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The scent of *Spiranthes spiralis*

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Abstract

HS-SPME-GC-MS analysis of the scent of *Spiranthes spiralis* showed the presence as main components of 2-(4-methoxyphenyl)ethanol, 4-methoxybenzaldehyde, tridecane, pentadecane, farnesal, heptadecane,1,4-dimethoxybenzene, and 1,2,4-trimethoxybenzene. All these compounds can have a role as attractive components towards pollinator insects. Furthermore, low amounts of several aldehydes (benzaldehyde, octanal, decanal, tridecanal, tetradecanal, and hexadecanal) have been detected. These compounds can be active compounds in sexual deceptive orchids.

Keywords: *Spiranthes spiralis*, scent, orchid, solid phase microextraction, gas chromatography, mass spectrometry, attractive compounds.

Introduction

Some years ago, we started a research project devoted to the study of the scent of spontaneous orchid species growing in Basilicata (Southern Italy) by using the same analytical procedure based on the use of SPME (Solid Phase MicroExtraction) coupled with gas chromatography and mass spectrometry. Until now, the study covered the aroma profiles of *Platanthera* [1,2], *Cephalanthera* [3], *Orchis* [4], *Serapias* [5], *Himantoglossum* [4], *Barlia robertiana* [4], *Dactylorhiza* [4], *Gymnadenia* [6], *Neotinea* [7], *Ophrys* [4], *Epipactis*, *Neottia*, *Limodorum* [4], *Anacamptis* species [8]. In this contribution the analysis of the scent of *Spiranthes spiralis* is presented.

Figure 1. *Spiranthes spiralis* (photo of V. A. Romano).

The genus *Spiranthes* belongs to the cosmopolitan family of *Orchidaceae*. There are about 40 species of this genus worldwide [9], four in Europe and 2 in Italy (*Spiranthes spiralis* (L.) Chevall. and *Spiranthes aestivalis* (Poir.) Rich.).

In Basilicata there is only *Spiranthes spiralis* [10] (Fig. 1), very common and widespread throughout the region in different environments, dry or temporarily humid, a typical heliophyte that prefers open habitats and well-lit ecological niches, characterized by low grassy vegetation such as that of olive groves, shrubs and forest clearings, occasionally it is found in marginal environments such as paths, roadsides, edges of crops and even urban environments.

Spiranthes spiralis, native to the British flora, is widely distributed in North Africa, southern Europe [11] and the Mediterranean region [9].

In the Mediterranean basin, the leaf rosette in *Orchidaceae* usually develops in winter, but may be delayed until early summer, depending on the species and vertical distribution in Europe. *Spiranthes spiralis* deviates widely from this pattern: both inflorescences and leaf rosettes appear above ground by late summer (August-September), but inflorescences may appear several days or even weeks before the rosettes, which usually appear in autumn and wither in late summer.

In Basilicata, the flowering of this species can be observed from late September to October depending on altitude.

It is a relatively small species, the spike inflorescence is 3–12(20) cm long, thin, with (6)10–25(30) with white flowers, very small (about 0.5 cm), arranged along a narrow left-handed or right-handed spiral along the flower stem, or more rarely they are all turned to one side. The labellum (3.3)4–5 x (1.7)2.4–3 mm, non-lobed, oblong, trough-shaped, forms, with the upper external and internal segments of the perianth (set of petals and sepals) joined, a trumpet-shaped tube of pale green color with a broad, jagged crystalline margin, which tightly embraces the column at the base, where there are two white, shiny, rounded glands, which secrete nectar much sought after by the few pollinators still active in the autumn

period, mainly bumblebees and honeybees including *Bombus pascuorum*, *B. lapidarius*, *B. terrestris* and *Apis mellifera* [12-14].

The floral biology of *Spiranthes spiralis* has been described in detail by Darwin [15] and summarized by Summerhayes [16] and Van der Cingel [17], its flowers are pollinated by insects, according to a highly evolved mechanism that effectively favors cross-fertilization.

The anthesis of the plant begins from the bottom to the top, the first flowers just opened, have the perianth very tight around the gynostemium, the pollinators in an attempt to reach the nectar remove the pollen masses, and immediately move to the higher flowers in search of more nectar and in a short time, once the inspection is finished, loaded with pollinia, they visit another ear, always starting from the lower flowers and, if these have already been visited, they can easily reach the nectar and deposit the pollinia taken from the other plants on the stigma because after the removal of the pollinia the perianth of the flowers tends to open facilitating access and the deposition of pollen on the stigma. Going up the inflorescence, the insect finds fresh flowers in which it cannot reach the nectar but loads itself with new pollinia that it will carry to the flowers of another plant. The flowers give off a sweet scent during the day, reminiscent of *Convallaria majalis* [18], vanilla [19] or almonds [20].

Results and Discussion

SPME analysis of the scent of *Spiranthes spiralis* is reported in Table 1. The main component of the scent was 2-(4-methoxyphenyl)ethanol (21.72%) and 4-methoxy benzaldehyde (12.14%). 2-(4-Methoxyphenyl)ethanol has an important aroma identified as fresh citrus juice. It has been reported as an attractive compound for *Euglossa nigropelosa* [21]. 4-Methoxybenzaldehyde It provides sweet, floral and strong aniseed odor. It has been reported as an active compound *vs. Bombus terrestris* [22].

Table 1. HS-SPME-GC-MS analysis of the scent of *Spiranthes spiralis.*

Compound	r.t [min.]	KI	Area [%]
4-Methyl-3-penten-2-one	5.41	782	2.14

2-Butoxyethanol	7.79	887	0.26
Benzaldehyde	8.84	939	0.50
Octanal	9.71	983	0.20
2,2,4,6,6-Pentamethyl-3-heptene	9.77	1004	0.22
1-Methoxy-4-methylbenzene	10.10	1020	0.26
Limonene	10.26	1031	0.26
Acetophenone	11.07	1065	0.04
2,2,6,6-tetramethyl-4-piperidinone	12.07	1103	0.44
1,4-Dimethoxybenzene	12.94	1163	2.80
Decanal	13.68	1204	0.84
4-Methoxymethylanisole	14.44	1250	0.80
4-Methoxybenzaldehyde	14.68	1275	12.14
Tridecane	14.98	1300	0.72
1,2,4-Trimethoxybenzene	16.62	1331	2.58
2-(4-methoxyphenyl)ethanol	16.83	1374	21.72
Tetradecane	16.99	1400	8.48
Methyl 2-phenylcyclopropanecarboxylate	17.76	1455	0.62
6,10-Dimethyl-5,9-undecadien-2-one	17.91	1453	0.50
2,6-Bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione	18.25	1474	0.90
Tridecanal	18.35	1484	1.56
Pentadecane	18.58	1500	5.88
Tetradecanal	20.30	1590	1.34

Benzophenone	20.72	1621	1.96
2,6,10-Trimethylpentadecane	20.77	1650	1.08
Heptadecane	21.50	1700	3.70
2,6,10,14-Tetramethylpentadecane	21.58	1710	1.88
Farnesal	22.20	1738	5.36
2-(Phenylmethylene)octanal	22.32	1750	1.74
Octadecane	22.85	1800	1.82
Phytan	22.98	1814	0.72
Hexadecanal	23.10	1819	0.58
6,10,14-Trimethyl-2-pentadecanone	23.46	1846	0.98

Other compounds found in the scent in relevant amounts were tetradecane (8.48%), pentadecane (5.88%), heptadecane (3.70%) and farnesal (5.36%). Alkanes can be sex pheromones in several insect species [23], Farnesal shows a floral minty odor. It is a pheromone for some hymenopter species. Furthermore, some other compounds were found in the scent: 4-methyl-3-penten-2-one (2.14%), 1,4-dimethoxybenzene (2.80%), and 1,2,4-trimethoxybenzene (2.58%). 4-Methyl-3-penten-2-one has a pungent earthy vegetable acrylic odor. 1,4-Dimethoxybenzene showed a sweet, aromatic, erbaceous, earthy, tobacco note odor. Both 1,4-dimethoxybenzene and 1,2,4-trimethoxybenzene have been reported as attractive compounds towards bee species [23]. Finally, it has to be noted that the presence of the scent of some aldehydes. The presence of 4-methoxybenzaldehyde and farnesal was just discussed above. However, benzaldehyde (0.50%), octanal (0.20%), decanal (0.84%), tridecanal (1.56%), tetradecanal (1.34%), and hexadecanal (0.58%) were present in the scent in low quantities. Benzaldehyde has a fruity odor. Octanal shows an intense citrus note. Decanal has a sweet, aldehydic, orange, waxy and citrus rind odor. Tridecanal odor is characterized by watery, citrus, and floral notes. The scent of tetradecanal has fatty, waxy,

dairy, creamy and fishy with a fruity, pear nuance properties. These compounds have been found in sexual deceptive orchids as active in electroantennography detection assays [23].

Conclusion

The SPMe analysis of the scent of *Spiranthes spiralis* showed the presence of a complex mixture of compounds where, starting from the main components until compounds present in very low amounts, most of the components are devoted to emit signal able to attract pollinator insect.

Materials and methods

Plant Material

The sample of *Spiranthes spiralis* was collected at Macchia Romana (Potenza), on September 15, 2017. The plants were collected by Vito Antonio Romano.

The plants were harvested taking all the clod of earth, taking care not to damage the root system. All the plants had closed flowers to avoid using flowers that were already fertilized but not visible because they were at the beginning of fertilization. The plants were planted in special pots in the greenhouse of the University of Basilicata (Potenza 650 m. a.s.l.), in closed boxes with transparent cloth to avoid fertilization (even if occasional). The correct classification of the species was carried out on flowering plants. The plants were tested when the flowers were all open.

The plants were tested, whole without being damaged, under a cylindrical glass bell (12cm x 45cm) in which only the inflorescence and the SPME probe are inserted.

To avoid contamination, the interior of the bell was isolated from the external environment with appropriate closing and sealing systems during the 24 hours of the test (from eight in the morning to 8 the following day).

In order to be sure that the internal environment of the bell was isolated from the external environment, various blank tests were carried out.

After the tests the plants remained closed in the boxes to verify that at the end of flowering there were no fertile ovaries and for this reason no herbarium samples were taken. The earthen bread with the bulbs were brought back to the site.

In view of the fact that the investigated taxa are rare wild plants, in order to preserve the species, we have chosen to use a single plant for our analysis.

Analysis of Volatile Organic Compounds

The SPME analysis of ten different samples of Ophrys has been performed. This way, the identified plants were collected and inserted in glass jar for 24 h where there was present also the fiber (DVB/CAR/PDMS) of and SPME syringe. After this time the fiber was de-sorbed in a gas chromatographic apparatus equipped with a quadrupole mass spectrometer detector. A 50/30-µm DVB/CAR/PDMS module with 1 cm fiber (57328-U, Supelco, Milan, Italy) was employed to determine VOCs. SPME fiber was maintained in the bell jar for 24 h. The analytes were desorbed in the splitless injector at 250 °C for 2 min. Analyses were accomplished with an HP 6890 Plus gas chromatograph equipped with a Phenomenex Zebron ZB-5 MS capillary column (30-m × 0.25-mm i.d. × 0.25 µm FT) (Agilent, Milan, Italy). An HP 5973 mass selective detector in the range 0-800 m/z (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. The EI source was used at 70 eV. The analyses were performed by using a splitless injector. The splitless injector was maintained at 250 °C and the detector at 230 °C. The oven was held at 40 °C for 2 min, then gradually warmed, 8 °C/min, up to 250 °C and held for 10 min. Tentatively identification of aroma components was based on mass spectra and Wiley 11 and NIST 14 library comparison. Single VOC peak was considered as identified when its experimental spectrum matched with a score over 90% that present in the library. All the analyses were performed in triplicate.

Declarations

Conflict of Interest

The Authors declare that there is no conflict of interest.

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Author Contributions

MD: Conceptualization, Writing - Review & Editing. MM: Data collection. **RR:** Investigation, Data Analysis. **RVA:** Investigation, Methodology, Writing.

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