintained in a steam-sterilized greenhouse substrate consisting of approximately a 2:1 mixture of recycled multi-purpose soil and brown peat. Plants were irrigated with 1 to 2 liters of water twice a week, allowing the soil surface to dry between waterings to maintain optimal moisture and avoid waterlogging. During initial repotting, a slow-release fertilizer (Osmocote 16-8-12 + 2MgO+TE) was applied into the substrate. Crucially, to avoid any nutrient-induced or stress-induced physiological variations, no additional fertilizers or chemical pesticides were applied to the plants leading up to and during the 72-hour experimental period. Prior to being enclosed in the PTFE chambers, all selected trees were visually inspected and confirmed to be completely healthy and free from any signs of pest infestation or diseases.

2.2 Experimental setup

The fig trees bearing 1–3 figs receptive were covered by a nylon bag to prevent pollinator entrance. The VOCs emitted by the fig trees were studied using a dynamic push-pull system. Plants were individually enclosed in approximately 25 L inert and odorless PTFE (polytetrafluoroethylene) frame closed by a 50 µm thick PTFE film (Figure 1A). Air was pushed into the chambers using a PTFE pump at 3500 mL min⁻¹ (KNF, N816.1.2K.18[®], Germany) and a PTFE fan ensured air mixing in the chambers. Air previously passed through a charcoal filter to scrub ambient VOC. Air flow was controlled by a flow meter and pulled directly from the experimental chambers into the PTR-ToF-MS at a flow rate of 100 mL min⁻¹, with all tubing lines being made of PTFE. The PTR-ToF-MS instrument was safely positioned inside the greenhouse facility, in close proximity to the experimental chambers, to minimize the length of the sampling lines and ensure high-resolution real-time measurements. For each series of measurements, we used three chambers: one empty chamber as blank control and two with a fig tree bearing receptive figs. The experiment was repeated five times, with each experiment lasting 72 hours, so there are 10 replications for the receptive fig trees.

During the 72 hours of the experiment, light intensity (photosynthetically active radiation, PAR) and temperature were recorded continuously every 10 min. Light intensity was measured on top of the chambers using a PAR sensor (SQ-100X-SS) connected to a

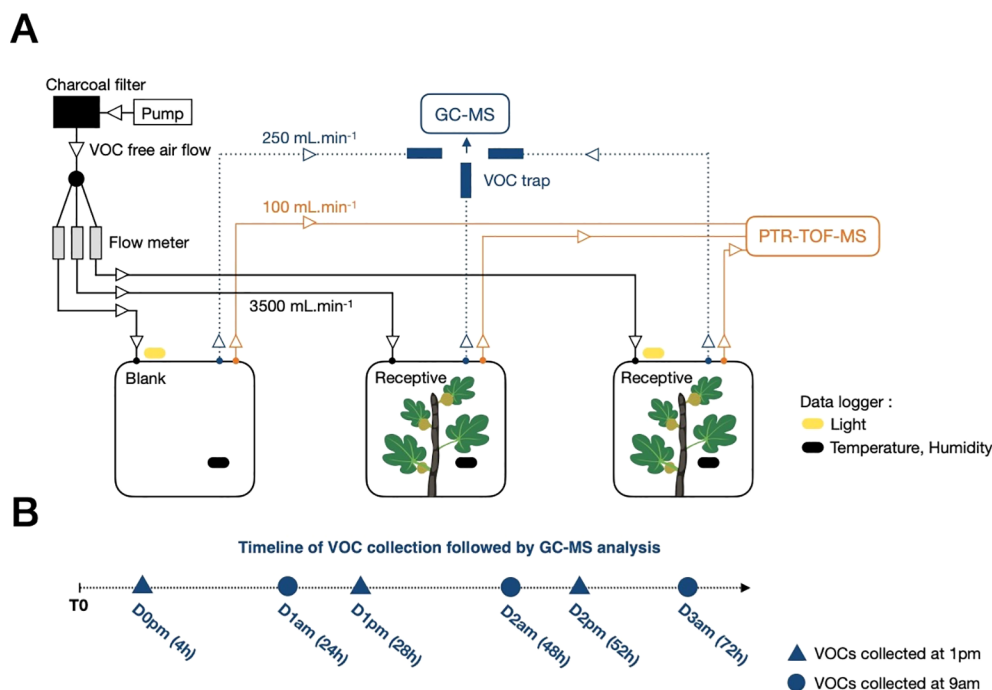


FIGURE 1

(A) Experimental setup for the study of VOC emission rates from *F. carica*. Three plants were individually enclosed in an inert and odorless polytetrafluoroethylene (PTFE) chamber. Air was pushed using a PTFE pump through a carbon filter into the chambers at 3500 mL min⁻¹. A PTFE fan ensured air mixing in the chambers. Air flow rates were controlled by flowmeters and all tubing lines were made of PTFE. Each chamber presented two outlets. One outlet was connected to the PTR-ToF-MS (positioned *in-situ* within the greenhouse) at a flow rate of 100 mL min⁻¹ and the other to VOC cartridges (at 250 mL min⁻¹) for species characterization by GC-MS in the laboratory. (B) Timeline of VOC collection in cartridges followed by GC-MS analysis. VOC of each plant were collected for 5 min twice per day (9 am and 1 pm UTC), at 4, 24, 28, 48, 52 and 72 hours post-bagging (corresponding to experimental time markers: D0pm, D1am, D1pm, D2am, D2pm, D3am). Triangles represent VOCs collected at 1 pm, circles illustrate VOCs collected at 9 am.

CR23X micrologger (Campbell Scientific, inc.). Temperature inside each chamber was measured using iButtons® data loggers (DS1923 iButton Temperature/Humidity Logger, 1-Wire).

After each series of measurement, the number of figs and leaves from each tree were counted. The numbered figs were dried in oven at 40 °C for at least 72 hours and dry weight was measured using an analytical balance (LS-120A SCS, Precisa, Swiss). The exact volume of the chamber was determined for each individual setup to standardize emission rates.

2.3 VOCs analyses in real-time by PTR-ToF-MS

Emission kinetics of the VOC fingerprint released by receptive figs were studied through continuous collection and analysis by PTR-ToF-MS (Vocus, ToFwerk, Switzerland) over several days. As soon as fig trees were enclosed into the PTFE chambers, PTR-ToF-MS analyses were launched. In the PTR-ToF-MS, the reaction chamber pressure was set at 0.99 mbar, the drift tube voltage at 300 V and the drift tube temperature at 60 °C corresponding to an electric field strength (*E*) allowing to buffer the gas density (*N*) ratio of ≈ 120 Td. Mass spectra were recorded up to *m/z* 502 at 30 seconds integration time. For each chamber, the PTR-ToF-MS performed 12 acquisitions over a 6-minute period.

The processing of the raw data from PTR-ToF-MS was performed with the R package *provoc* (Huguenin, 2022). The four

attractive compounds were identified by their major fragment weight plus 1. The intensities of ion peaks of the four attractive compounds for pollinators - 137 ((*S*)-linalool *m/z* 136 + 1), 109 (benzyl alcohol *m/z* 108 + 1) and 159 ((*Z*)-linalool oxide (furanoids) and (*E*)-linalool oxide (fur.) *m/z* 158 + 1) - were extracted. For comparison with the four attractive VOCs, the 205 peak (a mixture of sesquiterpenoids *m/z* 204 + 1) was used since it is not involved in attracting pollinators (Proffit et al., 2020). For each measurement, the intensity of each ion peak was estimated by averaging the last six acquisitions out of a total of 12 measured (to prevent possible interference from the previous sample). For each targeted ion peak, the background signal at every measurement point was strictly eliminated by subtracting the peak intensity of the corresponding empty control chamber (blank) from the sample chamber. The resulting net peak intensities were then normalized to calculate the final emission rate. This normalization was performed by dividing the net intensity by both the dry weight of the enclosed fig and the air turnover time of the chamber. The air turnover time averaged 10 minutes, which was calculated by dividing the exact volume of each individual chamber by the specific airflow rate entering it.

In addition to PTR-ToF-MS measurements, VOCs were collected by pulling the air out of the chambers at 250 mL min⁻¹ through adsorbent steel cartridges filled with 40 mg of Carbotrap and 80 mg of Tenax phases (20–40 and 60–80 mesh respectively, Sigma-Aldrich, Munich, Germany). We collected VOCs of each plant for 5 mins twice per day (9 am and 1 pm, UTC) (See Figure 1B).

These traps were stored at -20 °C until analysis by GC-MS (GC Trace 1310, Thermo Scientific, Milan, Italy; MS, ISQ QD Single Quadrupole, Thermo Scientific, Milan, Italy) equipped with an DB-wax capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness, Machery-Nagel, Doren, Germany). Analysis was performed using similar protocol as Proffit et al (2020) and Dubuisson et al. (2024). The injection system was composed of a Thermal Desorption Unit (TDU) and a Cold Injection System (CIS) (Gerstell, Molheim, Germany). Absorbent traps were handled with a Multi-Purpose Sampler (Gerstell, Molheim, Germany). The GC temperature program for the analysis was as follows: oven held at 40 °C for 3 minutes, then increased from 40 °C to 220 °C at 5 °C min⁻¹, and finally from 220 to 250 °C at 10 °C min⁻¹, temperature was maintained at 250 °C for 2 min. The GC-MS transfer line was set-up at 250 °C. The ion source of the mass spectrometer was at 200 °C. The MS acquisition was from 38 *m/z* to 350 *m/z* at 70 eV ionisation energy. The Xcalibur TM 266 software (Thermo Scientific TM, Milan, Italy) was used for data processing. Retention index (RI) was calculated by a series of *n*-alkanes (Alkanes standard solution, 04070, Sigma Aldrich, Munich, Germany). Compounds were identified based on computer matching of the mass spectra with library (NIST 2017 MS), and confirmation by comparison of RI reported in literature (Adams, 2007). Mass spectra and retention index were compared to those of reference compounds run in the same GC-MS with the same method. In addition, to precisely quantify the emission rate of VOCs detected by the pollinator in each sample, fixed amounts of pure standards of the reference compounds - linalool (Fluka purity: ~97%; 23.5, 9.4 and 0.9 ng), benzyl alcohol (Fluka, purity: >99.5%; 24.9, 9.9 and 0.9 ng), (*E*)-linalool oxide furanoid (Fluka, purity: >97%; 13.5, 5.4 and 0.5 ng), (*Z*)-linalool oxide furanoid (Fluka, purity: >97%; 13.5, 5.4 and 0.5 ng) were injected into an adsorbent trap with three replicates per amount and analyzed in the GC-MS system using the same method. A response factor was calculated for each VOC and corresponded to the slope of the regression between the peak area (integrated from Total Ion Chromatogram) of each VOC and the different amounts of the standard in the three replicate samples, with the intercept set to zero. We used the calibration factor for each compound to estimate the concentrations of each compound in the sample. VOCs analysis with PTR-ToF-MS and GC-MS were made on the Chemical Platform for Ecology, at CEFÉ, with the support of the LabEx CeMEB, an ANR “Investissements d’avenir” program (ANR-10-LABX-04-01).

2.4 Statistical analysis

VOC were characterized both qualitatively and quantitatively using R (v. 4.2.1; R Development Core Team¹) with the different packages *provoc* (Huguenin, 2022), *vegan* (Oksanen et al., 2022), *RVAideMemoire* (Hervé, 2023), *lme4* (Bates et al., 2015) and *gamm4* (Wood and Scheipl, 2020).

The proportions of the four attractive VOCs were calculated based on their absolute amounts (ng/mg of dry biomass/5 minutes)

derived from calibration curves (see above) for receptive fig trees at 4, 24, 28, 48, 52 and 72 hours post-bagging (corresponding to experimental time markers: D0pm, D1am, D1pm, D2am, D2pm, D3am) (Figure 1B). This quantification step was essential to correct for differences in MS detector response factors between compounds, ensuring that the calculated proportions accurately reflected the chemical signal composition available to the pollinator. The content of attractive volatile organic compounds was estimated based on calibration curves established using standard compounds. In order to compare the relative proportions of the four attractive VOCs between the different collection times, a distance matrix calculating the dissimilarity between all samples in pairs using a Bray-Curtis index (Bray and Curtis, 1957) was built. Effects of time were tested on the distance matrix, using permutational multivariate analysis of variance (PERMANOVA, Anderson, 2017) with 999 permutations.

Daily VOC kinetic (data collected from PTR-ToF-MS) and effect of time on the odor emissions were based on Generalized Additive Models (GAMs) by including the peak intensities of the following VOCs *m/z*: 137 ((*S*)-linalool), 109 (benzyl alcohol), 159 ((*Z*)-linalool oxide (fur.) and (*E*)-linalool oxide (fur.) MW) and 205 peak (a mixture of sesquiterpenoids). GAMs allow to determine whether the variability of the peak intensity is statistically related to parametric variables and to smooth the fit of the model in case the relationship is non-linear. In addition, the possibility to include smooth random effects in the GAMs allows us to consider the variability caused by individual responses and by the different experimental series.

To determine the effect of abiotic factors on VOC emissions from receptive figs in *F. carica*, statistical analyses were performed using the PTR-ToF-MS data. Models were built for data located between 4 am and 5 pm (UTC), as VOCs emission between 5 pm and 4 am (UTC) was negligible. To ensure consistency between abiotic factors and PTRMS data, we utilized light intensity and temperature readings recorded at half-hourly intervals.

We analyzed the log-transformed peak intensities using Generalized Additive Mixed Models (GAMMs) with the *mgcv* package in R. The model structure was specified as: $\log(Y) \sim s(T) + s(L) + te(T, L) + s(T, PlantID, bs = "re") + s(L, PlantID, bs = "re")$ where *Y* represents the VOC emission rate, *T* is the ambient temperature (°C), and *L* is the light intensity (µmol m⁻² s⁻¹). The term *s*(.) denotes smooth functions (thin plate regression splines) quantifying non-linear main effects, while *te*(*T*, *L*) represents the tensor product interaction between temperature and light. To account for repeated measurements and biological variability, random slopes for temperature and light were included for each plant individual (PlantID) using the random effect basis (*bs* = “re”). Additionally, to address the heavy-tailed distribution of the emission data and temporal dependencies, we utilized a Scaled-t distribution family (family = scat) and a first-order autoregressive correlation structure (AR(1)).

Finally, analyses of deviance (ANOVAs) were conducted, and fitted values were plotted against observed values to evaluate the model fit. To assess the significance of the abiotic factors and their interaction, we compared the full model to nested reduced models (e.g., removing the interaction term or specific main effects) using Likelihood Ratio Tests (LRT) via the ANOVA function with a Chi-square test.

¹ <http://www.R-project.org>

Given that independent GAM models were fitted for four distinct compound masses, we controlled the False Discovery Rate (FDR) using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). As the observed p-values for the abiotic effects were highly significant ($p < 0.001$), the adjustment did not alter the interpretation of significance.

It is important to note that under greenhouse conditions, PAR and temperature were positively correlated (Supplementary Figure 1). Therefore, our statistical models describe the response of VOCs to these coupled abiotic drivers as they occur in a natural diurnal cycle, rather than isolating their independent physiological effects.

In addition, we tested the dependence of emission rates to temperature alone or both temperature and light, using two classical algorithms typically used in plant ecophysiology and atmospheric sciences. These algorithms, described in Guenther et al. (1995), were applied for each mass (data located between 4 am and 5 pm). The temperature-dependence algorithm (C_T) is described as follows:

$$C_T = \exp[\beta(T - T_s)]$$

Where T is the experimental temperature (K) in the chamber, T_s is the standard temperature, i.e. 30 °C. where β is the coefficient representing the dependence of emissions on temperature. It can vary between 0.057 and 0.114 with an average value of 0.09 (Guenther et al., 1993), used here.

Temperature and light dependence algorithm ($C_L \times C_T$) was calculated using the following equations:

$$C_l = \frac{\alpha C_{L1} PFD}{\sqrt{1 + \alpha^2 PFD^2}}$$

$$C_t = \frac{\exp \frac{C_{T1}(T - T_s)}{RT_s T}}{1 + \exp \frac{C_{T1}(T - T_M)}{RT_s T}}$$

where α (0.0027), C_{L1} (1.066), C_{T1} (95000 J mol⁻¹), C_{T2} (230000 J mol⁻¹) and T_M (314 K) are empirical coefficients. L is the experimental light, R is the universal gas constant (3.814 K⁻¹ mol⁻¹), T is the experimental temperature, T_s is the standard temperature (30 °C).

For each of the four masses, Pearson's correlation coefficients (R) were calculated between measured emissions (y) and C_T or $C_L \times C_T$ values (x) to assess the linear association between observed and modeled data. These coefficients were used as descriptive statistics to evaluate whether VOC emission rates of m/z 137, 109, 159 and 205 showed a stronger alignment with the temperature-dependent algorithm or the temperature- and light-dependent algorithm.

3 Results

3.1 Daily kinetics of VOC emissions by fig trees bearing receptive figs

The intensity of the four masses (m/z 137, 109, 159 and 205) monitored during the 72h for the receptive fig trees of *F. carica* maintained a consistent rhythm throughout the monitoring with a

daily peak between 11 am and 1 pm, followed by a linear decline of the emission until the middle of the night (around 11 pm). This non-linear trend following a daily rhythmicity was further confirmed by the adaptive smoothing of GAMs with linalool (m/z 137) (GAM, $\chi^2 = 3537$, edf (the effective degrees of freedom) = 25, $p < 0.001$), linalool oxides (m/z 159) (GAM, $\chi^2 = 546$, edf = 18, $p < 0.001$), benzyl alcohol (m/z 109) (GAM, $\chi^2 = 5590$, edf = 28, $p < 0.001$) and sesquiterpenes (205) (GAM, $\chi^2 = 692$, edf = 18, $p < 0.001$) (Figure 2).

GC-MS analyses revealed no difference in the relative proportions of the four pollinator-attractive VOCs when they were collected at 9 am and 1 pm (PERMANOVA, $F_{1,53} = 0.7$, $df = 1$, $p = 0.774$) over a 72-hour period (PERMANOVA, $F_{4,53} = 0.7$, $df = 4$, $p = 0.681$) (Figure 3; Supplementary Table 1).

Each dot represents the peak intensity of VOCs of one individual. The black line represents the response of the VOC peak intensity over 72 hours according to the GAM modeling. The gray bands represent the standard error. Yellow areas correspond to the day (4 am–5 pm, UTC) and white areas represent the night (5pm–4am, UTC). The y axis represents the peak intensity of VOCs released by receptive figs (mg of DW.min⁻¹), and the x axis represents the time in hours post-bagging (where 0 h corresponds to the start of the experiment at 9:00 am UTC).

The proportions of the four attractive VOCs were estimated by the intensity of the total peak area of four attractive VOCs from GC-MS for receptive fig tree at 4, 24, 28, 48, 52 and 72 hours post-bagging (corresponding to experimental time markers: D0 pm, D1am, D1 pm, D2 am, D2pm, D3 am).

3.2 Effects of abiotic factors on VOC emissions from receptive figs in *F. carica*

The abiotic parameters recorded (temperature and light) had contrasting effects on the emission intensity of pollinator-attractive VOCs released by the receptive figs. However, it is important to first highlight that a significant positive correlation was observed between temperature and light ($R = 0.65$, $t = 20.425$, $df = 566$, $p < 0.001$) (Supplementary Figure 1). Tensor interaction product between temperature and light was the main fixed factor explaining variations of VOCs emitted by the trees bearing receptive figs (See Supplementary Table 2) including m/z 137 (linalool) (GAM, $\chi^{2T \times L1} = 80.1$, $edf_{TxL1} = 5.5$, $p_{TxL1} < 0.001$, [Supplementary Figure 2A]), m/z 109 (benzyl alcohol, GAM, $\chi^{2T \times Lb} = 70.9$, $edf_{TxLb} = 5.8$, $p_{TxLb} < 0.001$, [Supplementary Figure 2B]), m/z 159 (linalool oxides, GAM, $\chi^{2T \times Lo} = 42.4$, $edf_{TxLo} = 7.1$, $p_{TxLo} < 0.001$, [Supplementary Figure 2C]) and m/z 205 (sesquiterpenes, GAM, $\chi^{2T \times Ls} = 33.1$, $edf_{TxLs} = 7.2$, $p_{TxLs} < 0.001$, [Supplementary Figure 2D]). While temperature had a singular significant and non-linear effect on emission of m/z 137 (linalool, GAM, $\chi^{2T1} = 8.89$, $edf_{T1} = 1.0$, $p_{T1} = 0.003$) and linalool oxides (GAM, $\chi^{2To} = 98.5$, $edf_{To} = 1.0$, $p_{To} < 0.001$) (Figure 4), light had a significant and non-linear effect on emission of m/z 109 (benzyl alcohol, GAM, $\chi^{2Lb} = 28.1$, $edf_{To} = 5.6$, $p_{To} < 0.001$) and on m/z 137 (linalool) (GAM, $\chi^{2L1} = 30.7$, $edf_{L1} = 5.6$, $p_{L1} < 0.001$) (Figure 5). The χ^2 values associated with individual responses to either light and temperature (accounted as random effects) were extremely strong, revealing strong intraspecific variability in the quantitative emission and responses to abiotic factors

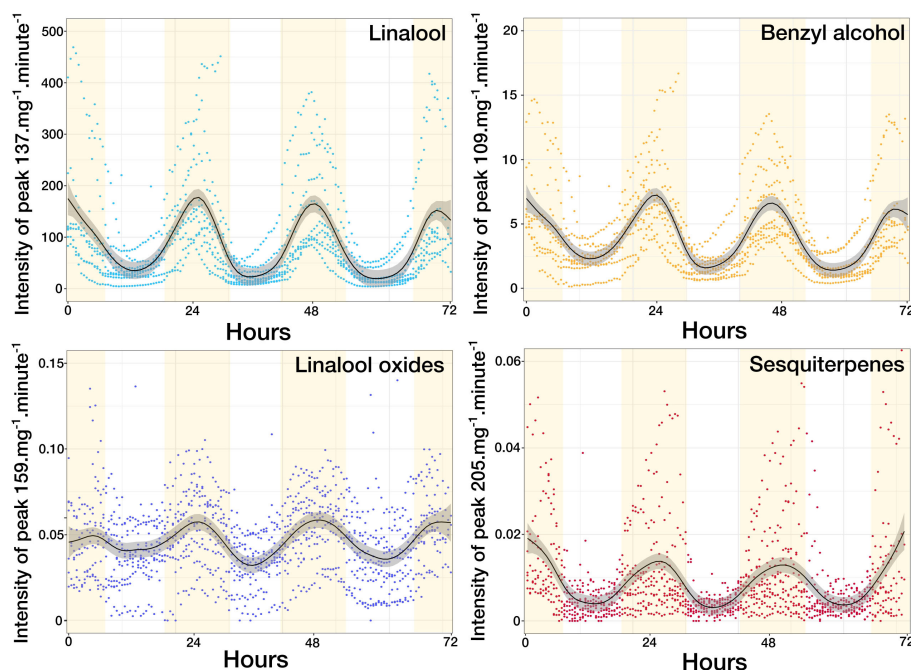


FIGURE 2

Dynamic emission of pollinator attractive VOCs (m/z 137, 159, 109) and sesquiterpenes (m/z 205) over 72 hours in fig trees bearing receptive figs. Each dot represents the peak intensity of VOCs of one individual. The black line represents the response of the VOC peak intensity over 72 hours according to the GAM modelling. The grey bands represent the standard error. Yellow areas correspond to the day (4 am–5 pm, UTC) and white areas represent the night (5 pm–4 am, UTC). The y axis represents the peak intensity of VOCs released by receptive figs ($\text{mg of DW} \cdot \text{min}^{-1}$), and the x axis represents the time in hours post-bagging (where 0 h corresponds to the start of the experiment at 9:00 am UTC).

(Supplementary Table 2). Emissions of linalool and linalool oxides increased with temperature and peaked at 22 °C. Thereafter, these emissions gradually decreased (Figure 4). However, emissions of linalool as well as benzyl alcohol were not observed until high light intensities are reached (Figure 5).

Each dot represents the peak intensity of VOCs of one individual. The line is the regression of GAM modeling of the peak intensity of VOCs with temperature over 72 hours. The gray bands represent the standard error. The vertical coordinate of dots represents the peak intensity of VOCs in receptive figs (mg/min), the horizontal coordinate of dots represents the temperature. *: the fixed effect of temperature is significant ($p < 0.001$) in the full GAM.

The line is the regression of GAM modeling of the peak intensity of VOCs with light intensity over 72 hours. The gray bands represent the standard error. The vertical coordinate of dots represents the peak intensity of VOCs in receptive figs (mg/min), the horizontal coordinate of dots represents the light intensity. *: the fixed effect of light is significant ($p < 0.001$) in the full GAM.

Based on algorithms commonly used in ecophysiology for leaf VOCs (Guenther et al., 1995), the emission of the four VOC attractive to the pollinator were negatively correlated to both C_T and $C_L \times C_T$. Correlations with C_T were slightly stronger than with $C_L \times C_T$. (linalool, $R = -0.251$ vs -0.241 ; benzyl alcohol, $R = -0.266$ vs -0.238 ; linalool oxide, $R = -0.331$ vs -0.317). By contrast, measured emission of sesquiterpenes responded positively to both C_T and $C_L \times C_T$, with a clearer and better fit to light and temperature combined (i.e., $C_L \times C_T$) ($R = 0.236$) than to temperature alone (i.e., C_T) ($R = 0.186$).

4 Discussion

This study represents one of the first high-resolution characterizations of floral VOC dynamics in a specialized mutualism. By combining the temporal precision of PTR-TOF-MS with the chemical identification of GC-MS, we demonstrated a critical ecological phenomenon: while the quantity of emissions is plastically driven by environmental fluctuations (tracking temperature and light), the *quality* (the specific ratio of attractive compounds) remains remarkably stable during the peak activity of pollinators. This suggests a robust physiological buffering mechanism that prioritizes signal fidelity.

While our GC-MS analysis detected a broader bouquet of VOCs typical of receptive *F. carica* figs (which may play general roles in plant defense or antimicrobial protection as described by Soler et al., 2012), our study strictly focuses on the temporal dynamics of the four targeted VOCs that are definitively responsible for pollinator attraction (Proffit et al., 2020), alongside sesquiterpenes as a comparative baseline.

4.1 Daily emission kinetic of VOCs from fig trees bearing receptive figs

As hypothesized, fig tree bearing receptive figs presented a consistent emission rhythm of VOCs throughout the day. Interestingly, the rhythmicity of floral emissions from receptive figs was very homogeneous during the 72 hours of measurements.

The diurnal emission peak observed in our study (Figure 2) aligns perfectly with the main activity time of *B. psenes* during the

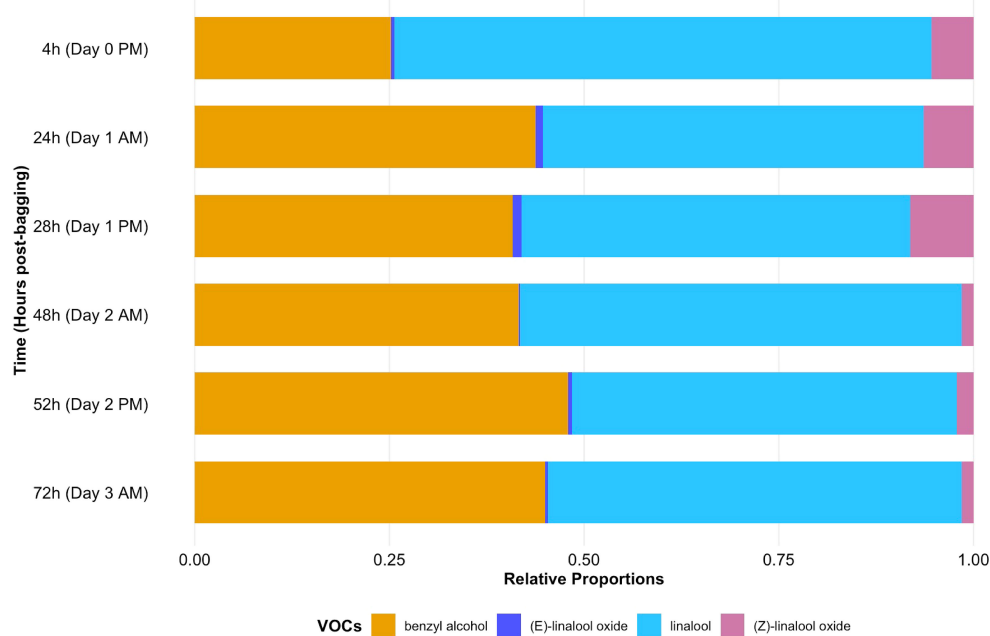


FIGURE 3

Relative proportions of the four pollinator-attractive VOCs of fig trees bearing receptive figs. The proportions of the four attractive VOCs were estimated by the intensity of the total peak area of four attractive VOCs from GC-MS for receptive fig tree at 4, 24, 28, 48, 52 and 72 hours post-bagging (corresponding to experimental time markers: D0 pm, D1am, D1 pm, D2 am, D2pm, D3 am).

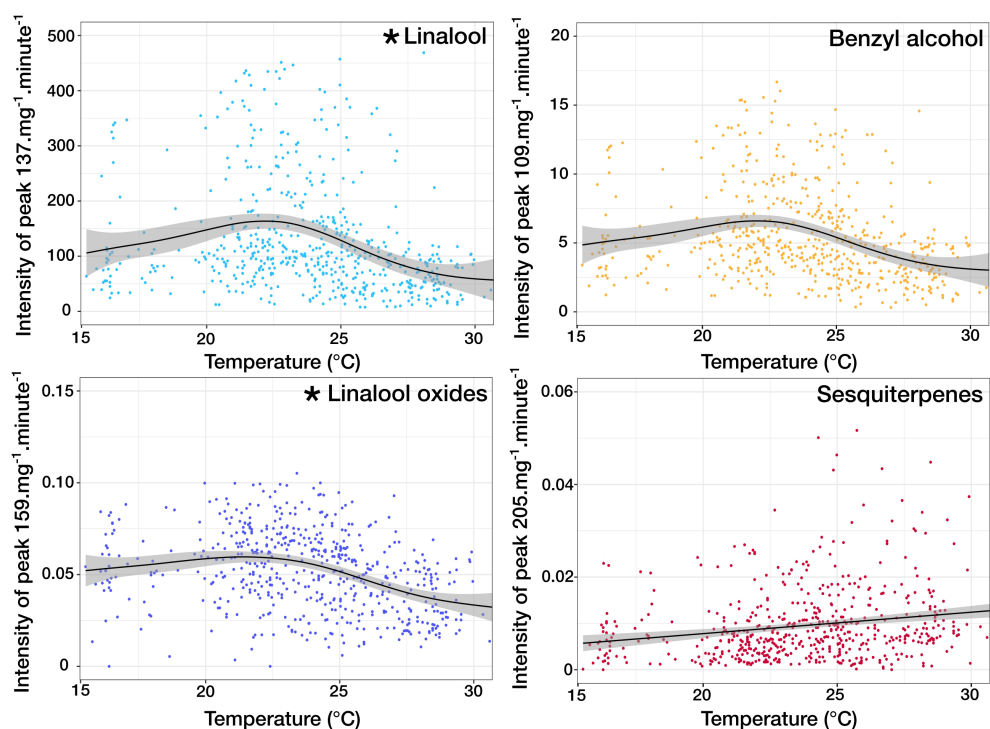


FIGURE 4

Effect of temperature on emission of VOCs attracting pollinators including linalool, benzyl alcohol and linalool oxides (m/z 137, 159, 109) and sesquiterpenes (m/z 205) in fig trees bearing receptive figs. Each dot represents the peak intensity of VOCs of one individual. The line is the regression of GAM modelling of the peak intensity of VOCs with temperature over 72 hours. The grey bands represent the standard error. The vertical coordinate of dots represents the peak intensity of VOCs in receptive figs (mg/min), the horizontal coordinate of dots represents the temperature. *: the fixed effect of temperature is significant ($p < 0.001$) in the full GAM.

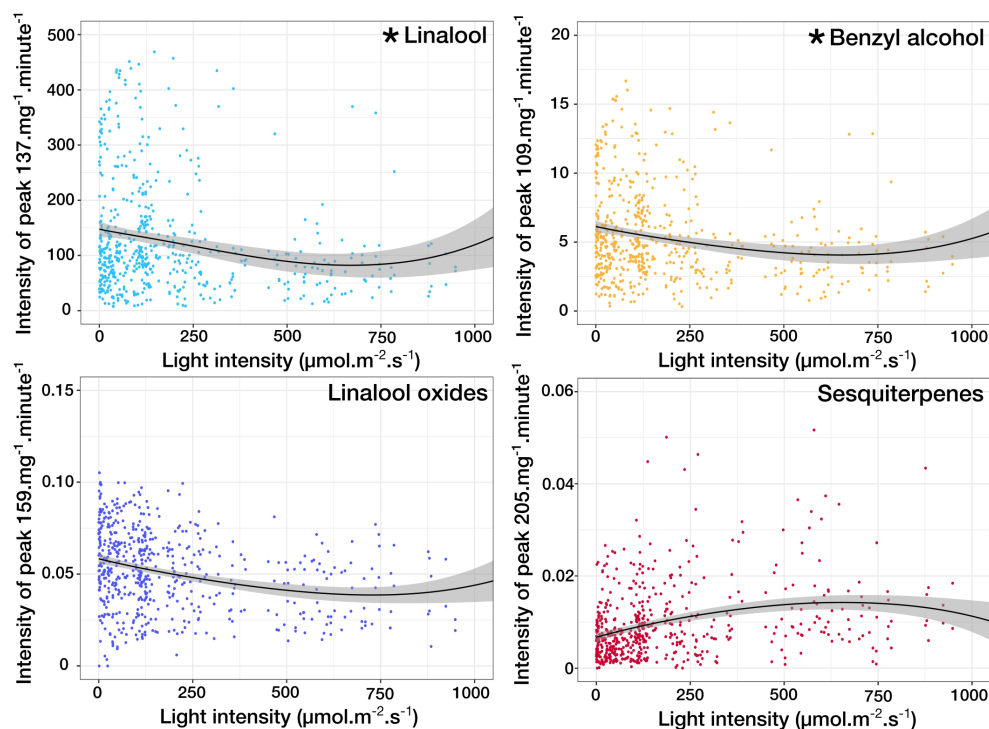


FIGURE 5

Effect of light (photosynthetically active radiation) on emission of VOCs attracting pollinators including linalool, benzyl alcohol and linalool oxides (m/z 137, 159, 109) and sesquiterpenes (m/z 205) in fig trees bearing receptive figs. Each dot represents the peak intensity of VOCs of one individual. The line is the regression of GAM modelling of the peak intensity of VOCs with light intensity over 72 hours. The grey bands represent the standard error. The vertical coordinate of dots represents the peak intensity of VOCs in receptive figs (mg/min), the horizontal coordinate of dots represents the light intensity. *: the fixed effect of light is significant ($p < 0.001$) in the full GAM.

spring season (Proffitt et al., unpublished data). In several other systems, it has been reported that the rhythmicity of floral scent emission agrees with pollinator activity (Hoballah et al, 2005; Powers et al., 2020; Steen et al, 2019). The timing of VOC emissions by *Petunia axillaris* is in tune with nocturnal hawk moth activity (Hoballah et al, 2005). Furthermore, the emission rate of VOCs involved in the attraction of pollinators seems to be controlled by an internal clock of the plant, that regulates diurnal rhythms of VOC emission in *Petunia* (Fenske et al., 2015). While the diurnal pattern observed in *F. carica* suggests a potential role for similar endogenous regulation, our study was conducted under light/dark cycles, preventing the disentanglement of circadian clock effects from direct responses to environmental cues. Several studies have shown that gene expression of key enzymes, final enzymes in biosynthetic pathways and their enzyme activity play a role in controlling the circadian rhythmic emission of plant VOCs (Zeng et al, 2017). For instance, in snapdragon flowers, the rhythmicity of terpenoid emission was controlled by the endogenous clock through the methylerythritol phosphate (MEP) pathway (Dudareva et al., 2005). Also, transcript levels of QH6, a β -pinene synthase gene (final enzyme), continue to fluctuate with circadian rhythms, resulting in diurnal patterns of β -pinene content and emissions, when *Artemisia annua* was cultivated under constant light or constant dark conditions (Lu et al., 2002). Another example showed that oscillations of salicylic acid carboxyl methyltransferase activity (enzyme activity) coincided with nocturnal methyl

salicylate emission in *Stephanotis* flowers (Pott et al., 2003). Additionally, the efficiency of VOC emission may depend on substrate availability (Benzoic acid's substrate regulates the rhythmic production and emission of Volatile Phenylpropanoids/Benzenoids (VPBs) such as methyl benzoate in petunia flowers), but also on transcription factors (Petunia hybrida Late Elongated Hypocotyl, PhLHY, regulates the production of floral volatiles in *Petunia*) (Fenske et al., 2015). To fully disentangle these physiological mechanisms, future studies must advance beyond semi-natural conditions. Evaluating VOC emission dynamics under strictly controlled, constant environmental conditions (e.g., continuous darkness or continuous light) is essential to confirm true endogenous circadian regulation. Furthermore, to understand the complete pathway from synthesis to release, future investigations should focus on the specific anatomical sites and mechanisms of VOC secretion within the enclosed fig syconium. Pairing real-time emission data with molecular analyses—specifically targeting the gene regulation and expression patterns of key terminal enzymes, such as terpene synthases—will provide a comprehensive mechanistic understanding of how these crucial ecological signals are orchestrated at the cellular and molecular levels.

Using data from GC-MS analyses, we also demonstrated that during the peak of floral VOC emissions, the proportions of VOCs attractive to pollinators were very homogeneous. This very high homogeneity in the proportion of these VOCs is probably the result of selection pressure to maintain the attraction of the specific

pollinator. Indeed, in *F. carica*, small variations in the proportions of the four attractive VOCs can lead to non-recognition of floral scents by pollinators (Proffitt et al., 2020).

In conclusion, our findings show that the fig tree employs a highly robust signaling strategy, characterized by both temporal precision in its daily emission rhythm and high fidelity in its chemical composition. This dual stability facilitates a reliable communication link with its obligate pollinator, which is crucial for its reproductive success.

4.2 Effects of abiotic factors on VOCs emission of *F. carica*

We used two different approaches to test the effects of temperature and light on VOC emissions from receptive figs of *F. carica*: one classically employed in plant eco-physiology to estimate VOC emissions from leaves, and one used in ecology to explore complex, non-linear relationships between variables. Both approaches, strongly suggest that temperature has a significant effect and is associated with VOC emissions from receptive figs. However, the effect of light is less straightforward.

The use of the classical Guenther's algorithms pointed out that temperature exerts a stronger influence than the combined light-temperature interaction effects with a negative correlation on the emission of the four VOC attractive to the pollinator. It was the opposite for sesquiterpenes. Despite the significant p-values of the results, the correlation between the estimated and measured values is low (absolute values of R range from 0.186 to 0.331). The Guenther's algorithms have been widely tested to assess VOC emissions from plant leaves (Guenther et al., 1999; Niinemets, 2010; Peñuelas and Llusia, 2001; Peñuelas and Staudt, 2010; Saunier et al., 2017; Wang et al., 2025) but have not been reported for estimating VOCs emissions from flowers. In addition, the Guenther algorithms do not allow to discern how light alone modulates emissions collected in field experiments which can be a complex question when measurements are carried out under light-saturated conditions (Hakola et al., 2006). Thus, the GAM model appears as a better alternative to test the effects (isolated or combined) of temperature and light on flower VOCs compared to the classical leaf-based Guenther algorithms. This statistical approach is especially important as our semi-controlled conditions do not allow us to independently test the single effects of light and temperature (which can be captured by the full GAM model).

GAM analyses show that temperature had a singular significant and non-linear effect on emission of linalool and linalool oxides. Emission of linalool and linalool oxides increases with increasing temperature to a threshold value around 22 °C and then decreases. This pattern of emission is coherent with what has been reported in other Mediterranean plants (Farré-Armengol et al., 2014). These results are also supported by Hu et al. (2013), who found an increase in floral emissions of *Lilium 'siberia'* from 10 to 30 °C, followed by a decrease at 40 °C. Our results are also similar to the well-characterized temperature response of leaf Biogenic Volatile Organic Compounds (BVOC) emissions (Guenther et al., 1999; Niinemets, 2010; Peñuelas and Llusia, 2001; Peñuelas and Staudt, 2010). The observed increase in VOC emissions with rising temperature up to a

threshold is likely driven in part by physicochemical processes. According to Henry's law, higher temperatures increase the volatility and vapor pressure of terpenoids (linalool and oxides), facilitating their diffusion from storage pools or synthesis sites into the atmosphere (Niinemets et al., 2004). However, as the optimal temperatures for VOC emission vary between several Mediterranean plants growing in the same area, it has been pointed out that the observed pattern cannot be solely due to differences in the physicochemical properties of specific compounds, but that specific characteristics of floral physiology probably play an important role in determining the responses of floral emissions to temperature (Farré-Armengol et al., 2014).

Light had a singular significant and non-linear effect on emission of linalool and benzyl alcohol, and no effect on linalool oxides and sesquiterpenes. Although the biosynthetic pathway of benzyl alcohol is currently unknown, the biosynthetic pathways associated with benzenoids are generally influenced by light and temperature (Lv et al., 2024). The light dependence of sesquiterpene emission after accounting for temperature response has been reported in some studies (Duhl et al., 2008; Geron and Arnsts, 2010). Helmig et al. (2006) found the best fit between modeled and experimental sesquiterpene emissions when these VOCs were assumed to be 80% temperature and light dependent and 20% temperature dependent only.

All GAM analyses demonstrate that interaction of temperature and light had a significant effect on emission of the four VOC attractive to the pollinator and sesquiterpenes. It is generally accepted that monoterpene emissions are driven by both temperature and light (Sanadze, 1991). The enzymatic activity of terpene synthases is not only affected by temperature (Holopainen and Gershenzon, 2010). Monoterpene production also requires photosynthesis and therefore light in order to synthesize their precursors (Loreto et al., 1996; Niinemets et al., 2002; Rasulov et al., 2009). Specifically, linalool and its derivatives are primarily synthesized via the methylerythritol phosphate (MEP) pathway in chloroplasts, which depends on the carbon supply (glyceraldehyde 3-phosphate and pyruvate) provided by photosynthesis (Dudareva et al., 2013). Thus, the correlation between light intensity and emission peaks may reflect the immediate availability of carbon substrates.

In contrast, sesquiterpenes (m/z 205) showed a distinct response pattern. While their emission was also significantly affected by temperature and light, sesquiterpenes are typically synthesized via the mevalonic acid (MVA) pathway in the cytosol and may rely more on stored carbon pools rather than immediate photosynthetic products (Dudareva et al., 2013). Consequently, their emission dynamics might be driven more by the physical effect of temperature on volatility than by light-dependent biosynthesis.

From a mechanistic perspective, it is noteworthy that the emission patterns of the primary pollinator-attractive VOCs, specifically linalool and benzyl alcohol, were highly synchronized (Figure 2). Although these two groups of compounds originate from different biosynthetic pathways, their synchronized diurnal rhythms and similar responses to abiotic factors suggest a shared physiological regulation of emission. This overall regulation is likely driven by physical processes, such as stomatal conductance or temperature-dependent volatility, rather than by the independent

upregulation of specific biosynthetic pathways for these attractive compounds.

From a methodological perspective, our absolute quantification of targeted VOCs achieved high accuracy through the use of compound-specific external calibration curves generated from pure synthetic standards. However, we acknowledge that future studies could be further optimized. In addition to the calibration curves, incorporating an internal standard during the VOC collection or thermal desorption phase would better account for potential matrix effects or minor analytical variations (e.g., thermal desorption fluctuations), thereby providing even greater accuracy and reliability in absolute VOC quantification.

5 Conclusion

This study is one of the few to have accurately described the daily dynamics of VOC emissions of flowering plants, particularly those responsible for attracting pollinators. We have shown that the VOCs emitted by *F. carica* figs follow a very consistent daily pattern, with emissions peaking in the middle of the day and the proportions of VOCs attractive to pollinators remaining very stable during this period. We were also able to demonstrate a non-linear relationship between temperature and light, and the emissions of these individual VOCs. In particular, emissions of VOCs attractive to pollinators all peak around 22 °C and then decrease thereafter. Future experimental studies on *F. carica*, as well as other species, are needed to confirm the non-linear relationships between floral VOC emissions and both temperature and light. Furthermore, given our finding that the emission of pollinator-attracting VOCs decreases at temperatures above 22 °C, rising temperatures associated with climate change could severely disrupt the obligate chemical communication in this mutualism. In the frame of ecology, such thermal disruption threatens the survival of specific fig wasp populations and the overall resilience of highly specialized plant-pollinator networks. In the frame of agriculture, these warming-induced pollination failures could drastically reduce fruit set and crop yields of Mediterranean figs, highlighting a direct economic risk to local agricultural systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LC: Validation, Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Writing – original draft, Project administration, Visualization, Data curation. VV:

Visualization, Writing – original draft, Formal analysis, Software, Data curation, Investigation, Writing – review & editing. JH: Writing – review & editing, Formal analysis, Software. BB: Software, Writing – review & editing. EO: Writing – review & editing, Methodology. AG-B: Supervision, Writing – review & editing. MP: Resources, Writing – original draft, Project administration, Supervision, Conceptualization, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work was supported by the program 80-PRIME CNRS (Li Cao's PhD Grant), the program FEDER-FSE-IEJ Languedoc-Roussillon 2014-2020 n°2014FR16MOOP006, the program National Natural Science Foundation of China (32261123001), the program Yunnan Key Research and Development Program (202503AP140027) and the IRP MOST (Figs and fig-wasps, a model system to investigate biotic interaction network responses to global change) co-raised by the Chinese Academy of Science (CAS) and the CNRS.

Acknowledgments

We thank the “Terrain d'Experience” (TE) and the “Plateforme d'Analyses Chimiques en Ecologie (PACE)” of the “Centre d'Ecologie Fonctionnelle et Evolutive” (CEFE) (LabEx CeMEB, the French National Research Agency under the “Investissements d'avenir” program ANR-10-LABX-04-01) for hosting the experiments and the chemical analysis. In particular, we would like to thank Pauline Durbin, Fabien Lopez and Thierry Mathieu for taking care of the fig trees used in this study, and David Degueldre and Pierrick Aury for designing the measurement chambers. We would also like to thank Jean Kempf for providing equipment for light measurements.

Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2026.1764436/full#supplementary-material>

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