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VOLATILES FROM THE FLOWERS OF FOUR SPECIES IN THE SECTIONS ARACHNITIFORMES AND ARANEIFERAE OF THE GENUS *OPHRYS* AS INSECT MIMETIC ATTRACTANTS

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Key Word Index—*Ophrys*; Orchidaceae; *Andrena*; *Colletes*; Hymenoptera; attractants; chemical mimetism; semiochemicals; 2-heptanol; 2-nonanol; α-pinene; citronellol; linalool; *E,E*-farnesol.

Abstract—Volatile compounds from flowering plants of *Ophrys sphecodes atrata*, *O. s. litigiosa*, *O. s. provincialis* and *O. splendida* were collected on Porapak Q and identified by GC/MS. Major compounds released were 2-heptanol, 2-nonanol, 2-heptanone, 2-nonanone and α-nonanone and α-pinene. Large amounts of mono- and diunsaturated tridecanes were found in *O. splendida*. Comparisons of flower scents and the cephalic secretions of their pollinators indicate chemical mimetism. Chemotaxonomic comparisons of the volatiles released by the flowering plants are also made.

INTRODUCTION

The species in the sections Araneiferae Rchb. f. and Arachnitiformes Nelson [1] of the orchid genus *Ophrys* L. (Orchidaceae) have large variations in flower morphology but with clear basic similarities. The distinctions between species, forms and the frequently formed hybrids are uncertain [1–5]. The sections are regarded as being under evolutionary speciation [6]. No separation is thus made between the two sections Araneiferae and Arachnitiformes [7] in this paper. In order to obtain information on the 'key' compounds involved in the pollination relationship between certain aculeate hymenopteran males and orchids of the genus *Ophrys* [2, 8], the volatiles released from four flowering plant species in the sections Araneiferae and Arachnitiformes: *O. sphecodes* Mill. subsp. *atrata* (Lindl.) E. Mayer, *O. s. litigiosa* (E. G. Cam.) Becherer, *O. s. provincialis* Nelson and *O. splendida* Gölz and Reinhard [9] were analysed by GC/MS. The nomenclature follows Nelson [1].

RESULTS AND DISCUSSION

The four *Ophrys* species investigated show rather similar composition of volatile flower constituents. α-Pinene, 2-heptanol, 2-nonanol, 2-heptanone and 2-nonanone and small amounts of linalool are present in all species analysed. However, there are certain specific differences between the four species (Table 1).

Ophrys splendida releases minor amounts of the 2-alcohols and the 2-ketones and the odour bouquet is constituted mainly of tridecene and tridecadiene isomers together with 2-heptanol. A characteristic compound is 2-phenylethanol present in substantial amounts. Citronellol and citronellal are also present. In addition to the substances mentioned above as being generally present, the odour bouquet of *O. s. litigiosa* contains small amounts of geraniol, geranial, citronellol, citronellal, *E,E*-farnesol and farnesyl octanoate. *O. s. litigiosa* and *O. lutea* Cav. release a similar odour bouquet as regards the oxygenated monoterpenes and *E,E*-farnesol. *O. s. atrata* and *O. s. provincialis* also contain a series of acetates (C-7–C-14) not found in the other two species.

There are chemical resemblances between the *Ophrys* species and certain possible pollinators of the genus *Andrena* F. The series of 2-alcohols, 2-ketones and acetates present in the *Ophrys* species are also found in the mandibular gland secretion of *A. nigroaenea*, pollinator of both *O. litigiosa* and *O. atrata* [6]. Geraniol, geranial, citronellol, citronellal and *E,E*-farnesol are found also in certain pollinator species of *Andrena* bees [10–15]. Farnesyl octanoate has been identified as one of the main compounds in the Dufour gland secretion of *Andrena* females [16]. Linalool is found in large amounts in the mandibular gland secretion of several species of *Colletes* bees [17] of which only *Colletes cunicularius* is known as a pollinator. Linalool is known to enhance the approach flight activity of *Colletes cunicularius cunicularius* males [18]. A combination of linalool and citral attracts *C. c. infuscatus* males [19].

The volatile odour pattern, especially the dominating aliphatic hydrocarbons in *O. splendida*, resembles the odour released by *O. scolopax scolopax* Cav. and *O. s. cornuta* (Stev.) E. G. Cam. [20]. The pollinator of *O. splendida*, *Andrena squalida* Per., is shown to be more attracted to scents of species in the Fuciflorae section according to odour attraction experiments [Borg-Karlson, A.-K., unpublished] than to the expected *Araneiferae* and *Fusci-Luteae* Nelson sections. Thus, both chemotaxonomically and regarding pollination behaviour, *O. splendida* seems to be related to the Fuciflorae section.

Several of the compounds identified are present in the mandibular gland secretions of the pollinating bee genera.

Table 1. Volatile compounds released by flowering *Ophrys* plants [the substances are listed according to their retention values on Superox FA (25 m, i.d. 0.15 mm)]

Substances	M_r	*O.a.*	*O.p.*	*O.l.*	*O.s.*
α-Pinene	136	X	X	XX	X
β-Pinene	136	X	X	X	X
Myrcene	136	X	X	X	X
2-Heptanone	114	X	X	X	X
Limonene	136	X	X	X	X
2-Heptanol	116	XX	XX	XX	XX
Tridecane	184	—	—	—	X
Tridecene I	182	—	—	—	XXX
Tridecene II	182	—	—	—	X
1-Hexanol	102	—	—	—	X
Tridecadiene	180	—	—	tr	X
Heptyl acetate	158	X	—	—	—
2-Nonanone	142	XX	XX	XX	X
1-Heptanol	116	X	X[1]	X	X
Monoterpene		X	—	—	—
Monoterpene		X	X	X	X
Linalool oxide	154	—	—	—	X
Octyl acetate	172	X	X	—	—
Citronellal	154	—	—	X	X
Pentadecane	212	X	X	X	X
Cyclosativene	204	X	X	X	X
α-Copaene	204	X	—	X	X
2-Nonanol	144	XXX	XXX	XXX	X
Pentadecene	210	—	—	—	X
Octenyl acetate	170	X	—	—	—
Linalool	154	X	tr	X	X
Benzaldehyde	106	X	X	X	X
1-Octanol	130	X	X	X	X
Monoterpene		X	X	X	—
Pentadecadiene	208	—	—	—	X
Nonyl acetate	186	X	X	—	—
Monoterpene		tr	tr	tr	—
2-Undecanone	172	—	—	X	—
Hexadecane	226	X	X	X	X
Monoterpene		tr	tr	X	X
Octenol	128	X	—	—	—
Caryophyllene	204	X	X	X	X
2-Decanol	158	—	—	X	—
1-Nonanol	144	X	X[1]	X	—
β-Santalene	204	X	X	X	—
1-Phenylethanone	120	tr	tr	tr	tr
Decanyl acetate	200	X	X[1]	—	—
Geranial	152	—	—	X	—
Heptadecane	240	X	X	X	X
Decenyl acetate	198	X	—	—	—
Dimethoxy benzene	148	X	X	—	—
Monoterpene		X	—	—	—
Citronellyl acetate	198	—	—	X	—
Citronellol	156	—	—	X	X
Geraniol	154	—	—	X	—
Dodecyl acetate	228	X	X[1]	—	—
2-Phenylethanol	122	—	—	—	X
E,E-Farnesol	204	—	—	X[1]	—
Farnesyl octanoate	346	—	X[1]	X[1]	—

XXX, Main compound; XX, compound in moderate amount > 5% of total; X, compound in minor amounts < 5% of total; tr, compound present in trace amounts; —, compound absent; [1] Enrichment method; enfleurage of *Ophrys* labella with Porapak Q. *O. a.*, *Ophrys sphecodes atrata*; *O. p.*, *O. s. provincialis*; *O. l.*, *O. s. litigiosa*; *O. s.*, *O. splendida*.

Linalool, citronellol, citronellal, geraniol, geranial, *E,E*-farnesol, the acetates, the 1- and 2-alcohols, and their respective 2-ketones are all present in the cephalic secretion of the genus *Andrena*. Linalool is present also in the mandibular gland secretion of the genus *Colletes*. These compounds are found to be generally attractive to males of several species of *Andrena* [10, 14, 15] and *Colletes* species [17, 19; Tengö, J., personal communication]. Thus, it is probable that these substances are 'key' compounds responsible for the insect males approach behaviour and their accomplishment of pollination.

EXPERIMENTAL

Plant material of *Ophrys sphecodes provincialis*, *O. s. litigiosa* and *O. s. atrata* was collected in Montbazin, and of *O. splendida* in Montferrier, Herault, S. France [cf. 7, 10].

The volatiles released from flowering plants were collected on Porapak Q, packed in a 50 × 5 mm glass tube (100 mg, 80–100 mesh), through suction (50–100 ml air/min in 24–48 hr), or through enfleurage of flower labella [21] using Porapak Q [14]. The odoriferous compounds were eluted with 4 ml of redistilled pentane (Merck) which was carefully evaporated at 42°.

The identifications were made on a Finnigan 4021 GC/MS instrument using a Superox FA-coated fused silica column (25 m, i.d. 0.15) with temp. programming (60° in 4 min followed by 5°/min up to 220°). Mass spectra and retention values were compared with available authentic samples.

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