

Can floral volatile organic compounds contribute to the taxonomy of the *Rhamnus* sect. *Alaternus*?

Leonardo Llorens¹, Pere Ferriol¹, Joan Tomàs², María Trinidad García³, Lorenzo Gil¹

1 *Interdisciplinary Ecology Group, Department of Biology, University of the Balearic Islands (UIB), Ctra. Palma-Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain* **2** *Department of Biology (Botany), University of the Balearic Islands (UIB), Ctra. Palma-Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain* **3** *Scientific and Technical Services, University of the Balearic Islands (UIB), Carretera de Valldemossa Km. 7,5, E-07122 Palma de Mallorca, Balearic Islands, Spain*

Corresponding author: Leonardo Llorens (lleonard.llorens@gmail.com)

Academic editor: L. Peruzzi | Received 27 November 2023 | Accepted 19 December 2023 | Published 29 December 2023

Citation: Llorens L, Ferriol P, Tomàs J, García MT, Gil L (2023) Can floral volatile organic compounds contribute to the taxonomy of the *Rhamnus* sect. *Alaternus*?. Italian Botanist 16: 149–164. <https://doi.org/10.3897/italianbotanist.16.116255>

Abstract

The chemistry of plants represents a taxonomic and phylogenetic value. Researchers have recently used volatile organic compounds (VOCs) for taxonomic studies. The present work analysed and determined, for the first time, the usefulness of floral volatile compounds in the taxonomy of two species of *Rhamnus* sect. *Alaternus*, as well as the hybrid between these taxa. The two species show significant quantitative and qualitative differences. The terpenes linalool, ocimene, caryophyllene, and green volatiles are exclusive to *Rh. alaternus*, while farnesene (terpene), methyl salicylate and methyl benzoate (benzoids) are obtained from *Rh. ludovici-salvatoris*. Both volatilomes were attractive to Hymenoptera pollinators; therefore, these pollinators could serve as the main hybridisation vector. In addition, *Rh. alaternus* shows greater chemical and genetic heterogeneity than *Rh. ludovici-salvatoris*. Hybrids between the two species, *Rh. ×bermejoi*, are closer in chemical similarity to *Rh. alaternus* than *Rh. ludovici-salvatoris*.

Keywords

Balearic Islands, hybrids, *Rhamnus*, taxonomy, VOCs

Introduction

Plant chemistry has repeatedly been used in systematics (Hegnauer 1986a; Tundis et al. 2014). It has long been known that chemical traits and characteristics, notably the secondary metabolites (such as alkaloids, terpenoids, and flavonoids) contained in vegetative parts of the plants, help to establish relationships between plant taxa (Cronquist

1977; Singh R 2016). Moreover, they have wide applications in plant systematics (see Seigler 1981; Roma-Marzio et al. 2017); however, their use presents some limitations (Hegnauer 1986b; Hadacek 2002). It is also known that the variability of volatile secondary metabolites in the vegetative parts of plants can be high (Ahmad et al. 2014). For this reason, several authors consider the construction of phylogenies on this basis too labile (Whitehead and Peakall 2009). The chemistry of floral biogenic volatile organic compounds (BVOCs) has been well studied as it relates to communication (with pollinators) and defence (mainly herbivore-related) (Bouwmeester et al. 2019). The analysis of BVOCs also enables the evaluation of their important role in the adaptation, environmental adequacy, and medicinal interest of plants (Goswami et al. 2016). Their stability and repeatability can also allow accurate identification of natural or cultivated closely-related species and varieties (Nogueira et al. 2001; Kong et al. 2012; Yang et al. 2014; Carta et al. 2015; Jaeger et al. 2016; Peruzzi et al. 2019). Recently, BVOCs have also been studied from the standpoint of taxonomy (e.g., Levin et al. 2003; Raguso et al. 2006; Meekijjaroenroj et al. 2007; Feulner et al. 2011; Tóth et al. 2016; Stocki et al. 2020), showing their utility as discriminating characteristics. BVOCs have also been used to recognise hybrids, in which new compounds, absent in the parents, can appear (Orians 2000; Cheng et al. 2011), together with others that are not taxonomically relevant (Georgescu et al. 2015). Additionally, some VOC-related phylogenetic patterns have been recognised in some taxa, such as *Ophrys* (Gögler et al. 2009).

Rhamnus is a wide-ranging genus of the Temperate and (sub)Tropical regions of the Northern Hemisphere that comprises more than 100 species (Hauenschild et al. 2016). In the Mediterranean region it is represented by 37 species, where *Rh. cathartica* L. and *Rh. alaternus* L. are the most widespread (Henning and Raab-Straube 2016). Molecular studies performed on *Rhamnus*, excluding *Oreoherzogia* W.Vent (Hauenschild et al. 2016), recognise the existence of at least two sections: sect. *Rhamnus* and sect. *Alaternus* (Mill.) DC. The latter includes different species of Mediterranean and Macaronesian distribution (such as *Rh. alaternus* L. and related taxa, *Rh. lojaconoi* Raimondo, *R. ludovici-salvatoris* Chodat for Mediterranean, as well *Rh. glandulosa* Ait. and *Rh. integrifolia* DC. for Macaronesia. Generally, hybrids are uncommon or rare; however, *Rh. cathartica* (Gil-ad and Reznicek 1997; Kurylo et al. 2007) and, occasionally, *Rh. alaternus* (Llorens 1979; Fraga and Bermejo 2008) have shown a notable capacity to hybridise.

Rhamnus alaternus is an evergreen tree native to the Mediterranean region and is often cultivated as an ornamental garden shrub in Mediterranean-climate regions. For this purpose, this tree was introduced to many areas of the Australasian-Pacific region, where it became an invasive tree along coastlines (Muyt 2001; GISD 2018). This wide distribution and the different types of plant communities in which it thrives agree with its remarkable morphological variability (López-González 2006; Rivas-Martínez and Pizarro 2011, 2013). On the other hand, although *Rh. alaternus* is a defined species, its differentiation from other species of the *Rh.* sect. *Alaternus*, especially the hybrids, is problematic (Ferriol et al. 2009). This differentiation occurs with *Rh. ludovici-salvatoris* (Fig. 1), from which it differs basically in its larger size and the arrangement of the leaf teeth (approximately perpendicular to the margin in *Rh. ludovici-salvatoris* and obliquely oriented upwards in

Rh. alaternus). This difficulty has taxonomic and nomenclatural consequences, especially in plants that develop in unfavorable conditions. This difficulty occurs with the definition and identification of some specimens, such as *Rh. jacobii-salvadorii* O.Bolòs & Vigo or *Rh. alaternus* var. *ferruginea* Pourr. ex Texidor, making it difficult to establish a more precise distribution range. This is the case, for example, in the confusing or erroneous indications of *Rh. ludovici-salvatoris* in the eastern Iberian Peninsula or the actual distribution of the hybrid of this species with *Rh. alaternus* (i.e., *Rh. ×bermejoii* Fraga & Rosselló).

The Balearic Islands are the largest archipelago of continental islands in Spain. These islands display a complex palaeogeography, although they have been isolated relatively recently. The eastern islands (Gymnesian) and western islands (Pityusian) have been separate groups from the early Pliocene. Nevertheless, the Gymnesian Islands (Majorca and Minorca) have only been completely isolated from each other since the Würm glaciation, approx. 15,000 ya (Cardona and Contandriopoulos 1979). In these islands, only two species from *Rh.* sect. *Alaternus* grow: the Mediterranean *Rh. alaternus* and the Gymnesian endemic *Rh. ludovici-salvatoris*. However, both species are related, and some hybrid plants have been identified in one locality of Minorca (Tirant) (Llorens 1979; Fraga and Rosselló 2008). Despite this, phenological and ecological barriers establish good isolation of the populations (Ferriol et al. 2009).

The chemical profile of the floral scent of the taxa of *Rhamnus* sect. *Alaternus* present in Majorca, Minorca, Ibiza, and eastern Iberian Peninsula was analysed in order to address the following three goals: 1) to recognise the volatile compounds of the two species, 2) to establish analogies and chemical differences between the hybrid plants and the parental species, and 3) to determine their value in taxonomy and their usefulness as discriminating characteristics in these species and their hybrids, as well as to establish their possible application to the taxonomy of other *Rhamnus* species.

Materials and methods

Plant material

The two species under study and their hybrids were collected from the Balearic Islands and the eastern Iberian Peninsula (Fig. 2, Suppl. material 1). Samples of natural hybrid plants reported as *Rh. ×bermejoii* (Llorens 1979; Fraga and Rosselló 2008) were collected in the locality of Tirant (N of Minorca), where they coexist with the parental species. Hybrid plants obtained by manual artificial hybridisation, cultivated and vegetatively propagated were also sampled. A total of 35 plants (14 female and 21 male) were screened for floral VOCs. The wild plants were collected from 13 sites, and the cultivated hybrids were collected from two sites.

Floral scent trapping

The volatiles emitted by flowers were obtained by the headspace solid phase extraction (HS-SPME) sampling technique, adapted from Friberg et al. (2013) and Tomas et al.

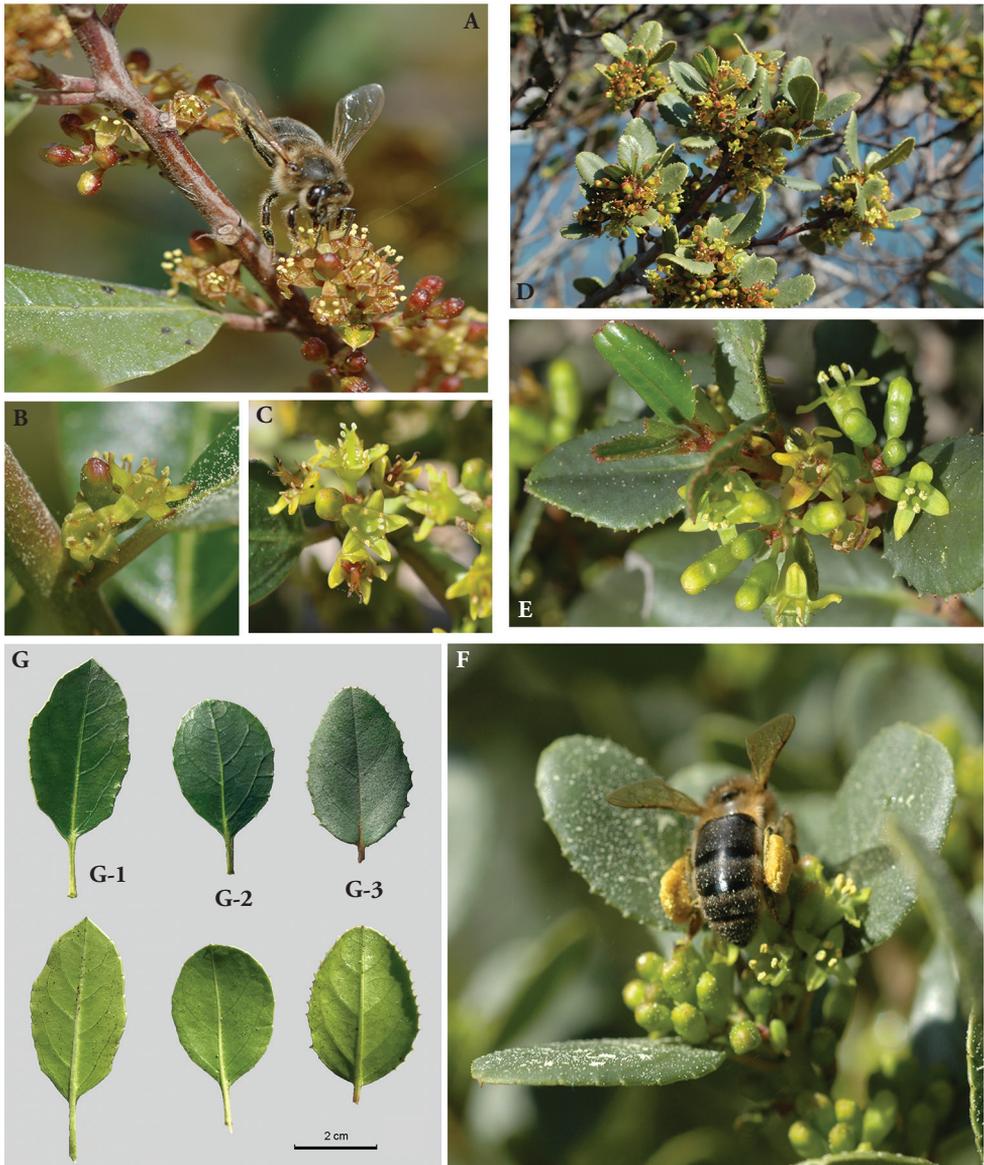


Figure 1. Inflorescences, flowers and major pollinator (*Apis mellifera*) of *Rhamnus* **A–C** *Rh. alaternus* **D, E** *Rh. ludovici-salvatoris* **F** *Rh. xbermejoii* **G** leaves of the three species.

(2022). Volatile compounds were extracted in a manual SPME holder together with 10 mL vials and PDMS-DVD fibres (Supelco Inc., Bellefonte, USA). First, ten flowers of each plant were placed in a vial. Next, these vials were placed at 25 °C for 20 min; then, the SPME fibre was exposed in the upper space (headspace) of the sealed vial for 30 min at 25 °C to adsorb the analytes. Three collections and their respective controls (empty vials) were prepared simultaneously. In addition, to distinguish between

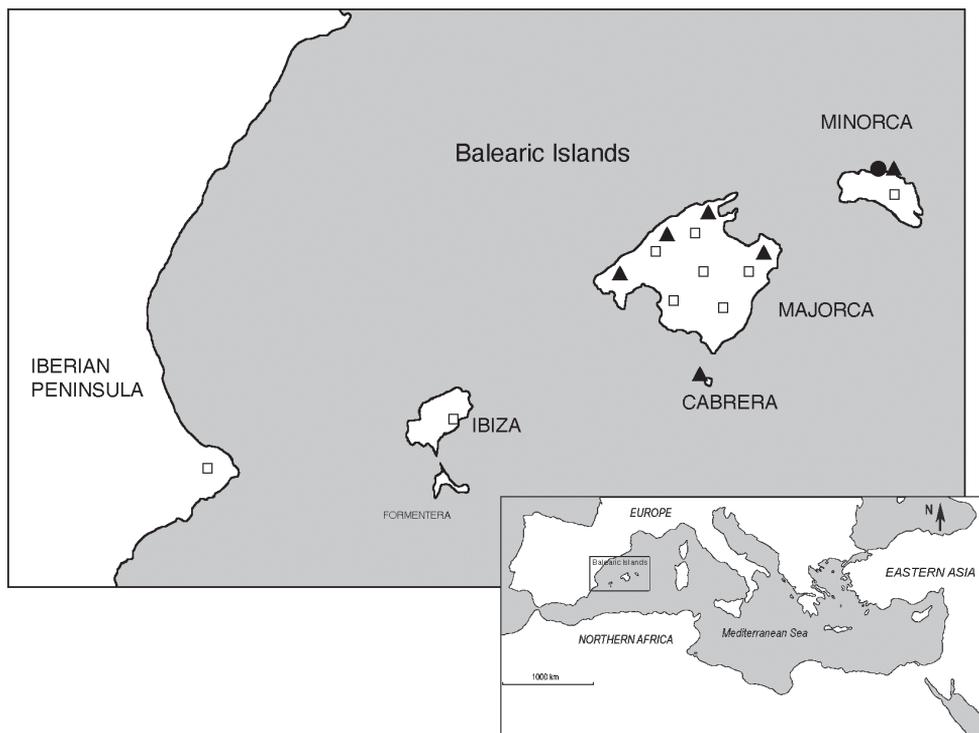


Figure 2. Location of the *Rhamnus* populations included in the study. (See Suppl. material 1 for population abbreviations). *Rh. alaternus* open squares; *Rh. ludovici-salvatoris* filled triangles; Hybrids filled circle.

BVOCs from flowers and those from flower pedicels, volatiles emitted by ten pedicels in both parental species were analysed.

Evaluation of scent composition

Analyses were conducted no longer than 24 hours after collection. HS-SPME-GC-MS analyses were performed on an Agilent 6980 GC - MDS 5975 inert XL (Agilent Technologies, USA), using a Supelcowax 10 gas capillary column (60 m × 0.25 mm × 0.25 mm; Supelco, Bellefonte, Pennsylvania, USA). The PDMS-DVB fibre of the SPME was aged for 3 min in the inlet of the GC. Helium was used as a carrier gas at a flow of 1.3 mL min⁻¹. The splitless sample injection mode was used. The injector temperature was 220 °C. The initial oven temperature was programmed as follows: 45 °C for 2 min, increasing to 250 °C at a rate of 5 °C min⁻¹, which was continued for 5 min. These settings were sufficient for the quantitative desorption of all analytes studied. Mass spectra (MS) were obtained in electron ionisation (EI) mode at 70 eV, while the ionisation source was 180 °C. The MS scan range was set between 45–300 amu. The chromatograms and spectra of the samples were processed using the GC-EM software Turbomass version 5.1 (Perkin-Elmer, Inc.).

The main isolated volatile compounds were annotated by comparing the mass spectra with mass spectral libraries (Wiley 7th edition and NIST08) and by comparing the calculated retention indices with those provided by NIST08, Adams (2007) and El-Sayed (2012). The annotation of the main compounds was verified using an in-house-developed mass spectra/RI library. The main compounds were verified by comparison with synthetic standards. All compounds that were not identified immediately as floral/plant volatiles were verified in the literature to determine if they were accepted as floral BVOCs or at least as potential volatiles.

Statistical analysis of data

Differences in BVOCs composition were analysed using the statistical software R version 4.1.2 (R Project for Statistical Computing). Since the total amount of volatiles released strongly fluctuated between individuals, we used the relative amounts taken as the peak area of each compound in relation to the total peak area. Those compounds considered as likely artefacts were excluded from the analysis. The volatiles from three plants were analysed for each population, averaged, and then treated as a single data set.

Data were transformed using the *decostand* function in the *vegan* library (Oksanen et al. 2019). To assess similarities between individual samples, a permutational multivariate analysis of variance (PERMANOVA) was performed with the *adonis2* function on the *vegan* library using the Bray-Curtis dissimilarity indices (Oksanen et al. 2019). Finally, as a post hoc test to detect differences in volatile composition between each of the species, a pairwise contrast for multilevel comparison (*pairwise.adonis* function, Martínez-Arbizu 2020) was used.

Significant *p*-values in a PERMANOVA indicate a significant difference between groups in the centroid or spread of objects in a multivariate space, so non-metric multidimensional scaling (NMDS, metaMDS function with Bray-Curtis distance, performing a Wisconsin double standardisation and also an sqrt transformation) was used. (Clarke et al. 2014).

To test whether there is a separation between groups in relation to the sex of the flowers, an analysis of similarities (*anosim* function, *vegan* package) was performed. To identify the percentage contributions of each volatile compound to the average dissimilarity amongst the three *Rhamnus* species, the similarity percentage analysis (SIMPER, the *simper* function in *vegan*) was used (Clarke 1993).

Results

Floral scent chemistry

No relevant traces of BVOCs were detected in floral pedicel samples. In the flowers, the entire GC–MS dataset allowed the detection of 41 compounds, of which 22 had greater presence and abundance (with percentages higher than 0.1%; Suppl. material

2). Chromatographic profiles of *R. alaternus* showed high heterogeneity. Thus, some plants exhibited the presence of certain components in high proportions (up to 89.9%), e.g., ionones (dihydro- β -ionone and α -ionone), 3-hexen-1-ol, acetate (*Z*), benzaldehyde 4-dimethoxy, caryophyllene, and linalools (cis-linaloxide, (*E*)-linalool oxide (furanoid), linalool, hotrienol). However, some of these components were absent in other plants. By contrast, despite including a larger number of samples, *R. ludovici-salvatoris* showed a remarkable uniformity. Thus, the major compounds, such as methyl salicylate, methyl benzoate, or α -farnesene, were found in 85–100% of the samples. Similarly, the largest number of minority compounds, such as ethyl benzoate, 1-butanol, and 3-methyl-benzoate, were present in more than 75% of the samples.

Floral scent species relations

The chromatographic profiles of *Rh. alaternus* and *Rh. ludovici-salvatoris* showed significant qualitative and quantitative differences between the species (Suppl. material 2 and Fig. 3). Thus, the following components were exclusive to *Rh. alaternus*: acetic acid hexyl ester, certain terpenoids and ocimene including *cis*- and *trans*- β -ocimene (6.73%); linalools including linalool, cis-linaloxide, trans-linalool oxide furanoid, linalyl formate, and hotrienol (9.10%); caryophyllenes including β - and α -caryophyllene (15.71%), and ionones including α -ionone and dihydro- β -ionone (48.28%). On the other hand, in *Rh. ludovici-salvatoris*, unique compounds were found, namely benzoic acid derivatives (methyl salicylate, methyl benzoate, 1-butanol, 3-methyl benzoate) and farnesenes (farnesene and α -farnesene). Among the quantitatively differential products, 3-hexen-1-ol acetate (*Z*) emerged because in *Rh. alaternus*, it was present in high amounts ($25.92 \pm 7.55\%$), while in *Rh. ludovici-salvatoris* it was only found in low quantities ($0.65 \pm 0.37\%$) and in less than half of the samples.

The results of the SIMPER analysis (Table 1 and Fig. 4) clearly highlight the differences in the aroma profile of the two species. They show that the two species have a distinct floral aroma profile and that four compounds (methyl salicylate, 3-hexen-1-ol acetate, ionone complex, and b-caryophyllene) account for 75% of these differences. On the other hand, no differences were found between the BVOC profiles in relation to sex (ANOSIM statistic $R = -0.03533$, $p = 0.8222$). According to the chemical composition of the hybrid samples and their uniformity, it is noteworthy that they exhibited a great affinity with *Rh. alaternus* (Table 1, Figs 3, 4, Suppl. material 2).

Discussion

Volatile compounds of the green parts of the plants have been used repeatedly as taxonomic characteristics (see Hegnauer 1986a, Nwafor et al. 2018). By contrast, the analysis of volatile biogenic compounds of flower aromas (floral BVOCs) has been scarcely studied. Despite this, most recently, the qualitative and quantitative variations of these compounds have shown their value as discriminating characteristics in related species or in ecotypes,

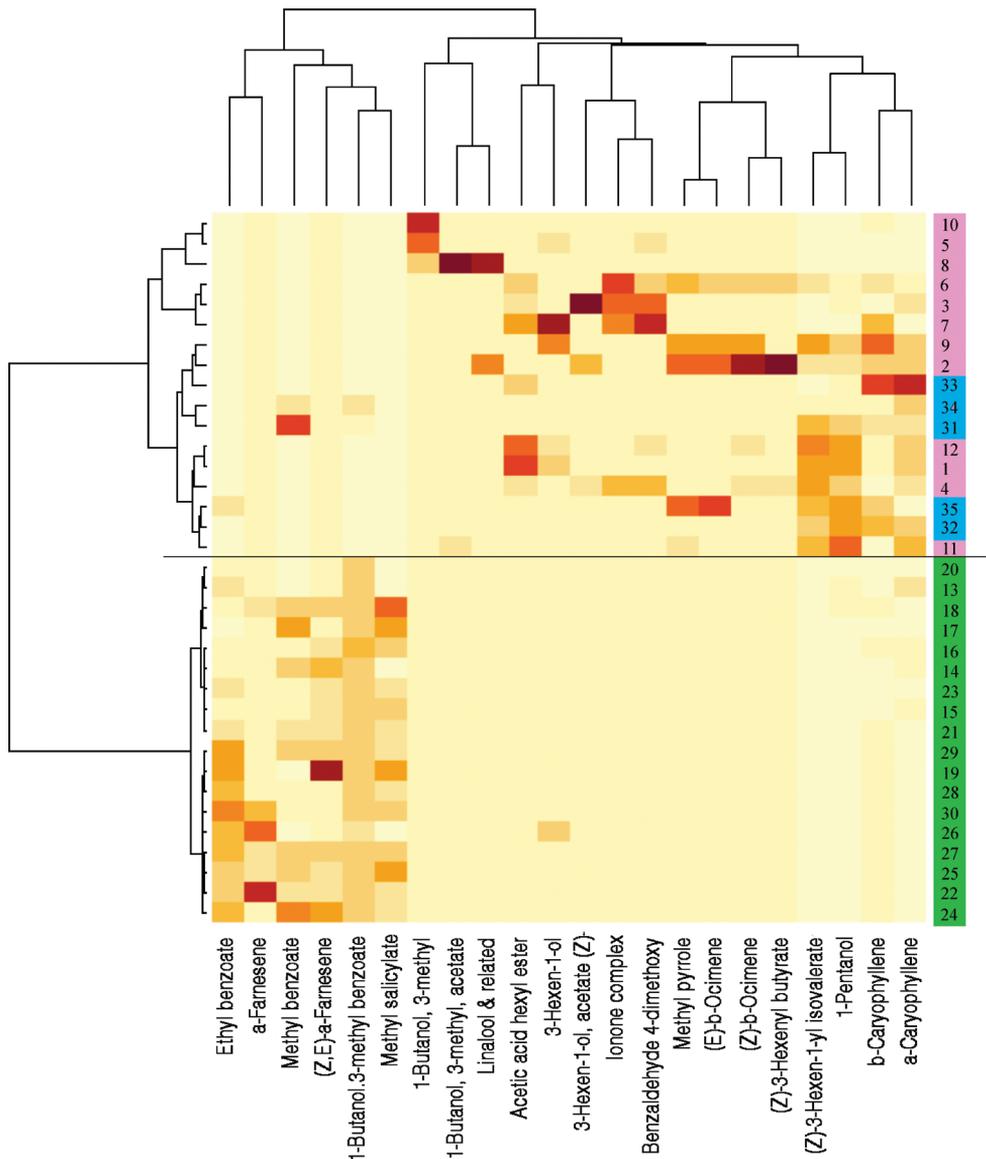


Figure 3. VOCs similarity histogram of *Rhamnus alaternus* (violet), *Rh. xbermejoi* (blue) and *Rh. ludovici-salvatoris* (green). Colour intensity is related to the level of presence of a component in each sample.

such as in the genus *Chusia* (Nogueira et al. 2001), *Nicotiana* (Raguso et al. 2006), *Lilium* (Kong et al. 2012) or *Artemisia* (Jaeger et al. 2016). The results obtained in *Rh. alaternus* and *Rh. ludovici-salvatoris* and their hybrids were consistent with these observations.

The main differences between *Rh. alaternus* and *Rh. ludovici-salvatoris* were found in the types of terpenoids and benzoids emitted, as well as in their emission rates (Suppl. material 2). Thus, among the terpenoids, *R. alaternus* exhibited the emission

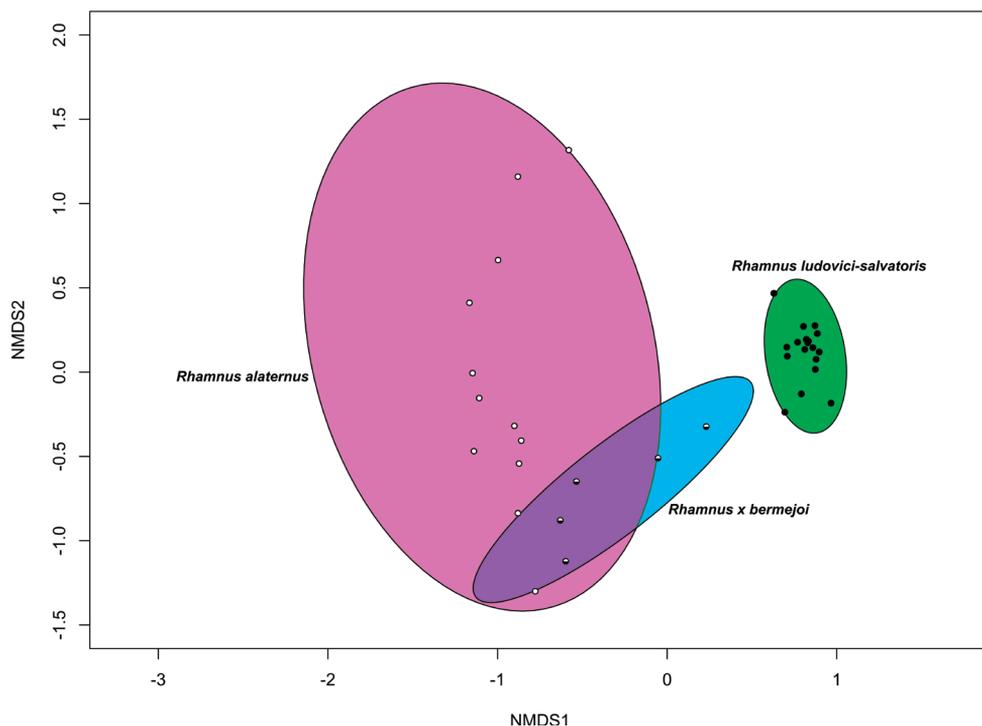


Figure 4. Non-metric multidimensional scaling ordination (NMDS) of floral scent profiles for *Rhamnus alaternus* (violet and empty circles), *Rhamnus x bermejoi* (blue and half black circles) and *Rhamnus ludovici-salvatoris* (green and black points).

Table I. Results of SIMPER analysis indicate the cumulative contributions of most influential compounds. AvA: Average in *Rhamnus alaternus*; AvB: Average in *Rhamnus ludovici-salvatoris*; Cumsum: cumulative contribution (in%).

	AvA	AvB	Cumsum
Methyl salicylate	0.00	82.32	44.4
3-Hexen-1-ol, acetate (Z)-	24.88	0.64	57.6
Ionone complex	16.09	0.00	66.4
b-Caryophyllene	15.18	0.00	74.6
Methyl benzoate	0.00	9.25	79.6
Linalool and related	9.11	0.00	84.4
Benzaldehyde 4-dimethoxy	5.23	0.00	87.1
(Z)-b-Ocimene	4.69	0.00	89.7
1-Butanol, 3 methyl, acetate	4.26	0.54	92.0

of monoterpenes ((Z)- and (E)- β -ocimene and linalool and related compounds) and ionones; all these products are absent in *Rh. ludovici-salvatoris*. There were also different types of sesquiterpenes, farnesenes being characteristic of *Rh. ludovici-salvatoris* and caryophyllenes being characteristic of *Rh. alaternus*. On the other hand, the uniformity and

richness of benzoids in *Rh. ludovici-salvatoris* (specifically, methyl salicylate and methyl benzoate) were very prevalent. This finding contrasts with its absence in *Rh. alaternus*, because in this species, only the rare presence of benzaldehyde 4-dimethoxy has been recognised. Methyl salicylate (83.3%) and methyl benzoate (9.2%) constitute the primary components of the floral aroma in *Rh. ludovici-salvatoris*. These compounds are also present in hybrids but notably absent in *Rh. alaternus*. Both esters are biosynthesised by the action of carboxyl methyltransferases and have been associated with pollinator attraction (Negre et al. 2003), but they also play an important role as aerial signals involved in inter- and intra-plant communication to activate disease (Shulaev et al. 1997) and, as also seems plausible in this case, signalling an abiotic stress situation for this species (Munné-Bosch and Peñuelas 2003; Karl et al. 2008; Liu et al. 2018; Gondor et al. 2022).

The high intra-population heterogeneity of chemical compounds of *Rh. alaternus* was evidenced by the fact that not all differential compounds were found in all the plants; however, some of them were always present. This observation stands in contrast with *Rh. ludovici-salvatoris*, which has a high chemical similarity with all plants, as evidenced by the presence of its main differential compounds, methyl salicylate and methyl benzoate. These chemical diversity profiles followed a pattern similar to that of genetic diversity (Ferriol et al. 2009; Hauenschild et al. 2016), which recognizes much higher levels of genetic variation in the populations of *Rh. alaternus* than in those of endemic *Rh. ludovici-salvatoris*.

The qualitative and quantitative patterns of VOC expression in hybrids are heterogeneous (López-Caamal 2014). In some species, hybridization could lead to the production of new secondary metabolites that are not present in parental species, due to the obstruction of biosynthetic pathways (Vereecken et al. 2010; Kong et al. 2012). In other hybrid species, highly variable proportions of several compounds are present. In some, the emission rates of some of these may be interpreted as transgressive with the parents; however, they are not always consistently intermediate. In others, only the emission of specific compounds was intermediate (Bischoff et al. 2014). In general, whenever hybridization occurs, a large qualitative variation in secondary metabolites is expected. In the studied hybrids, compounds shared with parental strains were produced at different levels, but no new products were recognized (Suppl. material 2). In the amounts of shared compounds, there was a clear tendency to be more like *Rh. alaternus* (Fig. 4).

Both personal observations and those of Canale et al. (2016) showed that the main pollinators of the two *Rhamnus* species were Hymenoptera (mainly *Apis mellifera* Linnæus, 1758). This finding was consistent with the fact that the two groups of BVOCs that are dominant in the flowers of the two species of *Rhamnus* attract honey bees: terpenoids for *Rh. alaternus* (Dobson 2006; Twidle et al. 2015) and benzoids for *Rh. ludovici-salvatoris* (Negre et al. 2003; Mallinger et al. 2011; Dötterl et al. 2014). Thus, this could be the pollination vector that mediates the hybridization process.

When the diversity of BVOCs is greater in hybrids than in the parentals, their heterogeneity and progression are encouraged, since this could help in attracting more pollinator species (Ayasse et al. 2011), breaking the reproductive isolation of hybrids. However, this increase does not occur in the studied hybrids; therefore, their VOC content, as in their parent *R. ludovici-salvatoris*, was not a factor that favours pollination and reproduction.

Under these conditions, compared to *Rh. alaternus*, lower photosynthetic efficiency of *Rh. ludovici-salvatoris* and its hybrids in the current climatic Mediterranean circumstances (Yll et al. 1997; Gulias et al. 2002) would be, together with the inability of regrowth, the main cause that would explain both the progressive decrease in the populations of this species as well as the rarity of hybrids and the relictual character of the current population of *Rh. ludovici-salvatoris* and its hybrids in Minorca (Fraga and Bermejo 2008).

Conclusions

The analysis of the floral volatile compounds in the studied taxa reveals both qualitative and quantitative differences in the chemical profile of *R. alaternus* and *Rh. ludovici-salvatoris*. Among them, the discriminatory nature of various compounds stands out, such as methyl salicylate and methyl benzoate (present in *Rh. ludovici-salvatoris* and absent in *Rh. alaternus*), linalool and related compounds, ionones, β -caryophyllene, 4-dimethoxy benzaldehyde, and (Z)- β -ocimene (present in *Rh. alaternus* and absent in *Rh. ludovici-salvatoris*). In contrast, the chemical profile does not allow differentiation between *Rh. alaternus* and the hybrid *Rh. \times bermejoi*. According to these results, it is suggested that floral volatile compounds could be an effective discriminatory trait among species in *Rh.* sect. *Alaternus*. This finding should be confirmed through the study of plants from other populations of *Rh. alaternus*, as well as samples from *Rh. lojaconoi* and the Macaronesian endemic species *Rh. glandulosa* and *Rh. integrifolia*.

Acknowledgements

The authors are especially grateful to L. Cortés and F. Roma-Marzio for their valuable comments on the manuscript, to S. Biondi for the English revision of the text, and to C. Cardona for providing plant material.

References

- Adams RP (2007) Identification of Essential oil Components by Gas Chromatography/MassSpectroscopy. Carol Stream, IL. Allured Publishing Corporation.
- Ahmad R, Baharum SN, Bunawan H, Lee M, Mohd Noor N, Rohani ER, Ilias N, Zin NM (2014) Volatile profiling of aromatic traditional medicinal plant, *Polygonum minus* in different tissues and Its biological activities. *Molecules* 19: 19220–19242. <https://doi.org/10.3390/molecules191119220>
- Aysse M, Stökl J, Francke W (2011) Chemical ecology and pollinator-driven speciation in sexually deceptive orchids. *Phytochemistry* 72: 1667–1677. <https://doi.org/10.1016/j.phytochem.2011.03.023>

- Bischoff M, Jürgens A, Campbell DR (2014) Floral scent in natural hybrids of *Ipomopsis* (Polemoniaceae) and their parental species. *Annals of Botany* 113: 533–544. <https://doi.org/10.1093/aob/mct279>
- Bouwmeester H, Schuurink RC, Bleeker PM, Schiestl F (2019) The role of volatiles in plant communication. *Plant Journal* 100(5): 892–907. <https://doi.org/10.1111/tpj.14496>
- Canale A, Benvenuti S, Raspi A, Benelli G (2016) Insect pollinators of the late winter flowering *Rhamnus alaternus* L., a candidate for honeybee-friendly scrubland spots in intensively managed agricultural areas. *Plant Biosystems* 150: 611–615. <https://doi.org/10.1080/11263504.2014.993742>
- Cardona MA, Contandriopoulos J (1979) Endemism and evolution in the islands of the western Mediterranean. In: Bramwell D (Ed.) *Plant and islands*. Academic Press, London, UK, 133–169.
- Carta A, Flamini G, Cioni PL, Pistelli L, Peruzzi L (2015) Flower bouquet variation in four species of *Crocus* ser. *Verni*. *Journal of Chemical Ecology* 41: 105–110. <https://doi.org/10.1007/s10886-014-0541-y>
- Cheng D, Vrieling K, Klinkhamer PG (2011) The effect of hybridization on secondary metabolites and herbivore resistance: implications for the evolution of chemical diversity in plants. *Phytochemistry Reviews* 10: 107–117. <https://doi.org/10.1007/s11101-010-9194-9>
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- Clarke KR, Gorley RN, Somerfield PJ, Warwick RM (2014) *Change in marine communities: an approach to statistical analysis and interpretation*, 3rd edn. PRIMER-E Ltd, Plymouth, 1–262.
- Cronquist A (1977) On the taxonomic significance of secondary metabolites in Angiosperms. In: Kubitzki K (Ed.) *Flowering Plants. Plant Systematics and Evolution / Entwicklungs-geschichte und Systematik der Pflanzen*, vol 1. Springer, Vienna. *Flowering Plants*, 179–189. https://doi.org/10.1007/978-3-7091-7076-2_12
- Dobson HEM (2006) Relationship between floral fragrance composition and type of pollinators. In: Duradeva N, Pichersky E (Eds) *Biology of floral scent*. CRC Taylor & Francis, 147–198. <https://doi.org/10.1201/9781420004007-8>
- Dötterl S, Glück U, Jürgens A, Woodring J, Aas G (2014) Floral reward, advertisement and attractiveness to honey bees in dioecious *Salix caprea*. *PLoS ONE* 9(3): e93421. <https://doi.org/10.1371/journal.pone.0093421>
- El-Sayed AM (2023) The Pherobase: Data base of Insect Pheromones and Semiochemicals. <http://www.pherobase.com>
- Ferriol M, Llorens L, Gil L, Boira H (2009) Influence of phenological barriers and habitat differentiation on the population genetic structure of the Balearic endemic *Rhamnus ludovici-salvatoris* Chodat and *Rh. alaternus* L. *Plant Systematics and Evolution* 277: 105–116. <https://doi.org/10.1007/s00606-008-0110-3>
- Feulner M, Schuhwerk F, Dötterl S (2011) Taxonomical value of inflorescence scent in *Hieracium* s. str. *Biochemical Systematics and Ecology* 39: 732–743. <https://doi.org/10.1016/j.bse.2011.06.012>
- Fraga P, Bermejo A (2008) Notes i contribucions al coneixement de la flora de Menorca (IX): característiques i estat de conservació de la població menorquina de *Rhamnus ludovici-salvatoris* Chodat. *Bolletí de la Societat d'Història Natural de les Balears* 51: 17–32.

- Fraga P, Rosselló JA (2008) *Rhamnus* × *bermejoi*, a new wild hybrid between *Rh. alaternus* and *Rh. ludovici-salvatoris*. *Flora Mont* 40: 47–79.
- Friberg M, Schwind C, Raguso R, Thompson JN (2013) Extreme divergence in floral scent among woodland star species (*Lithophragma* spp.) pollinated by floral parasites. *Annals of Botany* 111: 539–550. <https://doi.org/10.1093/aob/mct007>
- Georgescu L, Stefanakis MK, Kokkini S, Katerinopoulos HE, Pirintzos SA (2015) Chemical and genetic characterization of *Phlomis* species and wild hybrids in Crete. *Phytochemistry* 122: 91–102. <https://doi.org/10.1016/j.phytochem.2015.11.007>
- Gil-ad NL, Reznicek AA (1997) Evidence for hybridation of two Old World *Rhamnus* species -*R. cathartica* and *R. utilis* (Rhamnaceae)- in the New World. *Rhodora* 99: 1–22.
- GISD [Global Invasive Species Database] (2018) Species profile: *Rhamnus alaternus*. <http://www.iucngisd.org/gisd/species.php?sc=893> [Downloaded on 10-09-2018]
- Gögler J, Stökl J, Sramkova A, Twele R, Francke W, Cozzolino S, Cortis P, Scrugli A, Ayasse M (2009) Menage a trios: two endemic species of deceptive orchids and one pollinator species. *Evolution* 63: 2222–2234. <https://doi.org/10.1111/j.1558-5646.2009.00712.x>
- Gondor OK, Pál M, Janda T, Szalai G (2022) The role of methyl salicylate in plant growth under stress conditions. *Journal of Plant Physiology* 277: 153809. <https://doi.org/10.1016/j.jplph.2022.153809>
- Goswami P, Chauhan A, Verma RS, Padalia RC (2016) Chemical constituents of floral volatiles of *Plumeria rubra* L. from India. *Medicinal and Aromatic Plants Research Journals* S3: 005. <https://doi.org/10.4172/2167-0412.S3-005>
- Gulias J, Flexas J, Abadia A, Medrano H (2002) Photosynthetic responses to water deficit in six mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree Physiology* 22: 687–697. <https://doi.org/10.1093/treephys/22.10.687>
- Hadacek F (2002) Secondary metabolites as plant traits: current assessment and future perspectives. *Critical Reviews in Plant Sciences* 21: 273–322. <https://doi.org/10.1080/0735-260291044269>
- Hauenschild F, Favre A, Gerardo A, Salazar GA, Muellner-Riehl AN (2016) Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*. *Taxon* 65: 65–78. <https://doi.org/10.12705/651.5>
- Hegnauer R (1986a) Phytochemistry and plant taxonomy-an essay on the chemotaxonomy of higher plants. *Phytochemistry* 25: 1519–1535. [https://doi.org/10.1016/S0031-9422\(00\)81204-2](https://doi.org/10.1016/S0031-9422(00)81204-2)
- Hegnauer R (1986b) Comparative phytochemistry and plant taxonomy. *Gior Botany Italia* 120: 15–26. <https://doi.org/10.1080/11263508609428018>
- Henning T, Raab-Straube E von (2016): Rhamnaceae. In: Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. <http://ww2.bgbm.org/EuroPlusMed/> [Accessed 2023]
- Jaeger DM, Runyon JB, Richardson BA (2016) Signals of speciation: volatile organic compounds resolve closely related sagebrush taxa, suggesting their importance in evolution. *New Phytologist* 211: 1393–1401. <https://doi.org/10.1111/nph.13982>
- Karl T, Guenther A, Turnipseed A, Patton EG, Jardine K (2008) Chemical sensing of plant stress at the ecosystem scale. *Biogeosciences* 5: 1287–1294. <https://doi.org/10.5194/bg-5-1287-2008>

- Kong Y, Sun M, Pan H, Zhang Q (2012) Composition and emission rhythm of floral scent volatiles from eight lily cut flowers. *Journal of the American Society for Horticultural Science* 137: 376–382. <https://doi.org/10.21273/JASHS.137.6.376>
- Kurylo JS, Knight KS, Stewart JR, Endress AG (2007) *Rhamnus cathartica*: native and naturalized distribution and habitat preferences. *The Journal of the Torrey Botanical Society* 143: 420–430. [https://doi.org/10.3159/1095-5674\(2007\)134\[420:RCNAND\]2.0.CO;2](https://doi.org/10.3159/1095-5674(2007)134[420:RCNAND]2.0.CO;2)
- Levin RA, McDade L, Raguso RA (2003) The systematic utility of floral and vegetative fragrance in two genera of Nyctaginaceae. *Systematic Biology* 52: 334–351. <https://doi.org/10.1080/10635150390196975>
- Liu B, Kaurilind E, Jiang Y, Niinemets Ü (2018) Methyl salicylate differently affects benzenoid and terpenoid volatile emissions in *Betula pendula*. *Tree Physiology* 38(10): 1513–1525. <https://doi.org/10.1093/treephys/tpy050>
- Llorens L (1979) Nueva contribución al conocimiento de la flora balear. *Mediterranea* 3: 101–122. <https://doi.org/10.14198/MDTRRA1979.3.04>
- López-Caamal A, Tovar-Sánchez E (2014) Genetic, morphological, and chemical patterns of plant hybridization. *Revista Chilena de Historia Natural* 87: 16. <https://doi.org/10.1186/s40693-014-0016-0>
- López-González G (2006) Los árboles y arbustos de la Península ibérica e Islas Baleares. Mundi-Prensa, Barcelona, 533–536.
- Mallinger RE, Hogg DB, Gratton C (2011) Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in Soybean Agroecosystems. *Journal of Economic Entomology* 104: 115–124. <https://doi.org/10.1603/EC10253>
- Martinez-Arbizu P (2020) pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4. <https://github.com/pmartinezarbizu/pairwiseAdonis>
- Meekijjaroenroj A, Bessière JM, Anstett MC (2007) Chemistry of floral scents in four *Licuala* species (Arecaceae). *Flavour and Fragrance Journal* 22: 300–310. <https://doi.org/10.1002/ffj.1797>
- Munné-Bosch S, Peñuelas J (2003) Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* 217: 758–766. <https://doi.org/10.1007/s00425-003-1037-0>
- Muyt A (2001) Bush Invaders of South-east Australia. RG and FJ Richardson Meredith, Victoria, Australia, 213–214.
- Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, Wagner C, Clark DG, Dudareva N (2003) Regulation of methylbenzoate emission after pollination in snapdragon and *Petunia* flowers. *The Plant Cell* 15: 2992–3006. <https://doi.org/10.1105/tpc.016766>
- Nogueira PCL, Bittrich V, Shepherd GJ, Lopes AV, Marsaioli AJ (2001) The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). *Phytochemistry* 56: 443–452. [https://doi.org/10.1016/S0031-9422\(00\)00213-2](https://doi.org/10.1016/S0031-9422(00)00213-2)
- Nwafor FI, Orabueze IC (2018) Role of phytochemistry in plant classification: Phytochemotaxonomy. In: Egbuna C, Ifemeje JC, Udedi SC, Kumar S (Eds) *Phytochemistry: Fundamentals, Modern Techniques, and Applications* vol 1, Apple Academic Press, 198–224.
- Oksanen AJ, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, Hara RBO, Simpson GL, Solymos P, Stevens MHH, Szoecs (2019) E. vegan:

- community ecology package. R package version 2.5-6. <https://CRAN.R-project.org/package=vegan>
- Orians CM (2000) The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. *American Journal of Botany* 87: 1749–1756. <https://doi.org/10.2307/2656824>
- Peruzzi L, Roma-Marzio F, Dolci D, Flamini G, Braca A, De Leo M (2019) Phytochemical data parallel morpho-colorimetric variation in *Polygala flavescens* DC. *Plant Biosystems* 153(6): 817–834. <https://doi.org/10.1080/11263504.2018.1549615>
- Raguso RA, Schlumpberger BO, Kaczorowski RL, Holtsford TP (2006) Phylogenetic fragrance patterns in *Nicotiana* sections *Alatae* and *Suaveolentes*. *Phytochemistry* 67: 1931–1942. <https://doi.org/10.1016/j.phytochem.2006.05.038>
- Rivas-Martínez S, Pizarro JM (2011) Taxonomical system advance to *Rhamnus* L. & *Frangula* Mill. (Rhamnaceae) of Iberian Peninsula and Balearic Islands. *International Journal of Geobotanical Research* 1: 55–78. <https://doi.org/10.5616/ijgr110004>
- Rivas-Martínez S, Pizarro JM (2013) *Rhamnus* L. In: Muñoz Garmendia F, Navarro C, Quintanar A, Buirra A (Eds) *Flora Iberica* 9, Real Jardín Botánico, CSIC, Madrid, 36–44.
- Roma-Marzio F, Najar B, Alessandri J, Pistelli L, Peruzzi L (2017) Taxonomy of prickly juniper (*Juniperus oxycedrus* group): A phytochemical–morphometric combined approach at the contact zone of two cryptospecies. *Phytochemistry* 141: 48–60. <https://doi.org/10.1016/j.phytochem.2017.05.008>
- Seigler DS (1981) Secondary metabolites and plant systematics. In: Conn EE (Ed.) *The biochemistry of plants. Secondary plant products* vol 7, Academic Press, 139–171. <https://doi.org/10.1016/B978-0-12-675407-0.50012-9>
- Shul'ev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* 385: 718–721 <https://doi.org/10.1038/385718a0>
- Singh R (2016) Chemotaxonomy: A Tool for Plant Classification. *Journal of Medicinal Plants Studies* 4(2): 90–93.
- Stocki M, Banaszczyk P, Stocka N, Borowik T, Zapora E, Isidorov V (2020) Taxonomic implications of volatile secondary metabolites emitted from birch (*Betula* L.) buds. *Biochemical Systematics and Ecology* 92: 104132. <https://doi.org/10.1016/j.bse.2020.104132>
- Tomas J, Cardona C, Ferriol P, Llorens L, Gil L (2022) Floral traits and reproductive biology of two Mediterranean species of *Clematis*, asynchronous and sympatric, are key food sources for pollinator survival. *South African Journal of Botany* 151: 85–94. <https://doi.org/10.1016/j.sajb.2022.09.027>
- Tóth P, Undas AK, Verstappen F, Bouwmeester H (2016) Floral Volatiles in Parasitic Plants of the Orobanchaceae. Ecological and Taxonomic Implications. *Frontiers in Plant Science* 7: 312. <https://doi.org/10.3389/fpls.2016.00312>
- Tundis R, Peruzzi L, Menichini F (2014) Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: a review. *Phytochemistry* 102: 7–39. <https://doi.org/10.1016/j.phytochem.2014.01.023>
- Twidle AM, Mas F, Harper AR, Horner RM, Weish TJ, Suckling DM (2015) Kiwifruit flower odor perception and recognition by honey bees, *Apis mellifera*. *Journal of Agricultural and Food Chemistry* 63: 5597–5602. <https://doi.org/10.1021/acs.jafc.5b01165>

- Vereecken NJ, Cozzolino S, Schiestl FP (2010) Hybrid floral scent novelty drives pollinator shift in sexually deceptive orchids. *BMC Evolutionary Biology* 10: 103. <https://doi.org/10.1186/1471-2148-10-103>
- Whitehead MR, Peakall R (2009) Integrating floral scent, pollination ecology and population genetics. *Functional Ecology* 23: 863–874. <https://doi.org/10.1111/j.1365-2435.2009.01620.x>
- Yang L, Ren J, Wang Y (2014) Chemical investigation of volatiles emitted from flowers of three varieties of Damask rose cultivated in Beijing. *Horticulture Environment and Biotechnology* 55: 524–530. <https://doi.org/10.1007/s13580-014-0176-5>
- Yll E, Pérez-Obiol R, Pantaleon-Cano J, Roure JM (1997) Palynological evidence for climatic change and human activity during the Holocene on Minorca (Balearic Islands). *Quaternary Research* 48: 339–347. <https://doi.org/10.1006/qres.1997.1925>

Supplementary material 1

Geographical location and sexuality of *Rhamnus* sampled plants

Authors: Leonardo Llorens, Pere Ferriol, Joan Tomàs, María Trinidad García, Lorenzo Gil
Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/italianbotanist.16.116255.suppl1>

Supplementary material 2

Floral scent chemistry

Authors: Leonardo Llorens, Pere Ferriol, Joan Tomàs, María Trinidad García, Lorenzo Gil
Data type: docx

Explanation note: Floral scent chemistry of *Rhamnus alaternus* (blue), *R. ludovici-salvatoris* (yellow) and hybrids (green). For each species, compounds with presence in >10% of samples.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/italianbotanist.16.116255.suppl2>