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POLLEN AND FLOWER VOLATILES IN TWO *ROSA* SPECIES

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Key Word Index—*Rosa rugosa*; *R. canina*; Rosaceae; rose; flowers; pollen; pollenkitt; fragrance; odour; volatiles.

Abstract—GC/MS of headspace volatiles adsorbed separately from whole flowers and from pollen of *R. rugosa* shows distinct chemical profiles composed of aliphatics, terpenoids, and aromatics; only one-third of the identified compounds were detected in both samples. Analysis of pollenkitt extract indicates that most major pollen volatiles are included in this oily pollen coat. Volatiles from *R. canina* pollen contained few constituents in common with *R. rugosa*.

INTRODUCTION

Pollen from different plant species have characteristic odours that are often distinct from those of whole flowers, as perceived by both humans and insects [1–7]. Pollen odours are thought to represent evolutionarily ancient attractants to flower-visiting insects (8); in some primitive angiosperms, flower fragrance is produced exclusively by the androecium [9–12].

Evidence from behavioural and chemical studies point to the oily coating of pollen grains—'Pollenkitt' [13]—as the main source of volatiles. Indeed, pollen extracts containing primarily pollenkitt have been shown to be olfactorily attractive to pollen-foraging honey bees [14–17] and further investigations indicate that pollen-specific solitary bees can discriminate between plant species on the basis of pollenkitt odours (7). In terms of pollen chemistry, compound classes commonly found in essential oils (18, 19) have been reported in pollenkitt of *Ambrosia* [20] and various angiosperm species surveyed by TLC (21). In addition, typical floral fragrance compounds have been identified in *Vitis* pollen (22), and volatiles analysed from bee nest provisions most likely include pollen-originating chemicals (23, 24). Detailed investigations of pollen volatiles, however, are few and the constituents have not been well characterized.

Described here is a method for collecting pollen volatiles by adsorption-desorption, using *Rosa rugosa* L. and *R. canina* L. as models. In order to establish whether pollen odour components originate from the pollenkitt fraction and to determine the degree of similarity between pollen and flower fragrances, volatiles identified in *R. rugosa* pollen were compared with those in whole flowers and in pollenkitt extracts. Furthermore, since whole-flower samples included green structures, volatiles were also collected separately from green plant parts to evaluate their contribution to these samples. All volatile collections were analysed by GC/MS.

RESULTS AND DISCUSSION

A total of 31 compounds were identified in volatiles from the pollen and whole flowers of *Rosa rugosa*; these include terpenoids, aliphatics, and aromatics (Table 1). In addition, more than a dozen sesquiterpenes (unidentified) were detected in leaf and flower samples of *R. rugosa*, and one, probably farnesene, in flowers and pollen. Volatiles from flowers and pollen yielded similar numbers of identified compounds (*ca* 20). Of these, nine were restricted to flowers, 10 to pollen; 12 were found in both plant parts. Identification of volatiles from *R. rugosa* pollenkitt and *R. canina* pollen was limited to those chemicals found in common with *R. rugosa* flowers and pollen (Table 1).

The total number of compounds (identified and unidentified) in flower samples of *R. rugosa* is similar to the 22–30 essential oil constituents reported for this species by other workers (25, 26). Flowers contained predominantly terpenoids and aromatics, present as alcohols. One-third of the volatiles, primarily short-chain aliphatics detected in low to moderate amounts, were also found in green parts, but quantitative patterns suggest that several are produced by the non-green floral structures as well. These compounds represented most of those detected exclusively in whole flowers. Among the principal flower alcohols are β-phenylethanol and several terpenes which have been described as major components of rose flower essential oils (27–29). Three, citronellol, nerol, and geraniol, are among the chief chemicals contributing to interspecific variation in *Rosa* flower fragrance (28). In contrast to flowers, pollen showed equal numbers of aliphatic, terpenoid, and aromatic compounds. Chemicals not shared with flowers included long-chain aliphatics (C_{11}–C_{16}) and several terpenoids. There were striking differences in the terpenoids of pollen and flowers. Indeed, although the basic isoprenoid skeletons were the same in both samples, terpenoids in pollen were present as aldehydes, ketones, and acetates, while those in flowers were alcohols. Several of the aromatics detected in both samples have been found in flower fragrances of other rosaceous species (Bergström, J., in preparation).

Pollenkitt was similar to pollen in terms of the total number of chemicals detected. However, most were minor constituents; for those shared, pollenkitt contained proportionately more C_{16} acetate. The variability observed between pollen volatiles and pollenkitt extracts

Table 1. Chemicals identified* in volatiles from flowers, pollen, and pollenkitt of *Rosa rugosa* and *R. canina*

Compound	Flowers	*R. rugosa* Pollen	Pollenkitt†	*R. canina* Pollen†
Terpenoids				
trans-β-Ocimene	x			
6-Methyl-5-hepten-2-one	t	xx		x
Geranyl acetone		xxx	x	xx
Neral		xx		
Geranial	t	xxx	x	
3-Methyl-1-butanol	xx§			
Citronellol	xxx			
Nerol	xxx§	t		
Geraniol	xxx	xx		
Citronellyl acetate		xx	x	
Neryl acetate‡	t	xx		
Geranyl acetate		xxx	x	
Aliphatics				
Pentadecane	x§	xx	x	x
2-Undecanone		xxx		
2-Tridecanone	x	xxx	xx	
2-Pentadecanone‡		xx		
Tetradecanal‡		xxx	xx	xxx
Hexadecanal‡		x		xx
1-Pentanol	xx§			
1-Hexanol	x†			
3-Hexenol	x			
Acetic acid‡	t	t		
Hexyl acetate	x†			
3-Hexenyl acetate	xx†			
Tetradecyl acetate		xxx	xxx	
Hexadecyl acetate‡		t	xxx	
Aromatics				
Benzyl alcohol	xxx			
β-Phenylethanol	xxx	xxx		
Methyleugenol	xxx	xxx	x	
Eugenol	xx	xx		
β-Phenylethyl acetate	t	xx	x	

*Chemicals identified by comparing retention times and mass spectra with those of reference compounds analysed concurrently, except for chemicals marked by‡.

†Includes only compounds detected also in *R. rugosa* flower or pollen volatiles.

‡Identification is based on personal MS library and previous reference analysis (under comparable conditions).

t = trace quantities, identification tentative.

x = ⩽ 4%, xx = > 4%, ⩽ 20%, xxx = > 20% of largest peak.

§Detected in 'green' samples.

in the relative concentrations of components, as well as in the differential detection of chemicals, may in part result from the different natures of these two samples. Pollenkitt included half of the volatiles found in pollen (including major constituents), indicating that pollen volatiles do indeed occur in this surface oil fraction.

The pollen volatiles from *R. rugosa* and *R. canina* are generally different from each other. Indeed, although a comparable number of chemicals were detected in each species, only five were common to both. These included half of the major constituents in *R. canina*, most components of which were in minor amounts.

In summary, pollen produces a volatile profile that is distinct from that of whole flowers. Detection of different chemicals in flowers and pollen of *R. rugosa*, and in pollen of *R. rugosa* and *R. canina*, substantiate observations that bees can discriminate olfactorily between flowers and pollen of the same species, as well as between pollen of different species (1, 3, 5–7). The possibility, however, that some of the pollen volatiles may represent petal-originating chemicals which, subsequent to anther dehiscence, become adsorbed onto the surface (particularly pollenkitt) of pollen grains needs further clarification.

EXPERIMENTAL

All samples were obtained in July and August from plants growing near the Ecological Station on Öland, Sweden. Volatiles from flowers and pollen were collected by headspace adsorption-desorption using airflow onto Porapak®Q (150 mg, 80–100

mesh, packed into 50 × 5 mm glass tube sealed with silanized glass wool), followed by elution with 2 ml Et_2O and concentration to *ca* 20 μl (using 'Francke' vials in water bath) for GC/MS (30, 31).

Flowers. Blossoms attached to plants in the field were enclosed in a 35 × 43 cm polyacetate bag (Bosco trade-mark), leaving a small opening around the stem for air entry; one adsorbent plug was tightly placed in a hole of the bag through which air was drawn by suction, using a battery-run pump (air flow *ca* 100 ml/min, 24 continuous hr, ambient temp.). Background contributions of leaf and other non-flower structures (sepals and flower buds) were determined by concurrently collecting volatiles from all-green portions of the plant.

Pollen. 0.1 g (harvested from fresh flowers and used immediately or stored at −5° until use) was packed into a glass tube (13 × 5 mm) sealed with silanized glass wool. The pollen tube was fitted between two Porapak-filled adsorbent plugs, the first serving to clean incoming air (pure compressed air), the second to adsorb pollen volatiles (air flow 10 ml/min, 24 hr, room temp.).

Pollenkitt. 30 mg pollen, on a syringe-fitted millipore filter, were washed for *ca* 20 sec with 0.5 ml pentane [21]; the extract was concd to 50 μl for analysis.

GC/MS. Samples and reference compounds were analysed with a GC connected to a Finnigan Ion Trap Detector, injector temp. 220°, temp. programmed 55 to 210° at 8°/min, and with a quadrapole GC/MS system, temp. programmed 55 to 210° at 5°/min. Carrier gas He; capillary columns of fused silica coated with Superox FA.

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