South African Journal of Botany 2004, 70(2): 191–205
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SOUTH AFRICAN JOURNAL OF BOTANY
ISSN 0254–6299

Nectar sugar composition and floral scent compounds of diurnal and nocturnal *Conophytum* species (Aizoaceae)

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Received 10 February 2003, accepted in revised form 16 September 2003

The putative correlation between nectar sugar composition, floral scent compounds, and the pollination syndrome (diurnal vs nocturnal) was evaluated in the genus *Conophytum* (Aizoaceae). Nectar sugar compositions of 39 species, subspecies, and varieties of the genus *Conophytum* were analysed via high performance liquid chromatography (HPLC). Nectar contained mainly glucose and fructose and in several cases small amounts of sucrose. In all *Conophytum* species investigated nectar is hexose-dominant, or at least hexose-rich. An interesting variation in the fructose:glucose ratio was observed — samples of diurnal species had significantly higher fructose:glucose ratios than samples of nocturnal species. Floral scents of 27 diurnal and nocturnal *Conophytum* species from 11 sections were collected by headspace adsorption and analysed via gas chromatography-mass spectrometry (GC-MS). In total 33 compounds could be identified by their mass spectra as well as by their relative retention times. Most of the species were dominated by only a few (3–5) floral scent compounds. The species showed basic similarities in being dominated by benzenoids accompanied by fatty-acid derivatives, nitrogen-containing compounds, and terpenoids. The floral scent composition of all species was dominated by benzaldehyde and benzeneacetaldehyde. Compared to nocturnal species, diurnal species are characterised by a lower number of compounds, low amounts of aromatic esters, and an almost total absence of nitrogen-containing compounds. It is assumed that the relatively high amounts of aromatic esters in the scent of some nocturnal species are an adaptation to moth pollination.

Introduction

In southern Africa, there are numerous examples of genera that have radiated within a limited region (Cowling and Hilton-Taylor 1994, Johnson *et al.* 1998). The genus *Conophytum* N.E. BR. (Aizoaceae) is distributed in the winter-rainfall regions of South Africa and southern Namibia and comprises about 100 species in 16 more or less natural taxonomic sections (Hammer 2002). The winter-rainfall desert region (Cowling *et al.* 1999) is characterised by remarkable plant diversity (Cowling *et al.* 1998), particularly among leaf succulent plants (Van Jaarsveld 1987). No other genus in the Aizoaceae shows such a range of floral phenologies, flower opening times, and pollination syndromes, as does *Conophytum* (Vogel 1954, Haas 1976, Liede and Hammer 1990, Hammer 2002). A characteristic of flowering in *Conophytum* species is the synchronicity of a given population: the flowers are mostly presented during one concentrated period. This mass display may serve to attract specific pollinators.

The scant data about flower visitors show that these might be important agents for the radiation of the group. Liede *et al.* (1991) found that a small-sized bombyliid (*Euanthobates*) seems to be responsible for pollinating the narrow-tubed *C. pellucidum*, butterflies favour species of the blocked-tube group (e.g. *C. minutum*), and small wasps and beetles are attracted by diurnal species with exposed anthers. However, the most striking division in floral characters within *Conophytum* runs between the day- and night-flowering species (Liede and Hammer 1990). About one third of the species in the genus are described as nocturnal. Flowers of nocturnal *Conophytum* species are well-scented (Hammer 1993). The character of the aromas in *Conophytum* species has been described by Liede and Hammer (1990) as follows: almond (*C. achabense*), honey (*C. burgeri*), clover (*C. ficiforme*), and carnation (*C. obcordellum*). An analysis of the floral odour of 16 nocturnal species revealed benzaldehyde, benzeneacetaldehyde, and benzyl acetate as main compounds (Jürgens 2002).

Floral scent is an important factor in attracting pollinators (Proctor *et al.* 1996), and it has been shown that the floral scent composition may be typical for some pollination syndromes. Night-blooming, moth-pollinated plants often have oxygenated terpenoids and benzenoids (Kaiser 1993, Knudsen and Tollsten 1993, Miyake *et al.* 1998, Jürgens *et al.* 2002). In several nocturnal moth species, attraction to flowers is guided mainly by floral scent (Weisenborn and Baker 1990, Gabel *et al.* 1992, Zhu *et al.* 1993).

Studies of nectar sugar composition have shown that floral nectars normally contain only three major sugars (sucrose, glucose, fructose; but see Van Wyk and Nicolson 1995). The relatively constant (Baker and Baker 1977) species-specific nectar sugar composition can be classified into four classes based on the sucrose:hexose ratio of the three major sugars glucose, fructose and sucrose (Baker and Baker 1983b). The sugar ratios have been shown to be of ecological (Percival 1961, Baker and Baker 1975, 1983b) as well as of taxonomic (Van Wyk 1993, Van Wyk *et al.* 1993) significance. In some cases nectar sugar composition of a species can be used to predict pollinator types, in the absence of observable floral visitors (e.g. Lammers and Freeman 1986), but in other investigations the opposite was demonstrated (e.g. in *Erica* species, Barnes *et al.* 1995).

In the present publication floral scent data on 20 nocturnal and seven diurnal species is brought together, including the data on floral scent composition of 16 nocturnal *Conophytum* species published recently (Jürgens 2002). Objectives of the present evaluation of the floral scent composition were: (1) to summarise and compare the data on the floral scent composition of nocturnal species together with diurnal species, (2) to discuss the distribution of benzenoid esters in the floral scents among taxa and within sections. Based on the calculation of the Sørensen's index of similarity (Tollsten *et al.* 1994), nonmetric multidimensional scaling (MDS) was used to visualise similarities of the investigated species regarding their floral scent compounds. Nectar sugar composition of 27 species, including several subspecies and varieties, is presented for the first time for the genus *Conophytum*.

Material and Methods

Plant material

During September 1999, floral odour and nectar samples were taken from plants of known origin from the living collection of SA Hammer (San Diego, USA), cultivated in glasshouses. Additional samples were collected from plants in their natural habitat in South Africa in 2001 and 2002. A species list with origins and localities of the samples, and the classification of the species as day- or night-flowering are given in Table 1. The criteria for night-flowering species were: synchronised anthesis or repeated flower opening at night or in the evening, scent emission beginning in the evening or becoming more intense in the evening. The criteria for day-flowering species were: flowers open during the day (or day and night), no obvious change of the scent intensity between day and night.

Nectar sampling

Nectar was sampled in 39 species, subspecies, and varieties (Table 2) of the genus *Conophytum* during the main time of flower activity from flowers of unknown age. As much nectar as possible was extracted with a microcapillary (0.5µl) from single flowers or from a pool of flowers if nectar volume was low. Nectar was conserved with ethanol (70%) and kept until analysis.

Nectar analysis

The nectar samples were analysed by means of high performance liquid chromatography (HPLC). For analysis the HPLC equipment from Waters (described in Witt *et al.* 1999) was used. It included an isocratic pump 510, an autoinjector 717+, and a Waters High Performance Carbohydrate Column. Injection volume was 10µl, and elution took place with an acetonitrile:water mixture (72:28). Flow rate was 1.4ml min^{-1}, and temperature was 35°C. Sugars (glucose, fructose, sucrose) were detected with a refraction index detector 410 and quantified with the Millenium Software from Waters.

No sugars other than glucose, fructose and sucrose were found, with glucose and fructose being the dominant constituents. If traces of sucrose were detectable but the amount was not sufficient for quantification, the presence of sucrose was taken into account with 0.1µg $µl^{-1}$ for calculating the relative sugar composition and the sucrose:hexose ratio (Table 2, Figure 1). In some cases nectar samples were extremely low in concentrated sugars; the main ones, fructose and glucose, were detectable but amounts of one or both were just below the quantification limit. Such samples were excluded from further calculation of fructose:glucose ratios (Figure 1), although the visual evaluation of the chromatogram showed that the proportion of the hexoses was nearly equal (therefore given as 50% in Table 2).

Volatile sampling

Before scent samples were collected, a blank sample collection was done. Floral scent was collected following Jürgens (2002). Odour collection was started with the beginning of scent production. With a battery operated membrane pump, scent-containing air was sucked through glass cartridges filled with Tenax-TA (300mg) of mesh size 20–40. The flow rate through the cartridges was c. 150ml min^{-1}. Cartridges were conditioned by washing with acetone and heating at 250°C. After 2–3h the adsorbed scent substances were extracted with 1ml of acetone into glass vials.

Gas chromatography mass spectrometry

The samples were analysed by coupled gas chromatography and mass spectrometry (GC-MS) on a Varian Saturn 2000 System (Walnut Creek, USA), equipped with an 8200 CX autoinjector. The samples (1µl) were introduced using a 1079 Injector. A nonpolar fused silica GC-column was used (CP-Sil-8 CB-MS 30m long, inner diameter 0.25mm, film thickness 0.25µm). Electronic flow control (EFC) was used to maintain a constant helium-carrier gas flow of 0.8ml min^{-1}. The GC was programmed for 2min at 60°C, increased by 8°C per min for 35min, and maintained at 260°C; split ratio 20; injector temperature 200°C; interface heating 175°C; ion trap heating 200°C; mass spectra 70eV (in EI mode), scan range, 40–650amu at scan rate of 1 $scan^{-1}$. The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST mass spectral data base (NIST 1998) and confirmed by comparison of retention times with published data (Jennings and Shibamoto 1980, Davies 1990).

Table 1: Studied taxa and the locations where the studied plants grow or originated. Nomenclature and classification into sections is based on Hammer (2002). N = north, S = south, SE = southeast, SW = southwest. RSA = South Africa, NA = Namibia, hort. = out of horticulture, unknown origin. □ = diurnal, ● = nocturnal. Samples indicated with an asterisk (*) were included from Jürgens (2002)

Taxa/Species	Localities	Diurnal / Nocturnal
Barbata Schwantes ex S.A.Hammer		
C. pubicalyx Lavis	Scent: NE Kliprand, RSA	●
C. stephanii subsp. *helmutii* (Lavis) S.A.Hammer	Scent and nectar: Rosyntjieberg, RSA	●
Batrachia Opel & S.A.Hammer		
C. armianum S.A.Hammer	Scent*: Umdaus, NNW Steinkopf, RSA	●
Biloba N.E.Br.		
C. bilobum (Marloth) N.E.Br. subsp. *bilobum*	Nectar: Pokkiespramberg, N Richtersveld, RSA; hort.	□
C. bilobum subsp. *claviferens* S.A.Hammer	Nectar: Rietkloof, RSA	□
C. meyeri N.E.Br.	Nectar: Harrasberg, RSA	□
C. velutinum Schwantes subsp. *velutinum*	Nectar: Komaggas, RSA	□
Cataphracta Schwantes ex S.A.Hammer		
C. breve N.E.Br.	Scent*: Kamieskroon, E Kleinzee, RSA	●
C. calculus (A.Berger) N.E.Br. subsp. *calculus*	Scent* and nectar: Grootgraafwater, RSA	●
C. calculus subsp. *vanzylii* (Lavis) S.A.Hammer	Scent*: Kangnas, RSA; Scent* and nectar: E Naip, RSA	●
C. pageae subsp. *pageae* (N.E.Br.) N.E.Br.	Scent*: Bloedsuigersfontein, Calvinia, Kouberg, RSA	●
C. stevens-jonesianum L.Bolus	Scent* and nectar: Rooiberg E Kosies, W Gamoep, RSA	●
Cheshire-feles S.A.Hammer		
C. acutum L.Bolus	Scent*: SE Bitterfontein, RSA; Nectar: Kouvlei near Bitterfontein, RSA	●
C. burgeri L.Bolus	Scent: Aggeneys, RSA	□
C. hammeri G.Williamson & H.C.Kennedy	Scent: S Nababiep mountains, E Richterveld, RSA	●
C. maughanii N.E.Br. subsp. *maughanii*	Scent*: S Pofadder, RSA; Nectar: Smorenskadu, RSA	●
C. maughanii subsp. *armeniacum* S.A.Hammer	Scent* and nectar: N Augrabies, RSA; Nectar: Bontkoei, RSA	●
Conophytum N.E.Br.		
C. ficiforme (Haw.) N.E.Br.	Scent* and nectar: Lemoenpoort, RSA; Scent*: SE Robertson, RSA	●
C. obcordellum (Haw.) N.E.Br. subsp. *obcordellum* var. *obcordellum*	Scent* and nectar: Moweskop, Lamberts Bay, SSE Clanwilliam, Graafwater, RSA	●
C. obcordellum subsp. *obcordellum* var. *ceresianum* (L.Bolus) S.A.Hammer	Scent* and nectar: N Ceres, W Skitterkloof, Zoo Ridge, RSA; Kaaimansgat, RSA; Scent*: Hartnekskloof, RSA	●
C. obcordellum subsp. *rolfii* (De Boer) S.A.Hammer	Scent* and nectar: K'Taaibos, Elandsbaai, RSA; Scent*: Elandsbaai, RSA	●
C. obcordellum subsp. *stenandrum* (L.Bolus) S.A.Hammer	Scent* and nectar: Ottaspoort, RSA	●
C. piluliforme (N.E.Br.) N.E.Br. subsp. *piluliforme*	Scent: Between Ladismith and Montagu, RSA; Nectar: hort. Japan	●
C. truncatum (Thunberg) N.E.Br. subsp. *truncatum* var. *truncatum*	Scent* and nectar: Sandpoort, RSA; Scent*: Doornkloof, Uniondale, RSA	●
C. truncatum (Thunberg) N.E.Br. subsp. *truncatum* var. *wiggettiae*	Scent* and nectar: Hazenjacht, RSA	●
C. uviforme (Haw.) N.E.Br. subsp. *uviforme*	Scent* and nectar: Below Vanrhynspas, Grasberg near Nieuwoudtville, RSA; Scent*: Brakfontein, NW Loeriesfontein, RSA	●
C. uviforme subsp. *decoratum* (N.E.Br.) S.A.Hammer	Scent*: Kourkamberge, RSA	●
C. uviforme subsp. *rauhii* (Tischer) S.A.Hammer	Scent* and nectar: Messelpad, RSA	●
C. uviforme subsp. *subincanum* (Tischer) S.A.Hammer	Scent* and nectar: Wolwenes, S Quaggaskop, RSA	●
Costata Schwantes ex S.A.Hammer		
C. angelicae (Dinter & Schwantes) N.E.Br. subsp. *angelicae*	Scent* and nectar: Witsand, NA	●
C. angelicae subsp. *tetragonum* Rawe & S.A.Hammer	Scent* and nectar: Rosyntjieberg, RSA	●
Herreanthus (Schwantes) S.A.Hammer		
C. blandum L.Bolus	Nectar: Geselskapbank, RSA	□
C. herreanthus subsp. *rex* S.A.Hammer	Nectar: Little Hellskloof, RSA	□
C. marginatum subsp. *haramoepense* (L.Bolus) S.A.Hammer	Nectar: Aggeneys, RSA	□
C. regale Lavis	Scent and nectar: Ratelpoort, RSA	□
Minuscula (Schwantes) Tischer ex S.A.Hammer		
C. fulleri L.Bolus	Scent and nectar: Namies, RSA	□

Table 1 cont.

Taxa/Species	Localities	Diurnal / Nocturnal
Ophthalmophyllum (Dinter & Schwantes) Tischer		
C. lydiae (Jacobsen) Rowley	Scent: SE Springbok, RSA	□
Saxetana (Schwantes) S.A.Hammer		
C. carpianum L.Bolus	Scent* and nectar: Doornport, RSA	●
C. hians N.E.Br.	Scent* and nectar: Lekkersing, RSA	●
C. quaesitum subsp. *densipunctatum* (L.Bolus) S.A.Hammer	Scent* and nectar: Signalberg, Klein Karas, NA	●
C. quaesitum subsp. *quaesitum* var. *rostratum* (Tischer) S.A.Hammer	Scent* and nectar: Tatasberg, Richtersfeld, RSA	●
C. saxetanum (N.E.Br.) N.E.Br.	Scent* and nectar: N Buchuberg, NA; Nectar: Schwarze Kuppe, E Aurus, NA	●
Subfenestrata Tischer ex S.A.Hammer		
C. concavum L.Bolus	Scent and nectar: N Riethuis, RSA	□
C. subfenestratum Schwantes	Scent: Quaggaskop, RSA; Nectar: Rooiberg, RSA	□
Wettsteinia (Schwantes) Tischer ex S.A.Hammer		
C. jucundum subsp. *marlothii* (N.E.Br.) S.A.Hammer	Scent and nectar: Augrabies, RSA	□
C. jucundum subsp. *ruschii* (Schwantes) S.A.Hammer	Nectar: Rosyntjieberg, RSA	□
C. ricardianum Loesch & Tischer subsp. *ricardianum*	Nectar: Lorelei, NA	□

The data of scent samples from subspecies and varieties of the same species were pooled for further analysis. Sørensen's index of similarity (I_s) with a quantitative modification for relative values was used for the comparison of scent profiles between taxa (Tollsten *et al.* 1994):

$$I_s = \frac{2\ M_w \times 100}{M_a + M_b}$$

where M_w is the sum of the smaller values of compounds present in both taxa, and M_a and M_b respectively, are the sums of all compounds found in the individual taxa. We used (nonmetric) multidimensional scaling (MDS) in the STATISTICA 5.1 program to detect meaningful underlying dimensions and to visualise similarities between species (see Borg and Lingoes 1987). To evaluate how well (or poorly) the particular configuration produces the observed distance matrix the *raw stress* (or *raw Phi*) value (see Kruskal 1964) and the coefficient of alienation *K* (see Guttman 1968) are given. The smaller the stress value, the better is the fit of the reproduced distance matrix to the observed distance matrix.

Results

Nectar composition

Nectar sugar concentration and composition of 39 species, subspecies, and varieties investigated are presented in Table 2. Nectar sugar concentration was in general very low. It exceeded 100µg µl^{-1} only in 15 out of 50 samples, reaching a maximum of 352.4µg µl^{-1} in *C. bilobum* subsp. *bilobum.* No sugars other than glucose, fructose and sucrose were found. Glucose and fructose are the dominant constituents in the nectar of all investigated *Conophytum* species, with the proportion of sucrose ranging from 0% to 14.3%. To some degree variation is also detectable between samples of a single species (e.g. *C. bilobum*, *C. obcordellum*, *C. jucundum* and *C. saxetanum*). Using the terminology of Baker and Baker (1983b) nectar sugar composition in all *Conophytum* species is hexose-dominant (sucrose:hexose = <0.1), with the exception of *C. angelicae* subsp. *tetragonum*, *C. velutinum* subsp. *velutinum*, *C. jucundum* subsp. *ruschii*, and *C. saxetanum* (one out of two samples), which were hexose-rich (sucrose:hexose = 0.1–0.499, see Figure 1). The sucrose:hexose ratios do not differ significantly between the 14 day-flowering and 25 night-flowering species, subspecies, and varieties belonging to 11 sections that were analysed. In contrast to the relatively uniform sucrose:hexose ratios, the fructose:glucose ratios showed an interesting variation (Figure 1). Nectar samples of diurnal species had more or less equal amounts of fructose and glucose (Mean ± SD = 0.953 ± 0.12, min. = 0.807, n = 14) whereas samples of nocturnal species had significantly lower fructose:glucose ratios (Mean ± SD = 0.757 ± 0.164, min. = 0.432, n = 30) (Tukey HSD for unequal n, $P < 0.01$).

Floral scent compounds

The chemical composition and the relative amount of floral scent compounds are reported in Table 3a and 3b, where the species are grouped alphabetically within the taxonomic sections. In total 52 compounds were detected, of which 33 compounds could be identified from the floral odour of the 27 plant species. Floral fragrance data were organised by grouping the compounds into six different classes (Knudsen *et al.* 1993); within the groups compounds are listed in order of retention time. The number of compounds per species ranged from as low as five in the diurnal *C. concavum* and *C. burgeri* to 46 in the nocturnal *C. obcordellum*. Nocturnal species showed a higher number of compounds than diurnal species. Within diurnal species the richest scent profile was

Table 2: Nectar sugar concentration and relative amount of nectar sugars in 27 species within the genus *Conophytum*. S = sucrose, F = fructose, G = glucose, tr = traces of sucrose (considered as 0.1µg µl^{-1} for calculating the sucrose:hexose ratio), – = no data available, because amounts of both hexoses were just below quantification limit, (50[a]) = proportions of hexoses nearly equal but fructose amount was just below quantification limit; (50[b]) = proportions of hexoses nearly equal but glucose amount was just below quantification limit, (50[c]) = proportions of hexoses nearly equal but amounts of both were just below quantification limit

Taxa / Species	Sample number	Nectar volume (µl)	Sugar conc. (µg µl–1)	(%) Rel. amount fructose	(%) Rel. amount glucose	(%) Rel. amount sucrose	S/(F+G)	F/G
Barbata Schwantes ex S.A.Hammer								
C. stephanii subsp. *helmutii* (Lavis) S.A.Hammer	1	1.09	65.4	36.7	63.4	0	0	0.579
Biloba N.E.Br.								
C. bilobum subsp. *bilobum*	1	0.19	210.9	53.4	46.7	0	0	1.146
	2	0.69	352.4	42.1	49.5	8.5	0.093	0.851
C. bilobum subsp. *claviferens*	1	1.69	245.6	46.6	50.1	3.5	0.036	0.931
C. meyeri	1	0.78	316.2	45.9	48.0	6.2	0.066	0.957
C. velutinum subsp. *velutinum*	1	1.02	89.0	38.4	47.6	14.1	0.165	0.807
Cataphracta Schwantes ex S.A.Hammer								
C. calculus subsp. *calculus*	1	0.75	45.7	51.7	48.4	0	0	1.068
C. calculus subsp. *vanzylii*	1	0.34	23.1[a]	50[c]	50[c]	0	0	–
C. stevens-jonesianum	1	0.73	59.5	37.5	62.6	0	0	0.598
Cheshire-feles S.A.Hammer								
C. acutum	1	0.94	46.1	33.1	67.0	0	0	0.493
C. maughanii subsp. *maughanii*	1	1.31	57.7	40.2	59.7	0.2 (tr)	0.002	0.674
C. maughanii subsp. *armeniacum*	1	0.63	44.2	38.3	61.6	0.3 (tr)	0.003	0.622
	2	0.28	86.8	43.7	56.4	0	0	0.775
Conophytum N.E.Br.								
C. ficiforme	1	1.00	45.4	43.3	56.8	0	0	0.763
C. obcordellum subsp. *obcordellum* var. *obcordellum*	1	1.06	42.1	42.6	57.5	0	0	0.740
	2	0.67	30.5	48.6	51.5	0	0	0.945
	3	1.44	79.4	42.9	57.1	0.2 (tr)	0.002	0.752
	4	1.19	83.5	38.5	61.5	0.2 (tr)	0.002	0.627
C. obcordellum subsp. *obcordellum* var. *ceresianum*	1	1.06	77.7	45.9	54.1	0.2 (tr)	0.002	0.850
	2	0.73	77.6	48.2	51.9	0	0	0.929
	3	0.38	69.7	46.2	53.9	0	0	0.859
	4	0.69	129.8	46.0	46.5	7.7	0.083	0.989
C. obcordellum subsp. *rolfii*	1	1.03	76.1	48.9	51.2	0	0	0.954
C. obcordellum subsp. *stenandrum*	1	0.55	84.3	46.3	53.8	0	0	0.861
C. piluliforme subsp. *piluliforme*	1	0.19	110.9	51.2	48.9	0	0	1.048
C. truncatum subsp. *truncatum* var. *truncatum*	1	0.16	–	50[c]	50[c]	0	0	–
C. truncatum subsp. *truncatum* var. *wiggettiae*	1	0.88	62.6	42.0	58.1	0	0	0.722
C. uviforme subsp. *uviforme*	1	1.53	76.0	45.6	54.5	0	0	0.837
	2	1.63	110.1	48.6	51.4	0.1 (tr)	0.001	0.945
C. uviforme subsp. *rauhii*	1	0.22	32.6[b]	50[c]	50[c]	0	0	–
C. uviforme subsp. *subincanum*	1	0.13	–	50[c]	50[c]	0	0	–
Costata Schwantes ex S.A.Hammer								
C. angelicae subsp. *angelicae*	1	0.66	67.0	30.2	69.9	0	0	0.432
C. angelicae subsp. *tetragonum*	1	0.94	74.1	36.6	52.5	11.1	0.125	0.697
Herreanthus (Schwantes) S.A.Hammer								
C. blandum	1	0.69	65.8	44.9	55.0	0.2 (tr)	0.002	0.817
C. herreanthus subsp. *rex*	1	3.66	78.3	47.4	52.6	0.2 (tr)	0.002	0.901
C. marginatum subsp. *haramoepense*	1	0.70	223.9	52.1	47.9	0.1 (tr)	0.001	1.089
C. regale	1	0.25	218.1	54.5	45.6	0	0	1.194
	2	0.94	344.8	47.4	47.2	5.5	0.058	1.004
Minuscula (Schwantes) Tischer ex S.A.Hammer								
C. fulleri	1	0.16	58.5[b]	50[c]	50[c]	0	0	–

Table 2 cont.

Taxa / Species	Sample number	Nectar volume (µl)	Sugar conc. (µg µl–1)	(%) Rel. amount fructose	(%) Rel. amount glucose	(%) Rel. amount sucrose	S/(F+G)	F/G
Saxetana (Schwantes) S.A.Hammer								
C. carpianum	1	0.94	81.0	44.1	55.9	0.2 (tr)	0.002	0.790
C. hians	1	0.63	79.1	37.3	62.6	0.2 (tr)	0.002	0.596
C. quaesitum. subsp. *densipunctatum*	1	1.50	91.4	36.6	59.6	4	0.041	0.614
C. quaesitum. subsp. *quaesitum var. rostratum*	1	0.72	60.3	38.7	61.4	0	0	0.629
C. saxetanum	1	0.98	112.1	38.5	61.6	0	0	0.626
	2	0.94	93.8	37.1	53.2	9.9	0.109	0.696
Subfenestrata Tischer ex S.A.Hammer								
C. concavum	1	0.84	332.4	44.5	52.0	3.6	0.037	0.856
C. subfenestratum	1	0.50	155.8	47.5	52.6	0	0	0.904
Wettsteinia (Schwantes) Tischer ex S.A.Hammer								
C. jucundum subsp. *marlothii*	1	0.14	–	50[c]	50[c]	0	0	–
C. jucundum subsp. *ruschii*	1	0.75	157.5	42.7	43.2	14.3	0.166	0.989
C. ricardianum subsp. *ricardianum*	1	0.36	300.8	47.4	52.7	0	0	0.898

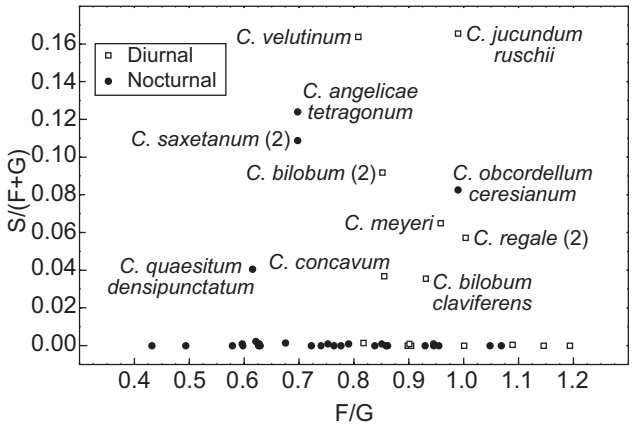

Figure 1: Sucrose:hexose ratios (S/[F+G]) *versus* fructose:glucose ratios (F/G) in 44 samples of 33 nocturnal and diurnal species, subspecies, and varieties of the genus *Conophytum*. Numbers in brackets refer to sample numbers of taxa that are present with two or more samples (see Table 2)

found in *C. jucundum* with 20 compounds. There is a predominance of benzenoid compounds in all sections. Other compounds present were fatty acid derivatives, isoprenoids, and nitrogen-containing compounds. The number of compounds found in the different compound classes was as follows: (1) fatty-acid derivatives 3, (2) benzenoids 26, (3) terpenoids 4, (4) nitrogen-containing compounds 4, (5) miscellaneous 1, (6) unknowns 14. The floral scent composition was dominated by only a few scent compounds. Benzaldehyde and benzeneacetaldehyde were found as main compounds with the highest relative abundance of all compounds in 19 species, and eight species respectively. Besides these two benzenoids, benzyl acetate was an important constituent in many species (e.g. *C. truncatum* 37.4%, *C. hammeri* 35.3%, *C. pageae* 32.0%, *C. calculus* 28.6%, *C. ficiforme* 21.1%, *C. uviforme* 20.5%). Methylbenzoate was a main compound in *C. stevens-jonesianum* (34.1%). Among the three fatty-acid derivatives, the aliphatic alcohols 3-nonen-1-ol, and 2-decen-1-ol were found in almost all species. The fatty-acid derivative 2-decen-1-ol was a main compound in the diurnal *C. regale* and accounted for 21.0% of the total scent of this species. Nitrogen-containing compounds were found in 18 species. The highest relative amount of nitrogen-containing compounds was found in the nocturnal *C. piluliforme* (8.0%). With the exception of *C. jucundum*, which contained 0.7% of indole, no diurnal species contained any nitrogen-containing compound. Fifteen species contained considerable amounts of compounds of isoprenoid origin. However, the mean relative amount of an isoprenoid never exceeded 2% in any species.

According to the chemical composition of their floral scent the species can be divided into two groups, (1) species that are characterised by the predominance of benzaldehyde and benzeneacetaldehyde, and (2) species with high relative amounts of benzenoid esters, such as benzyl acetate, methyl benzoate, and benzyl benzoate (Table 3a–3b, Figure 2). The first group contains many diurnal species and species from all nocturnal sections with the exception of the section *Conophytum*. The second group comprises only nocturnal species from the sections *Conophytum*, *Cheshirefeles*, and *Cataphracta*. These groups are supported by the results of the MDS (Figure 2). Some species, however, are intermediate regarding their floral scent composition. The MDS groups nocturnal species with a relative high amount of benzyl acetate (*C. truncatum* (37.4%), *C. hammeri* (35.3%), *C. pageae* (32.0%), *C. calculus* (28.6%), *C. obcordellum* (28.1%), *C. ficiforme* (21.1%), *C. uviforme* (20.5%), and *C. piluliforme* (9.1%)) on the right side in Figure 2. Closest to this group are species with benzaldehyde as main compound (*C. subfenestratum* (91.1%), *C. quaesitum* (79.0%), *C. carpianum* (72.3%), and *C. lydiae* (69.8%)). In the latter ones the relative amount of benzeneacetaldehyde never exceeded 30.0%. Species with rela-

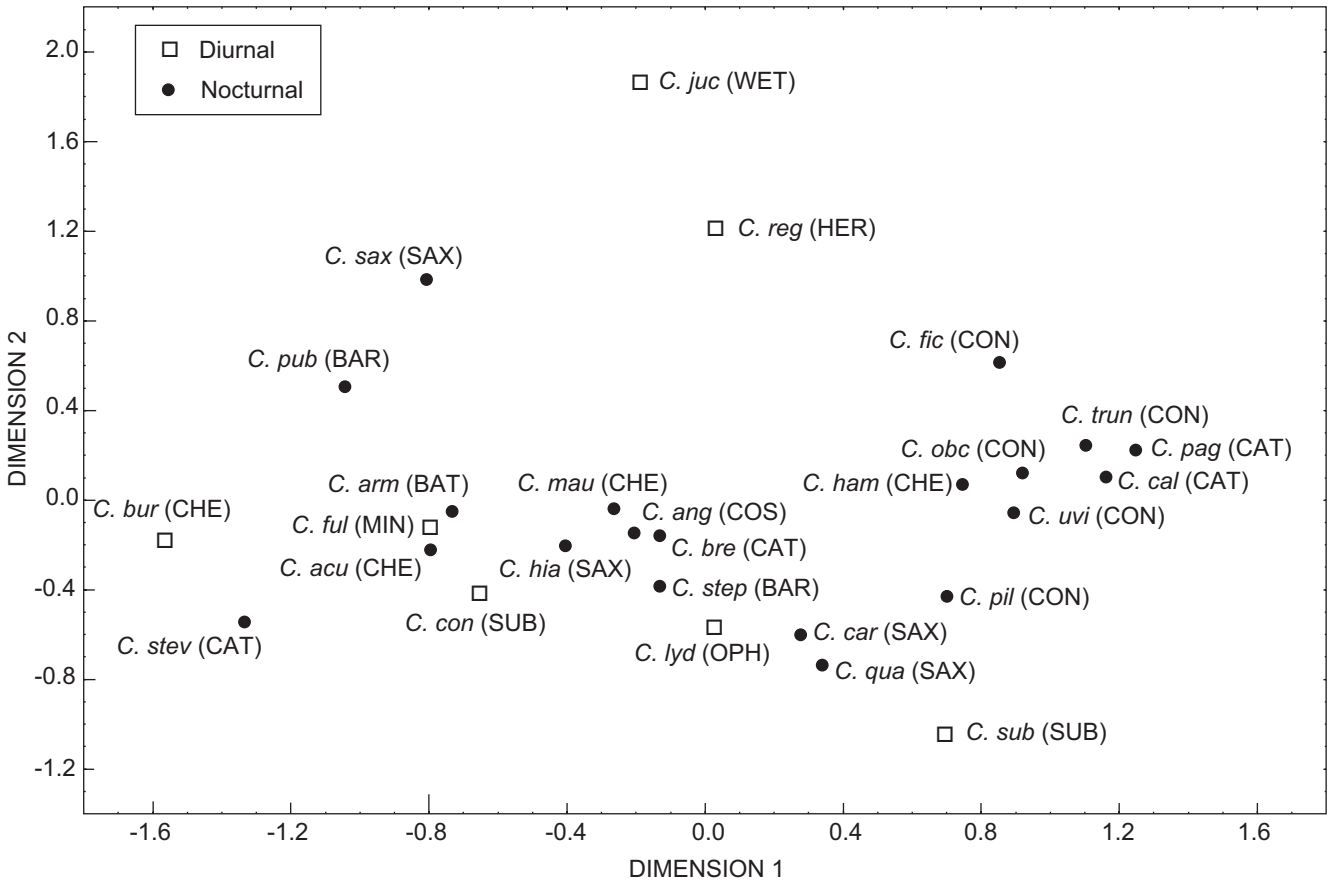

Figure 2: Multidimensional scaling of 27 diurnal and nocturnal *Conophytum* species. Stress value 0.109 (see Kruskal 1964); coefficient of alienation 0.122 (see Guttman 1968). Abbreviations of species and section names as in Table 3a and 3b

tive high amounts of benzeneacetaldehyde (*C. burgeri* (72.0%), *C. concavum* (64.3%), *C. acutum* (60.8%), *C. fullerii* (60.2%), *C. armianum* (50.1%), and *C. stevens-jonesianum* (44.6%)) are found on the left side of Figure 2. Some species are intermediate in their benzaldehyde and benzeneacetaldehyde content (e.g. *C. maughanii*, *C. angelicae*, *C. breve*, *C. stephanii*; see Table 3a–3b). The isolated position of *C. jucundum, C. regale*, *C. saxetanum*, and *C. pubicalyx* is due to relative high amounts of an unidentified compound (RR_t = 277, Table 3a–3b) and 1-ethyl-2-methyl benzene (Figure 2).

Discussion

Nectar sugar composition

Since the investigations of Percival (1961), and Baker and Baker (e.g. 1973a, 1973b, 1975, 1983a, 1983b, 1986) it is evident that there are some striking patterns of nectar sugar composition related to pollinator class. Flowers of some pollinator classes such as hawkmoths, long-tongued bees, and hummingbirds are characterised by high sucrose:hexose ratios, whereas others, such as short-tongued bees, flies, and passerine birds are characterised by low ratios (Baker and Baker 1983b). Exceptions to these trends have led Percival (1961) and Baker and Baker (1983b) to conclude that in some taxa phylogenetic constraints might play an important role. Species of Brassicaceae and Asteraceae, for example, tend to be typically hexose-dominated or hexose-rich, independent of the pollination syndrome, whereas Ranunculaceae and Lamiaceae are predominantly sucrose-dominant or sucrose-rich, respectively (Baker and Baker 1983b, Percival 1961). In the genus *Conophytum* similar constraints seem to occur. All species and subspecies offered hexose-dominated nectar, independent of the pollination syndrome or the section they belong to. Similar investigations of nectar sugar composition in larger taxonomic groups were done by Elisens and Freeman (1988) in 20 species of Anthirrhineae (Scrophulariaceae), by Van Wyk (1993) in 71 species of Fabaceae, by Van Wyk *et al.* (1993) in 82 species of Alooideae (Asphodelaceae), by Schwerdtfeger (1996) in Asteridae, by Galetto *et al.* (1998) in 14 species and 54 populations of *Lycium* (Solanaceae), by Nicolson and Van Wyk (1998) in 147 Proteaceae, and by Perret *et al.* (2001) in 45 species of Sinningieae (Gesneriaceae). Apart from Elisens and Freeman (1988) and Schwerdtfeger (1996) nearly all authors cited above concluded that the variability of the nectar sugar composition is mainly based on phylogenetic constraints, and ecological interactions play a minor role.

Table 3a: Chemical composition of the floral scent in 13 *Conophytum* species of the sections *Barbata (BAR)*, *Batrachia (BAT)*, *Cataphracta (CAT)*, *Cheshire–feles (CHE)*, and *Conophytum (CON)*. Average relative amounts (in %) of floral volatiles emitted by *C. pubicalyx (C. pub)*, *C. stephanii (C. step)*, *C. armianum (C. arm)*, *C. breve (C. bre)*, *C. calculus (C. cal)*, *C. pageae (C. pag)*, *C. stevens–jonesianum (C. stev)*, *C. acutum (C. acu)*, *C. burgeri (C. bur)*, *C. hammeri (C. ham)*, *C. maughanii (C. mau)*, *C. ficiforme (C. fic)*, *C. obcordellum (C. obc)*. The compounds are listed according to class and relative retention time order (RR_t). tr = trace amounts (<1.0%). Unknowns were included when present with more than 1.0% in any sample

Sample number	RR_t	*C. pub* *BAR* 1	*C. step* *BAR* 1	*C. arm* *BAT* 1	*C. bre* *CAT* 2	*C. cal* *CAT* 2	*C. pag* *CAT* 2	*C. stev* *CAT* 2	*C. acu* *CHE* 1	*C. bur* *CHE* 1	*C. ham* *CHE* 1	*C. mau* *CHE* 2	*C. fic* *CON* 2	*C. obc* *CON* 11
Fatty-acid Derivatives														
3-Nonen-1-ol	460	4.0	0.4	2.0	0.1	0.4	0.1	0.4	0.1	1.3	0.2	1.1	0.5	0.4
2-Decen-1-ol	552	5.3	0.4	3.0	0.1	0.6	0.3	0.6	2.6	8.2	0.9	3.3	1.3	0.9
Methyl hexadecanoate	1073	4.4	–	–	1.8	2.6	3.8	–	–	–	–	0.3	0.2	2.4
Benzenoids														
p-Xylene	258	–	0.3	0.3	2.0	0.2	0.1	0.8	0.2	–	0.2	1.0	0.6	0.2
Benzene, 1-ethyl-2-methyl	322	1.3	–	0.3	1.6	0.1	0.1	0.1	1.0	–	0.3	0.4	0.3	0.1
Benzaldehyde	326	18.3	50.4	28.4	46.9	40.5	39.6	14.1	28.3	9.3	44.5	39.5	32.5	43.1
Trimethylbenzene	355	5.5	2.5	0.7	2.3	0.2	0.1	1.2	1.7	9.3	0.3	3.6	1.1	0.5
Benzyl alcohol	395	–	–	–	–	0.5	3.9	–	–	–	0.4	0.4	0.1	0.3
Benzeneacetaldehyde	405	29.6	34.1	50.1	35.1	3.3	1.2	44.6	60.8	72.0	11.3	34.1	6.6	6.3
Phenyl benzoate	427	–	0.4	0.3	0.1	0.1	tr	0.2	0.1	–	0.2	0.6	0.2	0.1
Benzene 1-ethyl-2,3-dimethyl-	442	–	–	0.3	0.1	tr	tr	0.1	0.1	–	0.1	0.3	0.1	tr
Methylbenzoate	454	–	–	–	0.1	tr	tr	34.1	–	–	0.1	0.3	0.5	0.8
Phenyl ethyl alcohol	471	–	–	0.3	–	tr	tr	0.1	0.1	–	0.1	0.1	–	tr
Benzyl acetate	515	–	–	–	–	28.6	32.0	–	–	–	35.3	0.9	21.1	28.1
2,6-Dimethylbenzaldehyde	540	–	0.4	–	–	tr	tr	0.1	0.2	–	0.1	0.2	0.1	tr
Methyl Salicylate	546	–	–	–	0.1	–	–	–	–	–	tr	–	0.1	0.7
2-Phenyl ethyl acetate	598	–	–	–	tr	tr	tr	–	–	–	1.3	–	0.1	0.2
trans-Cinnamaldehyde	617	–	0.1	–	–	0.1	tr	–	–	–	–	–	–	tr
Cinnamyl alcohol	643	–	0.4	–	–	–	0.3	–	–	–	–	–	–	0.2
Dimethyl Salicylate	665	12.9	0.3	–	–	–	–	–	–	–	–	0.3	0.1	3.3
Isoeugenol	681	–	–	–	–	–	–	–	–	–	–	–	tr	tr
3-Phenylpropyl acetate	692	–	0.4	–	–	–	–	–	–	–	–	–	–	–
2-(4-Methoxyphenyl) ethanol	695	–	–	–	–	–	–	–	–	–	–	–	0.1	tr
Methyleugenol	718	–	–	–	–	–	–	–	–	–	–	–	9.8	0.2
Cinnamyl acetate	752	–	4.0	–	–	–	0.2	–	–	–	–	–	–	0.1
Benzene m/z: 220, 205, 189, 177, 161, 145, 133, 121, 105, 91, 77	797	–	3.2	–	–	–	–	–	–	–	–	–	–	–
Benzylbenzoate	986	–	–	–	–	19.5	8.1	–	–	–	0.2	–	0.3	0.5
Isoprenoids														
cis-beta-Ocimene	402	–	–	–	–	–	–	–	–	–	–	–	2.0	–
Sesquiterpene m/z: 204, 189, 175, 161, 147, 133, 119, 105, 91	706	–	–	–	–	–	0.1	0.2	0.2	–	0.2	tr	0.1	0.2
Sesquiterpene m/z: 204, 189, 175, 161, 147, 133, 119, 105, 91	732	–	–	0.3	–	–	–	0.2	0.1	–	0.1	0.1	0.1	0.1
Valencene	735	0.9	1.3	–	–	–	–	0.3	0.2	–	0.2	–	0.3	0.2

Table 3a cont.

Sample number	RR_t	*C. pub* *BAR* 1	*C. step* *BAR* 1	*C. arm* *BAT* 1	*C. bre* *CAT* 2	*C. cal* *CAT* 2	*C. pag* *CAT* 2	*C. stev* *CAT* 2	*C. acu* *CHE* 1	*C. bur* *CHE* 1	*C. ham* *CHE* 1	*C. mau* *CHE* 2	*C. fic* *CON* 2	*C. obc* *CON* 11
Nitrogen-containg Compounds														
Benzyl nitrile	495	–	–	0.3	4.3	0.1	0.7	0.1	–	–	1.4	0.3	0.2	0.4
Unknown nitrogen compound m/z: 117, 104, 90, 77, 63	593	–	–	–	0.1	0.1	1.1	–	–	–	0.1	–	–	tr
Indole	633	–	–	–	–	1.4	1.6	–	0.1	–	0.2	–	7.6	4.6
Methyl 2-aminobenzoate	674	–	–	–	–	tr	0.8	–	–	–	–	–	0.3	0.4
Miscellaneous														
Naphthalene	542	–	–	1.3	0.5	0.1	tr	0.1	0.3	–	0.1	0.7	0.3	0.1
Unknowns														
m/z: 55, 83, 99, 135	277	7.4	–	3.8	1.7	0.8	0.2	1.2	1.5	–	0.1	1.9	1.0	0.5
m/z: 121, 105, 93, 76, 66	570	–	–	–	–	0.1	0.1	–	–	–	–	–	tr	0.1
m/z: 85, 71, 57	607	–	–	1.3	–	–	–	0.2	0.2	–	0.3	–	0.2	tr
m/z: 147, 135, 122, 111, 97, 85	631	–	–	1.2	–	0.1	tr	0.2	0.9	–	0.1	0.2	1.1	0.2
m/z: 141, 115, 96, 81, 70	640	–	–	2.0	0.1	–	–	0.2	0.1	–	0.1	0.3	0.1	tr
m/z: 147, 119, 103, 90, 76, 63	653	–	–	–	–	tr	0.5	tr	–	–	0.2	–	0.7	1.0
m/z: 144, 131, 111, 97, 85, 71	712	–	–	0.3	–	0.1	0.1	0.1	0.1	–	0.1	0.2	0.8	0.2
m/z: 178, 149, 121, 93, 66	727	–	–	–	–	–	0.1	tr	–	–	–	–	0.1	0.1
m/z: 220, 205, 189, 177, 161, 145, 135	766	0.9	–	0.7	–	0.1	tr	0.2	0.4	–	0.2	0.1	0.3	0.1
m/z: 218, 203, 189, 175, 161, 147, 133, 119, 105, 91, 77	772	1.0	–	1.3	–	0.1	tr	0.3	0.2	–	0.2	0.1	0.1	0.3
m/z: 136, 122, 111, 97, 85, 71	789	0.9	0.8	0.7	2.5	1.3	4.9	0.2	–	–	0.2	–	8.3	2.1
m/z: 174, 146, 131, 118, 102, 76, 62	824	–	–	–	–	tr	0.1	–	–	–	–	0.1	–	0.1
m/z: 171, 157, 143, 132, 117, 105, 91	1036	–	0.4	0.7	0.2	0.1	0.1	0.1	0.1	–	0.1	0.5	0.3	0.1
m/z: 194, 181, 165, 152, 138, 124, 111	1122	4.4	–	–	–	–	–	0.1	–	–	0.9	–	0.6	0.5
m/z: 284, 269, 251, 231, 208, 194, 180	1365	–	–	–	0.6	–	–	–	–	–	–	9.3	–	–

Table 3b: Chemical composition of the floral scent in 14 *Conophytum* species of the sections *Conophytum* (*CON*), *Costata* (*COS*), *Herreanthus* (*HER*), *Minuscula* (*MIN*), *Ophthalmophyllum* (*OPH*), *Saxetana* (*SAX*), *Subfenestrata* (*SUB*), and *Wettsteinia* (*WET*). Average relative amounts (in %) of floral volatiles emitted by *C. piluliforme* (*C. pil*), *C. truncatum* (*C. tru*), *C. uviforme* (*C. uvi*), *C. angelicae* (*C. ang*), *C. regale* (*C. reg*), *C. fulleri* (*C. ful*), *C. lydiae* (*C. lyd*), *C. carpianum* (*C. car*), *C. hians* (*C. hia*), *C. quaesitum* (*C. qua*), *C. saxetanum* (*C. sax*), *C. concavum* (*C. con*), C. *subfenstratum* (*C. sub*), *C. jucundum* (*C. juc*). The compounds are listed according to class and relative retention time order (RR_t). tr = trace amounts (<1.0%). Unknowns were included when present with more than 1.0% in any sample

Sample number	RR_t	*C. pil* CON 1	*C. tru* CON 3	*C. uvi* CON 6	*C. ang* COS 2	*C. reg* HER 1	*C. ful* MIN 1	*C. lyd* OPH 1	*C. car* SAX 1	*C. hia* SAX 1	*C. qua* SAX 2	*C. sax* SAX 1	*C. con* SUB 1	*C. sub* SUB 1	*C. juc* WET 1
Fatty-acid Derivatives															
3-Nonen-1-ol	460	3.3	0.3	0.5	1.1	12.1	6.1	0.3	1.3	0.7	0.6	5.4	–	0.3	6.7
2-Decen-1-ol	552	6.6	0.4	0.5	0.5	21.0	1.1	0.3	2.0	0.8	1.2	2.1	0.2	1.4	1.4
Methyl hexadecanoate	1073	–	–	2.8	0.1	–	–	–	–	–	tr	–	–	–	–
Benzenoids															
p-Xylene	258	–	0.5	0.4	1.3	2.0	–	–	0.3	–	0.2	2.1	–	–	8.9
Benzene, 1-ethyl-2-methyl	322	–	0.3	0.3	0.6	2.9	–	–	0.1	0.6	0.1	2.1	–	0.1	23.5
Benzaldehyde	63.0	43.5	49.1	40.0	38.4	29.4	69.8	72.3	41.0	79.0	18.2	34.9	91.1	32.8	326
Trimethylbenzene	355	1.0	0.7	1.0	0.7	2.4	1.1	–	0.5	4.2	0.2	11.8	–	0.1	2.4
Benzyl alcohol	395	–	tr	0.2	0.1	–	–	–	1.4	0.1	0.6	–	–	–	–
Benzeneacetaldehyde	405	8.1	2.0	4.9	36.3	4.4	60.2	29.0	16.7	48.3	16.1	19.3	64.3	–	0.7
Phenyl benzoate	427	–	0.1	0.1	0.5	–	1.1	0.3	0.1	0.1	tr	2.1	0.2	0.2	3.9
Benzene 1-ethyl-2,3-dimethyl-	442	–	0.1	0.1	tr	–	–	0.3	–	0.1	tr	2.0	–	0.1	0.3
Methylbenzoate	454	–	0.3	1.9	–	–	–	–	0.8	0.1	tr	2.0	–	0.3	0.7
Phenyl ethyl alcohol	471	–	0.1	0.1	tr	–	–	–	1.8	0.1	0.6	2.1	–	–	–
Benzyl acetate	515	9.1	37.4	20.5	–	–	–	–	0.1	0.1	tr	0.4	–	0.2	0.7
2,6-Dimethylbenzaldehyde	540	–	tr	tr	tr	–	–	–	tr	–	tr	–	–	–	–
Methyl Salicylate	546	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–
2-Phenyl ethyl acetate	598	–	0.1	0.1	–	–	–	–	–	–	tr	–	–	–	–
trans-Cinnamaldehyde	617	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4-Methoxy Benzaldehyde	604	–	–	–	–	–	–	–	–	–	–	–	–	1.8	–
Dimethyl Salicylate	665	–	–	1.5	–	–	–	–	0.1	–	tr	–	–	–	–
Isoeugenol	681	–	0.3	tr	–	–	–	–	–	–	–	–	–	–	–
2-(4-Methoxyphenyl) ethanol	695	–	–	–	–	–	–	–	tr	–	0.2	–	–	–	–
Methyleugenol	718	–	1.2	1.3	–	–	–	–	–	–	–	–	–	–	–
Benzylbenzoate	986	–	1.1	5.4	15.7	–	–	–	–	–	0.3	–	–	–	–
Isoprenoids															
cis-beta-Ocimene	402	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
Sesquiterpene m/z: 204, 189, 175, 161, 147, 133, 119, 105, 91	706	–	–	tr	0.3	–	–	–	–	–	tr	–	–	–	0.7
Sesquiterpene m/z: 204, 189, 175, 161, 147, 133, 119, 105, 91	732	–	tr	tr	0.1	–	–	–	–	–	tr	–	–	–	–
Valencene	735	–	tr	0.1	0.4	–	–	–	–	–	0.1	–	–	–	–

Table 3b cont.

Sample number	RR_t	*C. pil CON* 1	*C. tru CON* 3	*C. uvi CON* 6	*C. ang COS* 2	*C. reg HER* 1	*C. ful MIN* 1	*C. lyd OPH* 1	*C. car SAX* 1	*C. hia SAX* 1	*C. qua SAX* 2	*C. sax SAX* 1	*C. con SUB* 1	*C. sub SUB* 1	*C. juc WET* 1
Nitrogen-containing Compounds															
Benzyl nitrile	495	–	tr	0.6	0.1	–	–	–	0.4	–	0.3	2.1	–	–	–
Unknown nitrogen compound m/z: 117, 104, 90, 77, 63	593	–	–	0.1	0.1	–	–	–	–	–	tr	–	–	–	–
Indole	633	8.0	tr	2.9	–	–	–	–	–	–	–	2.1	–	–	0.7
Methyl 2-aminobenzoate	674	–	–	1.3	–	–	–	–	–	–	–	0.4	–	–	–
Miscellaneous															
Naphthalene	542	–	0.1	0.2	0.2	1.0	–	–	tr	0.1	tr	1.1	–	–	0.7
Unknowns															
m/z: 55, 83, 99, 135	277	–	0.5	1.0	0.7	19.8	–	–	1.7	3.3	0.3	17.7	–	3.5	15.0
m/z: 121, 105, 93, 76, 66	570	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–
m/z: 147, 135, 122, 111, 97, 85	631	–	0.3	0.6	0.4	–	–	–	0.3	–	tr	–	–	–	0.6
m/z: 141, 115, 96, 81, 70	640	–	–	0.1	0.1	–	–	–	–	–	tr	2.1	–	–	–
m/z: 147, 119, 103, 90, 76, 63	653	–	–	0.9	tr	–	–	–	tr	–	tr	–	–	–	0.3
m/z: 144, 131, 111, 97, 85, 71	712	–	0.5	0.1	0.1	–	–	–	–	–	tr	–	–	–	0.7
m/z: 178, 149, 121, 93, 66	727	–	–	0.1	–	–	–	–	–	–	tr	–	–	–	–
m/z: 220, 205, 189, 177, 161, 145, 135	766	–	0.1	tr	0.3	–	–	–	–	–	0.1	1.0	–	–	–
m/z: 218, 203, 189, 175, 161, 147, 133, 119, 105, 91, 77	772	–	tr	tr	0.2	–	–	–	–	–	tr	1.0	–	–	–
m/z: 136, 122, 111, 97, 85, 71,	789	–	7.9	0.5	tr	–	–	–	–	–	tr	–	–	–	2.9
m/z: 174, 146, 131, 118, 102, 76, 62	824	–	0.1	tr	–	14.1	1.1	–	tr	0.1	tr	–	0.2	0.1	9.6
m/z: 171, 157, 143, 132, 117, 105, 91	1036	1.0	0.2	0.1	tr	1.2	–	–	0.1	0.1	0.1	2.1	–	–	0.7
m/z: 194, 181, 165, 152, 138, 124, 111	1122	–	1.1	0.1	–	–	–	–	–	–	tr	–	–	–	–
m/z: 284, 269, 251, 231, 208, 194, 180	1365	–	–	tr	–	–	–	–	–	–	–	–	–	–	–

Alternatively, it may be argued that there are no differences in sucrose:hexose ratios in *Conophytum* species simply because all their pollinator groups prefer hexose-rich to hexose-dominant nectar. The data about flower visitors of *Conophytum* species (Liede *et al.* 1991) show that in day-flowering species bee-flies, butterflies and small wasps and beetles are attracted. Personal observations (Jürgens unpublished) suggest that nectar-drinking wasps (*Quartinioides*, Masaridae), which also use the flowers as mating places, are frequent flower visitors in many diurnal species (e.g. *C. bilobum*, *C. marginatum*, *C. subfenestratum*, *C. jucundum* subsp. *ruschii*). Despite their small body size (2.5–5.0mm in length), many *Quartinioides* species are especially long-tongued, indicating an adaptation to the nature of flowers they visited (Gess and Gess 1989). But even these long-tongued wasps have to insert themselves deeply into the narrow flower tubes to reach the nectar of *Conophytum* flowers, and usually female individuals are deeply hidden in the floral tube while waiting for males (Jürgens pers. obs.). According to Baker and Baker (1983b) the occurrence of normally short-tongued pollinator guilds as for example, small bees and wasps, or flies, is correlated with hexose-dominated nectars, whereas long-tongued pollinator groups are correlated with high sucrose contents. The observed wasps are both especially small and extremely long-tongued, and therefore are not the 'typical' wasp pollinator, as mentioned by Baker and Baker (1983b). Further it has to be objected that in the investigations of Baker and Baker (1983b) plant species number pollinated by wasps was low, maybe too low to draw general conclusions on preferences of wasp pollinators.

Besides Masaridae, Bombyliidae (Diptera) were observed as flower visitors of diurnal *Conophytum* species (e.g. *C. fulleri*, Jürgens pers. obs.). With their extremely long proboscis adapted to nectar-foraging in long-tubed flowers, they do not correspond morphologically with the short-tongued flies that typically feed on open flowers. However, nectar-foraging of Bombyliidae on hexose-dominated nectars is in accordance with the preferences of Diptera presented by Baker and Baker (1983b).

In night-flowering *Conophytum* species moths are predicted (and in several cases are found, Jürgens pers. obs.) as pollinators. Baker and Baker (1983b) showed that moths and long-tongued butterflies tend to prefer high sucrose contents, but the present investigation shows that neither diurnal nor nocturnal *Conophytum* species offer sucrose-dominated nectars.

Besides direct phylogenetical and ecological constraints, Percival (1961) observed a relationship between long-tubed flowers and sucrose-dominated nectar. After Corbet (1978) sucrose-dominated sugar solutions tend to evaporate faster than hexose-dominated sugar solutions due to their lower osmotic potential (compare also Nicolson 2002, Plowright 1987). If the viscosity of nectar solutions is too high potential nectar-foraging pollinators are not able to extract the nectar and possibly avoid these flowers. Consequently, it might be more advantageous for plants offering sucrose-dominated nectars to decrease evaporation by producing their nectar in long-tubed flowers, flowers that are for functional reasons associated with long-tongued pollinators. Especially in *Conophytum* species the selective pressure to keep nectar viscosity low is assumed to be high. Most species live in arid semideserts with high daily temperatures and *Conophytum* plants are known for their adaptations to reduce water loss. Species grow in rock crevices of the hills or between the stones on flats in order to reduce irradiation and water stress (Hammer 2002). Offering hexose-dominated nectar with a high osmotic potential in 'long-tubed' flowers, which rise from deep inside the bodies that themselves are hidden in the rocks or stones may be interpreted as a further adaptation against evaporation. The observed tendencies to restrict the daily flowering period to some hours not only during the day, but also during the afternoon, evening or night (Liede and Hammer 1990), might be another strategy to escape water stress and to keep nectar viscosity sufficiently low for potential pollinators.

In contrast to the uniformity of sucrose:hexose ratios of nocturnal and diurnal species, fructose:glucose ratios show significant differences between both ecological groups with diurnal species having on average higher fructose:glucose ratios than nocturnal species. However, due to the small sample number and variable conditions before and during nectar sampling the results have to be interpreted with caution. The variability of the sugar ratios is obvious, for example in eight samples of the nocturnal *C. obcordellum* subsp. *obcordellum* with fructose:glucose ratios ranging from 0.627 to 0.989, in two samples of *C. saxetanum* with 0% and 9.9% relative sucrose content, and in two samples of two subspecies of *C. jucundum* with differing sucrose:hexose and fructose:glucose ratios. Especially in the latter case these differences may indicate taxonomical differences, as *C. jucundum* subsp. *ruschii* was formerly considered as a subspecies of *C. wettsteinii* (Hammer 2002). Van Wyk *et al.* (1993) proved the value of fructose:glucose ratios for distinguishing taxonomical groups in Alooideae (Asphodelaceae). However, the generic discontinuities found in Alooideae are not completely comparable to those occurring in *Conophytum*, as differing fructose:glucose ratios occurred in Alooideae genera producing sucrose-dominated nectar, whereas all conophytes studied offer hexose-dominated nectar. Further, in conophytes the delimitation does not run between genera but between diurnal and nocturnal conophytes, although it has to be admitted that section designations in *Conophytum* are somehow congruent with the occurrence of the diurnal and nocturnal pollination syndrome (see Hammer 2002).

After all it can be argued that conophytes, due to ecological constraints, have the main priority to save water and therefore secrete hexose-dominant nectar in order to reduce evaporation. The possible sugar preferences of pollinators seem to be of lower importance. Certainly, more detailed investigations on pollinator assemblages and nectar production of conophytes are needed to confirm these first findings.

Floral scent composition

Excellent reviews of the useful literature on floral scents were given by Knudsen *et al.* (1993) and Dobson (1994). Several studies have shown that floral scent can play an important role as an attractant for flower-visiting animals

(Gerlach and Schill 1991, Yasukawa *et al.* 1992, Honda *et al.* 1998, Knudsen *et al.* 1999, Ayasse *et al.* 2000). A similar floral scent composition has been found in species with the same pollination syndrome in plants pollinated by moths (Knudsen and Tollsten 1993), butterflies (Andersson *et al.* 2002), and bats (Knudsen and Tollsten 1995). Thus, together with other flower features (e.g. morphology, colour) the floral scent composition can be an additional indicator of the pollination syndrome. To some extent, however, chemical profiles of floral scent may reflect the taxonomy rather than the pollination biology (Azuma *et al.* 1999).

The floral scent of *Conophytum* mainly includes compounds that occur widely in many plant species (see Knudsen *et al.* 1993). Compared to nocturnal *Conophytum* species diurnal species showed a lower number of compounds. In two diurnal species, *C. wettsteinii* and *C. subfenestratum,* observation on pollination showed that small wasps (*Quartinioides* sp.) are the main flower visitors (Liede *et al.* 1991). The relatively faint odour of diurnal *Conophytum* flowers suggests that flower volatiles can only be effective in orienting day-active flower visitors at short range. It has been shown by Kugler (1932) that flower odours even when weak to humans can enhance landings by bees because olfactory stimuli become increasingly important at closer range. However, for long distance attraction the flower colour as a visual cue is likely more important in the showy *Conophytum* flowers.

In contrast to the weakly scented flowers of diurnal *Conophytum* species the flowers of nocturnal *Conophytum* species are strongly scented. A sweet penetrating floral fragrance characterises many moth-pollinated flowers (Baker 1961, Raguso and Pichersky 1995). Volatile compounds from moth-pollinated flowers have been shown to release visual search behaviors in sphingid moths and function as nectar guides for noctuid moths (Brantjes 1973, 1978). As in other moth-pollinated species nocturnal *Conophytum* flowers close during the day following anthesis, and also show a fragrance periodicity (Hammer 1993).

Nocturnal *Conophytum* species can be divided into two groups: the first group has a scent composition similar to that of diurnal species. These species are dominated by benzaldehyde and benzeneacetaldehyde and contain relatively low amounts of aromatic esters. The second group of nocturnal species are characterised by high contents of aromatic esters (benzyl acetate, benzylbenzoate) accompanied by low amounts of nitrogen-containing compounds. Especially the characteristics of the second group are pointing to moth pollination (Jürgens 2002).

Knudsen and Tollsten (1993) found that benzenoid compounds and nitrogenous compounds are common in floral scents of many moth-pollinated species. In particular, the aromatic esters benzyl acetate and benzyl benzoate found in some nocturnal *Conophytum* species are ubiquitous floral scent compounds in moth-pollinated plants.

It is striking that this differentiation in scent compounds is consistent with the distribution of the species. Floral scents of species from the Cape Floristic Region (definition according to Jürgens 1991) seem to be more adapted to moth-pollination, whereas floral fragrances of species distributed in the northern regions of the Succulent Karoo tend to be more generalistic. Considering the taxonomy of the species investigated, species with a high content of aromatic esters are mainly found in the sections *Conophytum*, *Cheshire–feles*, and *Cataphracta*. Therefore it is not possible to judge if floral scent composition is governed by ecological selection or phylogenetic constraints.

In summary, variation within the genus *Conophytum* occurs in floral scent composition and fructose:glucose ratios of floral nectars but not in sucrose:hexose ratios. It may be speculated that nectar secretion underlies more ecological constraints due to water stress, whereas floral scent production shows adaptations to possible pollen vectors. However, more data on the pollination biology and the phylogeny are needed to clarify if differences are due to phylogenetic constraints or due to an adaptation to different pollinator types. Besides a phylogenetic analysis of the genus *Conophytum* based on molecular data to investigate if night-flowering species have evolved several times independently from diurnal ancestors, additional field observations on the pollination biology, especially of the night-flowering species, will be needed to confirm the nature of the pollinators involved in the pollination of *Conophytum* species.

Acknowledgements — Northern Nature Conservation Service and Western Nature Conservation Service for the readiness of their staff to help, and for the fast and friendly issuing of collection permits. Dr Steve Johnson (School of Botany and Zoology, University of KwaZulu-Natal, South Africa) for his kind support. All hospitable farmers in Namaqualand and Bushmanland, who gave permission to work on their land. For the analysis of nectar samples, Dr Gerhard Gottsberger and his staff (Department of Systematic Botany and Ecology, University of Ulm, Germany). Steven Hammer, Tom Jacobs, Matthew Opel, Terry Smale and Phillipp Desmet for their kind support and fruitful discussions about Conos. Two referees, Steven Hammer and Ben-Erik Van Wyk, helped to improve the manuscript. This study has been financially supported by the Deutsche Forschungsgemeinschaft (DFG). Grant No. JU 418/3-1.

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Edited by JC Manning