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VOLATILE COMPOUNDS FROM EXTRACTS OF FIGS OF *FICUS CARICA*

MARC GIBERNAU, HANS R. BUSER,* JÜRG E. FREY* and MARTINE HOSSAERT-MCKEY†

CEFE/CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France; *Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, CH-8820, Wädenswil, Switzerland

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Key Word Index—*Ficus carica*; Moraceae; figs; inflorescence volatiles; pollinator attraction; linalool; sesquiterpenes; GC-MS.

Abstract—Pentane extracts from figs of *Ficus carica*, the common fig tree, were analysed by GC-mass spectroscopy. Four series of extracts were prepared from receptive male figs, non-receptive male figs, receptive female figs and non-receptive female figs. Extracts from non-receptive figs are characterised by furanocoumarins (tentatively identified as angelicin and bergapten), sesquiterpene hydrocarbons (i.e. *trans*-caryophyllene, and a compound tentatively identified as germacrene D), oxygenated sesquiterpenes (i.e. hydroxycaryophyllene) benzyl alcohol and benzylaldehyde. Extracts from receptive figs of both sexes are characterised by benzyl alcohol, linalool and linalool oxides (furanoid), cinnamic aldehyde, cinnamic alcohol and indole. Extract from female receptive figs has in addition large amounts of pyranoid (linalool oxides), whereas an extract from male receptive figs contains eugenol and an unidentified sesquiterpene hydrocarbon. Differences between extracts from male and female figs appear to be mainly qualitative due to pyranoid compounds, sesquiterpenes 1, 2 and 3 for female figs and eugenol and sesquiterpene 5 for male figs. © 1997 Elsevier Science Ltd

INTRODUCTION

Insect pollination (entomophily) is widespread among angiosperms [1]. Entomophilous flowers use a great variety of cues, including olfactory cues, in order to signal their presence [1, 2]. Volatile compounds have been documented as pollinator-attractants in a diversity of pollination syndromes [3] including pollination by bats [4], beetles [5], and bees [6, 7].

However, surprisingly little is known about the chemical aspects of one of the most specialised pollination systems: the mutualism between figs and fig-wasps [8]. Figs are the urn-shaped inflorescences of *Ficus* species (Moraceae). The entrance of the fig, the ostiole, is tightly closed by bracts. Only specific insects (Hymenoptera: Agaonidae) enter and pollinate female flowers enclosed in figs [9]. Each of the circa 750 species of *Ficus* is only pollinated by a single species of fig-wasp. Beyond the demonstration that pollinator attraction to figs is due to uncharacterised volatile chemicals emitted from receptive figs [10–14], nothing is known of the chemical basis of pollinator attraction and specificity.

Olfactory cues are particularly important to the reproductive success of both fig and wasp due to various constraints affecting encounter of the mutualists such as the low densities of fig trees [15], the small proportion of individual trees in the population at the appropriate reproductive phase stage for wasp transfer or receipt [16], the presence of several other fig species within the same site [17] and finally the short life span of the adult wasp [18].

In a previous study, we have shown that pentane extracts of fig; (1) exert long-range attraction of the pollinator of *F. carica*, and (2) act as contact chemostimulants [14]. In bioassays, our extracts proved to be as attractive as receptive figs. They thus contained all the chemical information necessary for the pollinators to recognise a receptive fig. Several chromatograms of receptive figs have been published [10, 13, 19], but so far no analysis of the volatile compounds has been available.

We present here results of GC-mass spectrometric analyses of pentane extracts of receptive and non-receptive figs of *F. carica*. The purpose of this study was to identify the fig fragrance compounds responsible for fig-wasp attraction, in order to know (1) if the volatile compounds present a complex blend, as in other specialised pollination systems, and (2) if the volatile compounds implicated in the attraction of fig pollinators are numerous or few.

† Author to whom correspondence should be addressed.

Table 1. Volatile compounds extracted from figs of *Ficus carica* at different stages. Names of compounds between quotation marks are tentative identifications according to MS library

Compounds	Scan	M^+	Non receptive male figs	Receptive male figs†	Non receptive female figs	Receptive female figs
Benzyl aldehyde (2 isomers)	45,55	106	—	*	—	—
Benzyl alcohol	60	108	*	**	*	***
Furanoid (*cis*) linalool oxide	100	170	—	****	*	***
Furanoid (*trans*)	120	170	—	***	—	*
Linalool (2 isomers)	130	136	—	base peak	*	***
Pyranoid (*cis*) linalool oxide	200	170	—	*	—	***
Pyranoid (*trans*)	230	170	—	—	—	**
Unknown 1	210	180	—	—	*	—
Cinnamic aldehyde	330	132	—	*	—	*
Indole	380	117	—	**	—	*
Cinnamic alcohol	395	134	—	**	—	*
Eugenol	430	164	—	*	—	—
trans-Caryophyllene	530	204	*	—	***	—
Sesquiterpene 1	560	204	—	—	*	*
Sesquiterpene 2	570	204	—	—	*	*
Sesquiterpene 3: 'Germacrene D'	615	204	*	—	base peak	*
Sesquiterpene 4	640	204	*	—	*	—
Sesquiterpene 5	725	204	—	****	—	—
Hydroxycaryophyllene	750	220	—	—	*	—
Oxygenated sesquiterpene 1	830	220	—	—	*	—
Oxygenated sesquiterpene 2	850	220	*	—	*	—
Unknown 2	900	208	*	—	*	—
Unknown 3	910	292	—	—	**	—
'Angelicin'	1050	186	base peak	***	***	base peak
'Bergapten'	1310	216	***	*	**	**

Approximate relative concentrations compared to the principal compound in the volatile fraction (base peak) as determined by GC/MS; —, absent; *, <25%; **, 25–50%; ***, 50–75%; ****, >75%.

† Fraction 4 (70% *n*-hexane, 30% Et_2O) of the extract of receptive male figs.

RESULTS AND DISCUSSION

GC-MS analysis of extracts

The GC-mass spectral data are summarised in Table 1. Peak identification of linalool, linalool oxides (furanoids), indole, cinnamic aldehyde, eugenol, and *trans*-caryophyllene were made by comparison of mass spectra and retention times to those of pure reference compounds. Moreover, tentative identifications (see Table 1) have been made for germacrene D, angelicin and bergapten according to a mass spectrum library and published data [20, 21]. Linalool oxides (pyranoids) and cinnamic alcohol have been identified according to the mass spectrum library.

Fig fragrance

Differences in chemical composition are observed between extracts obtained from receptive and non-receptive figs (Table 1). Extracts from non-receptive figs are characterised by furanocoumarins ('angelicin', 'bergapten'), sesquiterpene hydrocarbons (i.e. *trans*-caryophyllene, 'germacrene D'), oxygenated sesquiterpenes (i.e. hydroxycaryophyllene), benzyl alcohol and benzyl aldehyde.

Extracts from receptive figs of both sexes are characterised by benzyl alcohol, linalool and linalool oxides (furanoid), several aromatic compounds (cinnamic aldehyde, cinnamic alcohol) and a nitrogen-containing compound (indole). Extract from female receptive figs has in addition large amounts of linalool oxides (pyranoid), whereas extract from male receptive figs contains an additional aromatic compound (eugenol) and an unidentified sesquiterpene hydrocarbon. These results have been confirmed by GC analysis of non-fractionated extracts (unpubl. data), suggesting that there is no bias due to experimental technique.

Differences between extracts from male and female figs appear to be mainly qualitative due on one hand to pyranoid compounds, sesquiterpenes 1, 2 and 3 for female figs and, on the other hand, eugenol, and sesquiterpene 5 for male figs. Such difference in volatile composition should be important in selection for wasp discrimination between male and female figs in dioecious species [22, 23].

Volatiles from ripe figs (i.e. long after receptive phase) were analysed by Jennings, but only for the very volatile components (M_r < 130) [24]. He found 19 compounds, principally fatty acid derivatives,

which are common in ripe fruits. These compounds were not present in our extracts of receptive figs.

Some volatile compounds have also been isolated from fig leaves by Buttery *et al.* [20]. The major compound found was the sesquiterpene germacrene D. Other compounds were fatty acid derivatives (hexene alcohols and esters) typical of the 'green leaf' odour. In the extract from non-receptive female figs, a sesquiterpene related to germacrene D is the principal compound. It was also found in lesser amount in the extract from non-receptive male figs and in the extract from female receptive figs.

Among the less volatile compounds (elution temperature >202°; retention time >24 min 30 sec) present in our extracts were benzyl esters of *n*-alkanoic acids, the free fatty acids and the corresponding long chain hydrocarbons (unpubl. data). This suggests that the benzyl alcohol and benzyl aldehyde may be products of the degradation of these benzyl esters.

All the identified compounds are already known from flower volatiles. Benzaldehyde and benzyl alcohol are among the most common benzenoids, and they are found in more than 40 genera of plants [25]. Phenylpropanoids are rare compounds in flower odours, but the three compounds found in figs (cinnamic compounds and eugenol) are among the most frequent. Isoprenoids are abundant constituents of floral odours. One of the most common is linalool, present in at least 66 genera; but linalool oxides seem to be rarer. Nitrogen-containing compounds are uncommon in flower odours. Among those volatiles, indole, present in fig odours, is the most frequent [25].

Fig odours responsible for pollinator attraction appear to be composed of a few volatile compounds. Despite the fact that the fig pollination system is a highly coevolved and species-specific mutualism [8], the compounds involved in pollinator attraction are quite common in flower fragrances (except perhaps for the unidentified sesquiterpenes). Only the combination of these compounds may be novel and ensures the specific attraction of fig pollinators.

EXPERIMENTAL

Plant material. The trees of *Ficus carica* used for this work were taken from the experimental collection of the CNRS in Montpellier (France). This species is functionally dioecious. Figs on 'male' trees produce wasps and pollen, whereas figs on 'female' trees produce only seeds. The annual cycle of *F. carica* in southern France is seasonal, male trees bearing receptive figs mainly in spring and female trees in summer [26]. Branches bearing figs were enclosed in gauze bags prior to natural pollination in order to prevent pollinator visits. Receptive 'male' figs were collected on May 2 and 3, 1995. The non-receptive figs were collected on June 6, 1995, several days after natural pollination occurred. After pollination, a fig quickly loses its attractiveness [27]. Receptive female figs were collected on June 28 and 29, 1995 and non-receptive ones on July 7, 1995.

Solvent extracts. Entire figs were soaked in pentane during 1 hr for extraction of the volatiles. In a previous study, pentane was found to be the best solvent for fig volatiles [14].

Four different extracts were performed, using male and female, receptive and non receptive figs. For the 'male' extracts, 110 receptive figs were put in 1000 ml of pentane, and 73 non-receptive figs in 900 ml of pentane. For the female extracts, 108 receptive figs were soaked in 850 ml of pentane and 87 non-receptive figs in 750 ml of pentane. After that, the figs were removed and the extracts were concd by rotary evapn at 45–50° to approximately 5 ml.

Male receptive extract was then fractionated on a silica gel column (200 × 14 mm i.d. glass column, silica gel 60–210 μm, Prolabo®, Paris, France). The column was eluted sequentially with 50 ml of each of; (1) 100% *n*-hexane; (2) 97% *n*-hexane, 3% Et_2O; (3) 94% *n*-hexane, 6% Et_2O; (4) 70% *n*-hexane, 30% Et_2O; (5) 100% Et_2O; and (6) 100% MeOH. The frs were then concd to approximately 15 ml. The fr. 4 (70% *n*-hexane, 30% Et_2O) was as attractive as the total fig extract in our bioassays with fig wasps [14]. Female receptive extract was also fractionated but fr. 4 was less attractive than the total extract. This GC-MS analysis was performed done on the total extract.

Gas chromatographic-mass spectrometric (GC-MS) analysis. A VG Tribrid double focussing magnetic sector GC-MS instrument and a 25-m SE54 fused-silica (0.32 mm i.d.) high resolution column were used. The ion source was operated in the electron-impact mode (El, 70 eV, 180°). Full-scan mass spectra (*m/z* 35–535; 1.16 s/scan, resolution M/M = 500) were recorded. The column was temp. programmed as follows: 50°, 2 min isothermal, 20°/min to 80°, then at 5°/min to 280°. Female and male fig extracts (or frs thereof) were analysed using on-column injection (1–2 μl) at 50°.

Reference compounds. The following reference compounds were available: linalool (Fluka®, Buchs, Switzerland), linalool oxide (Roth®, Karlsruhe, Germany), indole (Fluka®), cinnamic aldehyde (Fluka®), eugenol (Fluka®), and *trans*-caryophyllene (Fluka®).

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