Abstract: *Rhipsalis juengeri* was described in 1995 as an unusual representative of epiphytic cacti, forming more than 3 m long curtains, hanging from the canopy of the Atlantic Rainforest in eastern Brazil. At the apex of thin, pendant shoots, green-brownish berries are formed. We report here as a novelty for *R. juengeri* is dominated by ketones, some of which are responsible for the characteristic blackcurrant-like scent, as is shown by GC-olfactometry. The odour and inconspicuous colour stand out among fruits of other epiphytic cacti that are thought to be consumed by birds. Fruit characters of *R. juengeri* and the flagellicarpic presentation indicate adaptation to chiropterochory.

**Key words:** Bats, volatiles, *Rhipsalis*, fruit scent, frugivory, chiropterochory, Cactaceae.

Introduction

Epiphytic flowering plants need to have seed dispersal mechanisms that ensure deposition of diaspores within the canopy or onto the stems of trees. While wind is one efficient mechanism, e.g., for very small, light seeds as in orchids (Murren and Ellison, 1998), many plants rely on birds (Nadkarni and Matelson, 1989) and bats (Sazima et al., 2003). To ensure attractiveness to animals, fruits must fit the respective sensory abilities and preferences, i.e., especially colour and scent.

Spread over most of the American continent and – secondarily – parts of the Old World, cacti are adapted to a wide range of habitats, from deserts to rain forests, from sea level to alpine zones. As a consequence, numerous adaptations in morphology and physiology are found. Among them, cactus fruits and seeds adapted to different biotic and abiotic dispersal agents such as wind (Barthlott and Hunt, 1993; Anderson, 2001), birds (Silvius, 1995; Wolf and del Rio, 2000), bats (Naranjo et al., 2003), terrestrial mammals (Montiel and Montaña, 2000), reptiles (Cortes Figueira et al., 1994), and ants (González-Espinosa and Quintana-Ascencio, 1986; cited in Montiel and Montaña, 2000; Barthlott and Porembski, 1996). As a most conspicuous mechanism, adaptation to water dispersal was found in seeds of an epiphytic cactus native to Amazonian inundation forests (Barthlott et al., 1997).

Although the almost 1400 species of the Cactaceae are often cultivated and well studied, no scented fruits are known. To date, only volatiles extracted from fruit pulp have been described in two cactus species commercially grown as fruit crops: the prickly pear *Opuntia ficus-indica* (L.) Miller and the columnar *Cereus peruvianus* (L.) Miller. The volatile blend of the former consisted mainly of alcohols and esters, as well as aldehydes, ketones, and hydrocarbons (Flath and Takahashi, 1978; Weckerle et al., 2001). In the latter species, linalool and derivatives were the major compounds (99%) of pulp of ripe fruits (Ninio et al., 2003; Sitrit et al., 2004). Further, Naranjo et al. (2003) mentioned “dehiscent fruits that have an odour” for two species of Venezuelan columnar cacti. However, odour source and chemistry remain unknown and it is likely that this odour is emitted by the pulp, as in the cactus fruits mentioned above. Fruit volatiles emitted from intact cactus fruits, i.e., emitted through the epidermis, have so far never been described.

In 1995 a most peculiar slender epiphytic cactus was described as *Rhipsalis juengeri* Barthlott and Taylor, named after the writer Ernst Jünger. This species grows in the Mata Atlântica, Minas Gerais, Brazil, 1500 to 1600 m above sea level (Taylor and Zappi, 2004). It belongs to the “bell-flowered” subgenus *Erythroarthropsalis* and is, among other features, characterized by its enormous size: the multi-branched thin stems hang from the high canopy like green curtains, more than 3 m long. When it fruited for the first time in cultivation, another unique feature within the cactus family became apparent: the mature, small, dull greenish-brown fruits are strongly scented, reminiscent of blackcurrants.

Here, we present analysis data on the composition of fruit volatiles by *ex situ* headspace analysis followed by GC-MS analysis and determination of the key olfactory compounds by GC-olfactometry (GC-O). An olfactory survey on fruits of various species of *Rhipsalis* and related genera was performed in order to assess if fruit scent also occurs in related species. Further,
we discuss the potential function of this unusual case of fruit odour in the Cactaceae in the context of morphology and habitat of the plant.

Materials and Methods

Plant material

Fruits of *R. juengeri* were obtained from the Botanic Gardens of the University of Bonn. Most olfactory observations in other species were performed at the Botanic Gardens Bonn; additional data were obtained in the Botanical Garden München-Nymphenburg and in private collections. As only one clone of *R. juengeri* – the type specimen – is in cultivation in Europe, all samples were taken from one individual. Intraspecific variation of fruit scent could therefore not be studied.

Olfaction

The olfactory screening comprised all four genera of the tribe Rhipsalideae, including 37 of all 58 species. The screening focused on the genus *Rhipsalis*, with 30 species and 5 subspecies tested (plus six geographically distinct accessions of the widespread *R. baccifera* Miller [Stearn]). Additionally, fruits of 3 related genera were included in the survey: *Lepismium* (4 spp.), *Hatiora* (2 spp., 1 hybrid), *Schlumbergera* (1 hybrid).

Fruit odour was assessed on ripe, undamaged fruits on cultivated plants.

Volatile extraction and analysis

Intact fruit-bearing stems were picked and dispatched for analysis. In the lab, the berries were removed from the stems and approximately 40 of the fresh berries were sealed in a 20-ml headspace vial at room temperature. Clean, filtered nitrogen was supplied through a fused silica capillary at 20 ml/min for 20 min and the flushed headspace collected in a pre-conditioned trap containing PDMS and Carbopak in sequence. The trap was connected to the vial by a short length of uncoated, fused silica capillary. A second identical trap was connected for a further 20 min and a second sample collected in the same manner.

The traps were analyzed by direct thermal desorption; the first into the GC-MS system and the second into the GC-O system.

GC-MS and GC-O

The GC-MS analysis was carried out on a Varian 3400 GC fitted with an OPTIC® programmable injector and attached to a Finnigan ITS 40 ion trap mass spectrometer. The column employed for analysis was an HP ultra 2 (Hewlett Packard) (50 m × 0.32 mm), film thickness 0.33 μm. Helium was used as the carrier gas with a flow rate of 1.6 ml/min. The temperature was programmed from 50°C to 270°C at 2°C/min. The compounds were identified by matching the mass spectra against commercial libraries, Wiley, NIST, (Adams, 1995) and in-house libraries and comparing their retention indices with those of reference compounds measured on the same system.

Direct thermal desorption of the traps was carried out in the OPTIC® injector with an initial temperature of 5°C ramped at 16°C/s to 220°C (5 min) with a 1-min splitless injection time.

Results

In *R. juengeri*, flowers and fleshy, non-dehiscent berries develop on the terminal ends of the stem segments, a typical characteristic for the subgenus *Erythrorhapisal*. Weight and dimensions of the fruits were measured and averaged; mean weight was 0.11 g (n = 10), mean equatorial diameter was 6.22 mm (se = 0.07), mean diameter from pole to pole was 5.3 mm (se = 0.08) (each n = 10). When ripe, fruit colour was translucent light green to light beige, with some red around the apex. Later, berries may darken to a dull brown. The fruits were found to contain from one to seven seeds (mean: 3.35 per fruit, n = 20). In cultivation, the ripe berries were observed to remain on the plant for about 3 months, remaining scented the entire time. Long-lasting fruits are a common feature in Rhipsalideae.

Olfactory screening in tribe Rhipsalideae

The survey of 52 different taxa, hybrids, and geographical variations of *Rhipsalis* and related genera indicates that the fruit odour in *R. juengeri* is an exceptional phenomenon in the genus, if not in the entire tribe Rhipsalideae. Besides *R. juengeri*, only the fruits of *R. aff. pachyptera* Pfeiffer seemed to emit a very faint odour, at the limit of perceptibility. The fruits of all other species were scentless (Table 1).

Analysis of volatile compounds and odour perception

The composition of volatiles from fruits of *R. juengeri* is dominated by ketones that make up 80% of total volatiles (Table 2). The odour profile of fruits, with their characteristic fruity, slightly soapy odour, is as well dominated by two of those ketones, undecan-2-one and 10-methylundecan-2-one. Another more volatile ketone, 6-methylheptan-2-one contributes a cassis/blackcurrant note to the odour, as determined by GC-olfactometry (Fig. 1). Apart from the ketones, other compounds contributing significantly to the odour were linalool with a floral, fruity character and the mushroom note of oct-1-en-3-ol. Several other compounds with characteristic odours, such as the terpenes, aliphatic aldehydes, benzyl acetate, and methyl salicylate, were detected by GC-MS, but these were either present at low concentrations or have a high odour threshold and thus make only a minor contribution to the overall odour.
Discussion

While the scent bouquet of *Rhipsalis juengeri* fruits was dominated by ketones, the conventional fruity aliphatic esters of the C₄ – C₈ acids were notably absent. Most other compounds contributed little to the odour as perceived by the human nose, but may be important for animals with more sensitive olfactory systems.
The ketones dominating the odour of *R. juengeri* fruits have also been reported in some tropical fruit volatiles (Binder and Flath, 1989; Arriaga et al., 1997) but are more abundant in leaf essential oils such as rue, *Ruta graveolens* L. (Yaacob et al., 1989). The most characteristic feature of the fruit scent of *R. juengeri* is the strong blackcurrant note, and GC-olfactometry uncovered 6-methylheptane-2-one as the source of this flavour.

As only one clone of *R. juengeri* was in cultivation when our studies were performed, no data on intraspecific variation could be acquired.

**Possible dispersal biology of the scented fruits of *R. juengeri***

The most common dispersal mechanism for cactus fruits is by birds (Anderson, 2001), and all known epiphytic cacti are supposed to be ornithochorous. Especially the members of the tribe Rhipsalideae are characterized by small, white to reddish fruits, reminiscent of mistletoes (hence the common name "mistletoe cactus" for *Rhipsalis*).

The most likely function for fruit scent is the attraction of frugivorous animals that help in dispersing the seeds. Given the fact that most birds have a poorly developed olfactory sense and have to assess ripeness of fruits visually (Wheelwright and Janson, 1985), bats are the most likely target for scented fruits. According to van der Pijil (1982), fruits dispersed by bats are often specified by characters such as strong odour, hanging position (flagellichory), and greenish or whitish colour, all of which are present in *R. juengeri*. This species is an extremely large representative of the genus, growing as an epiphyte with hanging stems more than 3 m in length (flowers begin to appear at a length of 2 m) (Fig. 2A), but having stem diameters of only 3 mm in extension shoots, and 1.25 – 1.75 mm in flower-bearing segments (Barthlott and Taylor, 1995). We therefore suggest that the apically produced berries (Fig. 2B) are most likely consumed by flying animals, i.e., by bats, combining all typical characters described for bat-dispersed fruits.

A clear correlation of greenish fruits, fruit scent, and bat dispersal was shown for several fig species in Panama, contrasting with sympatric fig species adapted to ornithochory (Korine et al., 2000). The importance of olfaction for fruit-eating bats was shown for Old World bats, some of which do not echolocate (e.g., Luft et al., 2003), and for New World frugivorous bats (Fleming, 1988; Rieger and Jakob, 1988; Mikich et al., 2003). It was shown that fruit odours not only serve as an attractant, but also enable the bats to assess the ripeness of fruits (Laska, 1990). Furthermore, flagellicarpic presentation of fruits not only allows easy approach for bats, but was also demonstrated to be of importance for finding fruits by echolocation (Kalko and Condon, 1998). However, the chemistry of volatiles of fruits known to attract bats olfactorily is unknown, and most fruit scent studies are for fruit crops.

Our olfactory screening included the other 6 species of the subgenus *Erythrorhipsalis* to which *R. juengeri* belongs, and all proved to be unscented. Also, these species have pink to red, in one case white, berries as in most other *Rhipsalis* species. This
indicates that the scented fruits of *R. juengeri* have evolved from a group of bird-dispersed species with unscented fruits of conspicuous colouration.

In cacti, bat dispersal was mentioned for fruits of some species of columnar cacti in northern South America and southern North America (Soriano and Ruiz, 2002; Valiente-Banuet et al., 2002; Naranjo et al., 2003). However, their fruit odour, as well as the fruit volatiles of most other bat-dispersed plants, is unknown, leaving opportunities for future studies to understand the role of olfactory attraction in co-evolution of frugivorous bats and chiropterochorous plants. After the recent rediscovery of *R. juengeri* in nature, field studies are needed to prove our hypothesis on bat dispersal, and the role of fruit scent for bat attraction.

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**References**


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