

Glandular Hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): Morphology, Histochemistry and Composition of Essential Oil

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Received: 3 July 2001 Returned for revision: 14 September 2001 Accepted: 13 December 2001

Long-stalked glandular hairs of outer and inner involucre bracts of *Sigesbeckia jorullensis*, which are important for epizoid fruit propagation, were investigated using light and scanning electron microscopy. The essential oil secreted by the hairs was analysed by chromatographic methods including gas chromatography/mass spectrometry and with a laser microprobe mass analyser. The glandular hairs consisted of a large multicellular stalk and a multicellular secreting head. The apical layer of glandular head cells was characterized by leucoplasts and calcium oxalate crystals. Below the apical cells there were up to six layers of cells containing many chloroplasts around the nucleus and surrounded by vacuoles filled with flavonoids and tannins. The essential oil originating in the head cells was secreted into the subcuticular space and may be liberated by rupture of the cuticle. It was mainly composed of sesqui- and diterpenes, with the sesquiterpene hydrocarbon germacrene-D as the main component. Monoterpenes, *n*-alkanes and their derivatives as well as flavonoid aglycones were also detected. The stickiness of the essential oil is probably associated with the high content of oxygenated sesqui- and diterpenes. In addition to long-stalked trichomes, small biserial trichomes occurred, secreting small quantities of essential oil into a subcuticular space.

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Key words: *Sigesbeckia jorullensis* Kunth, Asteraceae, glandular hairs, fruit propagation, histochemistry, morphology, essential oil, flavonoids, LM, SEM, GC-MS.

INTRODUCTION

The Asteraceae (Compositae) are the largest family of plants, comprising approx. 23 000 known species (Bremer, 1994), some of which, e.g. *Bidens* and *Arctium* species, disperse their fruits by epizoochory, which is commonly accomplished by means of hooked hairs or other projecting organs. Instead of hooked hairs, *Sigesbeckia* and *Carpesium* species produce sticky substances by which their fruits adhere to animals. This method of epizoid fruit dispersal is unusual within the Asteraceae (Wagenitz, 1979).

Plants of the genus *Sigesbeckia* are annual herbs of warm areas throughout the world, and several species are cultivated as medicinal plants. *Sigesbeckia pubescens* is used in Japan as a drug against arthritis. Extracts of its aerial parts contain diterpenes, mostly from the kauran derivative series (Cameron *et al.*, 1969; Murakami *et al.*, 1973; Kim *et al.*, 1979; Liu and Röder, 1991) and other terpenoids, especially sesquiterpene lactones, have also been reported (Baruah *et al.*, 1979, 1980; Zdero *et al.*, 1991). The bitter taste of *S. orientalis*, which is used as a panacea in Madagascar, is due to darutoside (C₂₆H₄₄O₈), a substance that can be split into the diterpene darutigenol and glucose by emulsin and elaterase. *Sigesbeckia orientalis* and *S. jorullensis* are not indigenous to Europe, but both species are naturalized (Tutin *et al.*, 1976; Wagenitz, 1979). *Sigesbeckia jorullensis*, an annual herb growing up to 1.5 m tall and originating

from subtropical America, grows in Europe in the undergrowth of bushes, in gardens and on banks. Its outer and inner involucre bracts are covered with glandular hairs whose sticky excretion is important for epizoid fruit dispersal and propagation (Wagenitz, 1979). This paper deals with the morphology, anatomy, histochemistry and biochemistry of the glandular hairs of *S. jorullensis*.

MATERIALS AND METHODS

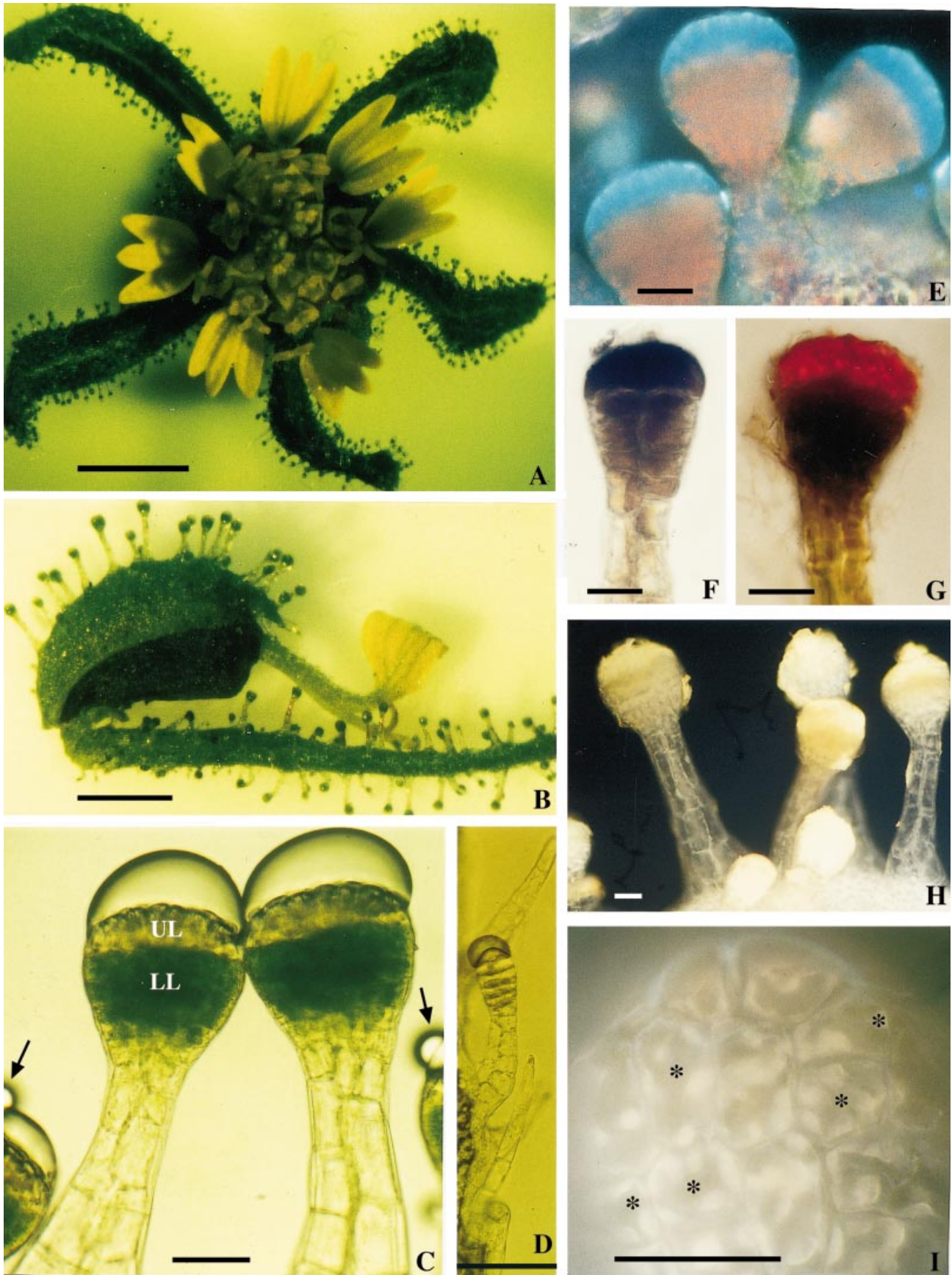
Plant material

Plants of *Sigesbeckia jorullensis* Kunth in Humb., Bonpl. and Kunth (*S. cordifolia* Kunth), grown from seeds collected in the north-east of Hamburg (Germany), were cultivated in the Botanical Garden of the University of Graz, Austria. Involucre bracts, sampled before (June/July), during (August) and after (September/October) flowering were investigated.

Light microscopy (LM) and histochemistry

For anatomical and histochemical observations, 2–5 mm long involucre bracts were used. Lipids were localized by Sudan black B, Sudan III and Nile blue A (Cain, 1947). Tannins were detected using ferric chloride and the nitroso reaction (Reeve, 1951; Stafford *et al.*, 1987). For the detection of flavonoids, 5 % aqueous AlCl₃ solution and 0.05 % Naturstoffreagenz A (β-aminodiethylester of diphenylboric acid) dissolved in 10 % methanol were used.

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Naturstoffreagenz A, a well-known reagent used in thin layer chromatography to visualize flavonoid compounds (Wollenweber, 1982), reacts with hydroxyl groups causing a yellow-green fluorescence, depending on the structural features of the flavonoid. Tissues were cleared using chloral hydrate or sodium hypochlorite, and observations were made using a Zeiss Axioplan epifluorescence microscope equipped with the following filter systems: exciter filter 365, dichroic mirror 395 and barrier filter 420. Fujicolor Superia 200 film was used for colour prints and Ilford FP 4, 125, for black and white prints.

Scanning electron microscopy (SEM)

For SEM, bracts were fixed with 2.5 % glutaraldehyde in 50 mM cacodylate buffer at pH 7 for 3 h, dehydrated in a graded series of acetone solutions and critical point dried with solvent-substituted liquid carbon dioxide. The dried bracts were coated with a thin layer of gold using an AGAR Sputter and examined in a Philips SEM XL 30 ESEM. An SE-detector (Everhart-Thornley, Philips Electron Optics, Eindhoven, The Netherlands) was used in the high vacuum mode for the investigation of fixed and dried samples, and a GSED-detector (gaseous secondary electrons) in the ESEM-mode (cooling stage 5 °C, 3–5 torr chamber pressure) was used to observe fresh samples without preparation.

Extraction of essential oils and gas chromatography (GC)

Samples of fresh inflorescences (30 g) of *S. jorullensis*, randomly collected during flowering, were placed in a round-bottom flask containing 500 ml deionized water. The samples were then steam distilled for 4 h in a Karlsruhe-type apparatus (Stahl, 1953), which is similar to a Clevenger-type circulatory steam distillation apparatus. The essential oil was trapped in 1 ml analytical grade *n*-pentane and analysed by GC and by GC-mass spectrometry (GC-MS).

GC was performed using a DANI 8400 Capillary Gas Chromatograph (DANI, Monza, Italy) equipped with a Programmed Temperature Vaporiser (PTV) injection system, a flame ionization detector (FID) and a LDC/Milton Roy CI-10 B integrator (LDC/Milton Roy, Riviera Beach, FL, USA). The samples were analysed on a fused silica 50 m CP-SIL 5 CB (dimethylpolysiloxane) capillary column (Chrompack, Middelburg, The Netherlands) with an internal diameter of 0.25 mm and a film thickness of 0.12 µm.

The hydrogen carrier gas had an average linear velocity of 40 cm s⁻¹. Oven temperature programming was 40 °C during injection and for an additional 3 min, and was then increased from 40 to 300 °C at the rate of 4 °C min⁻¹. The temperature of the PTV was 40 °C during injection, followed by very rapid heating (approx. 900 °C min⁻¹) to 250 °C. One microlitre of each sample was injected in the split mode (1 : 30). The detector temperature was 300 °C.

GC-MS was performed on a Hewlett Packard G1800A GCD system (Electron impact voltage, 70 eV; injector temperature, 280 °C; detector temperature, 320 °C; foreline pressure, 4 Pa; mass range, 30–425 amu). Samples were analysed on a DB-1 (50 m × 0.20 mm i.d.; film thickness 0.33 µm) capillary column. The helium carrier gas had a delivery rate of 1 ml min⁻¹ and the column temperature programming was as follows: 40 °C initial temperature maintained for 5 min, then the temperature was increased from 40 to 300 °C at a rate of 4 °C min⁻¹.

Compounds were identified using both chromatographic and mass spectroscopic criteria. The WILEY275 database was used for automatic identification of GC-MS peaks; and linear retention indices (van den Dool and Kratz, 1963), obtained on a polar and an apolar column, were compared with published data (Davies, 1990). Whenever possible, mass spectra and retention indices were also compared with data obtained from authentic compounds (Fluka Chemie GmbH, Buchs, Switzerland). Quantitative results were achieved from GC-FID profiles on a non-polar column using the area per cent method without consideration of calibration factors (i.e. F = 1.0) for all compounds.

Analysis of flavonoid aglycones

A sample of fresh inflorescences (28 g) of flowering *S. jorullensis* was dipped for 1–2 s in analytical grade chloroform. After evaporation of the solvent, 0.42 g of a dark green viscous material was obtained (yield 1.5 %). The material was dissolved in warm (50–60 °C) methanol, stored overnight at –25 °C, and wax components were removed by filtration. Five microlitres of the remaining extract was analysed by thin layer chromatography (TLC) according to Wollenweber (1982). The conditions were: stationary phase polyamide 11 F₂₅₄ (Merck), and the mobile phase toluene/ethyl methyl ketone/methanol, 12/5/3 (v/v/v). The chromatogram was sprayed with 0.5 % methanolic Naturstoffreagenz A.

FIG. 1. LM micrographs of capitula and long-stalked hairs of *Sigesbeckia jorullensis*. Bars = 1 mm (A and B), 50 µm (C–I). A, The pauciflorous capitulum consists of outer female florets with short ligules, inner hermaphrodite florets without a pappus, and five outer involucre bracts, up to 2 cm long, possessing long-stalked glandular hairs. B, Fruit dispersion unit consisting of a female floret that has been torn out of the receptacle together with the outer and inner involucre bracts. C, Long-stalked hairs showing a subcuticular space filled mainly with essential oil, yellow coloured cells of the upper layer (UL), green cells of the lower layers (LL) and a long stalk. Lipophilic droplets (arrows) are visible on the cuticle of the subcuticular space. D, Long-stalked hair with only one row of head cells. E–I, Long-stalked hairs with damaged cuticle. E, Hairs under UV light (365/395/420). Lower layers of the head and stalk cells show red autofluorescence of chloroplasts. Even in young hairs the apical layer did not contain chlorophyll, and the bright blue autofluorescence indicates lipophilic material. F, Upper head cells stained more intensely with Sudan black B than lower ones. G, Head cells stained with Sudan III. H and I, Reaction with Naturstoffreagenz A. H, Yellow secondary fluorescence indicates the presence of flavonoids in the head cells, and of flavonoid aglycones in the secretion products on the surface of head and stalk cells. I, Higher magnification indicates the presence of flavonoids in vacuoles, whereas the inner part of the cells (asterisks), where the nucleus is surrounded by plastids, is free of flavonoids, as indicated by the lack of secondary fluorescence.

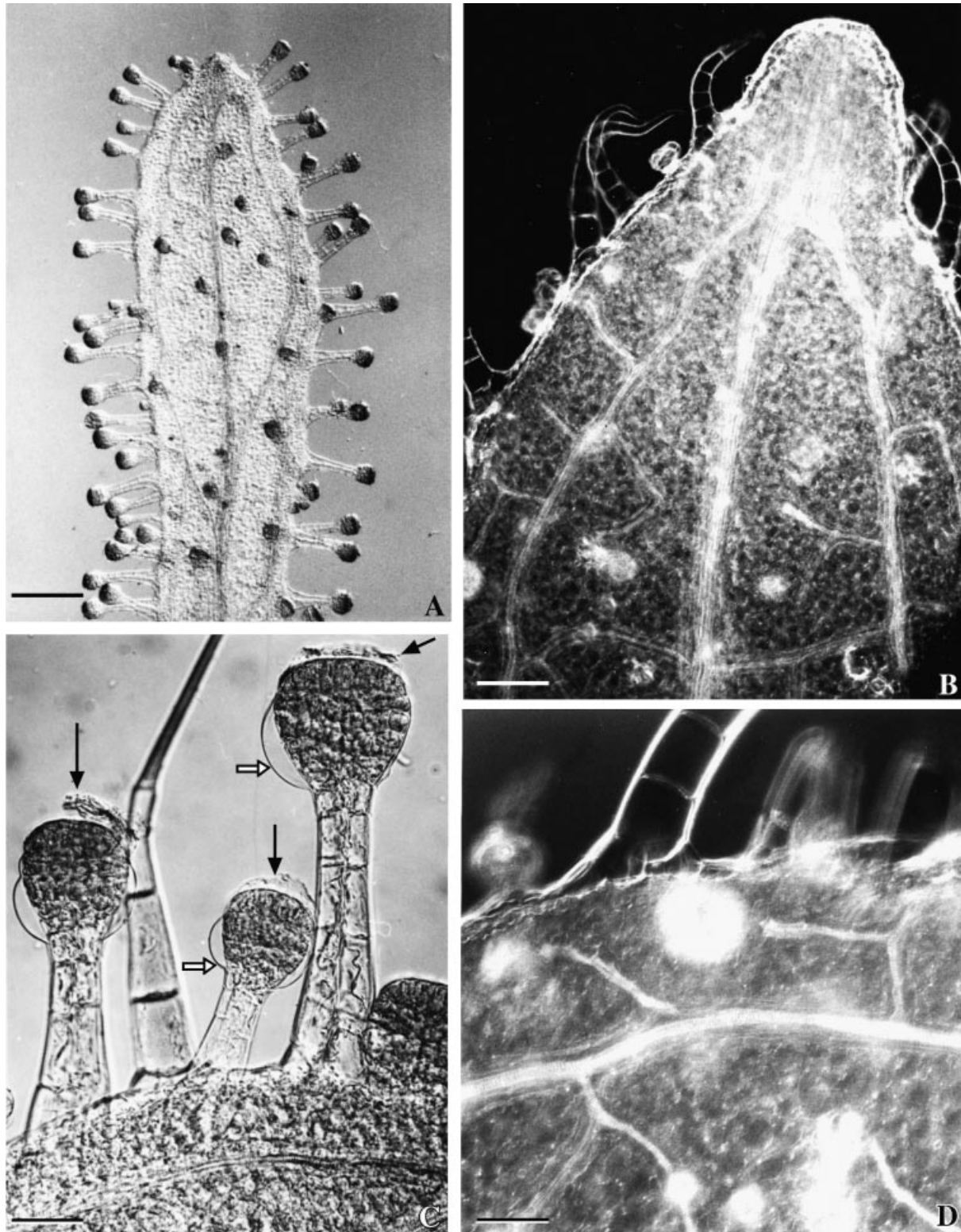


FIG. 2. Vascularization of the outer bracts and long-stalked glandular hairs of *Sigesbeckia jorullensis*. A, Outer involucre bract with a large median vein, two lateral veins, smaller veins, and many long-stalked glands. Bar = 1 mm. B, The three veins fuse to an epithem hydathode. Bar = 200 μ m. C, Hairs treated with glycerol-ethanol and chloral hydrate show the remnants of the cuticle forming the subcuticular space (black arrows) and the girdle between the upper and lower layers of cells, where the cuticle of the lower parts of the head (white arrows) is fixed to the cell wall. Bar = 100 μ m. D, Small veins, mainly composed of tracheids, lead to the base of gland cells. Bar = 100 μ m.

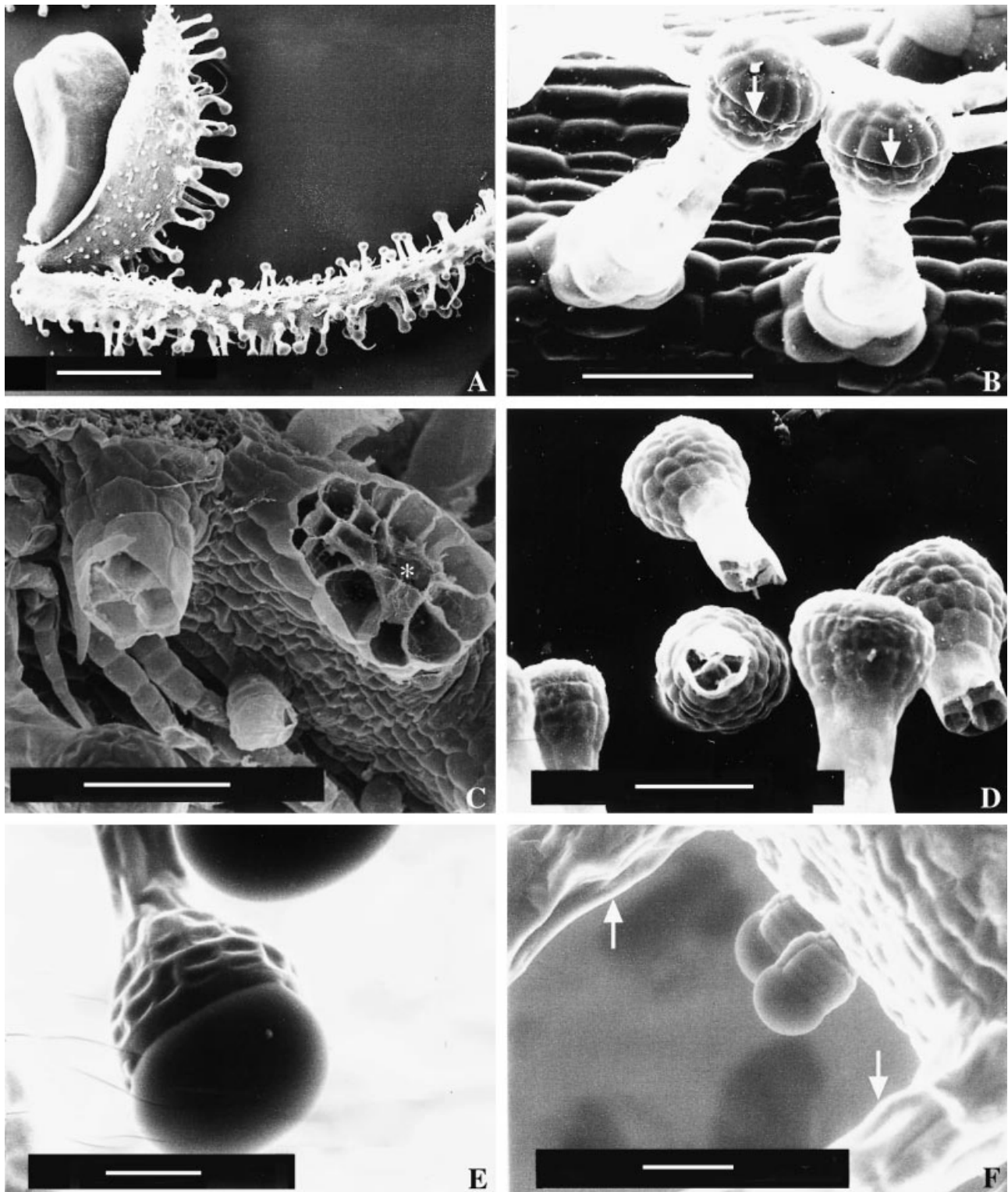


FIG. 3. SEM micrographs of *Sigesbeckia jorullensis*. A, Fruit dispersion unit consisting of an achene that had been torn out of the receptacle together with the outer and inner involucral bracts. Bar = 1 mm, magnification 22 \times . B, Young long-stalked glandular hairs showing the line (arrows) where the cuticle is detached from the wall. Bar = 100 μ m, magnification 359 \times . C, Broken hairs. The base of the hair on the right-hand side (asterisk) shows 16 cells, whereas the stalk of the hair on the left-hand side is composed of four cells. Bar = 100 μ m, magnification 249 \times . D, Broken hairs showing four to six stalk cells. Bar = 100 μ m, magnification 307 \times . E and F, ESEM-mode. E, Long-stalked hairs without preparation, showing the filled subcuticular space. Bar = 50 μ m, magnification 397 \times . F, Two small ten-celled hairs and stalks of long hairs (arrows). Bar = 50 μ m, magnification 376 \times .

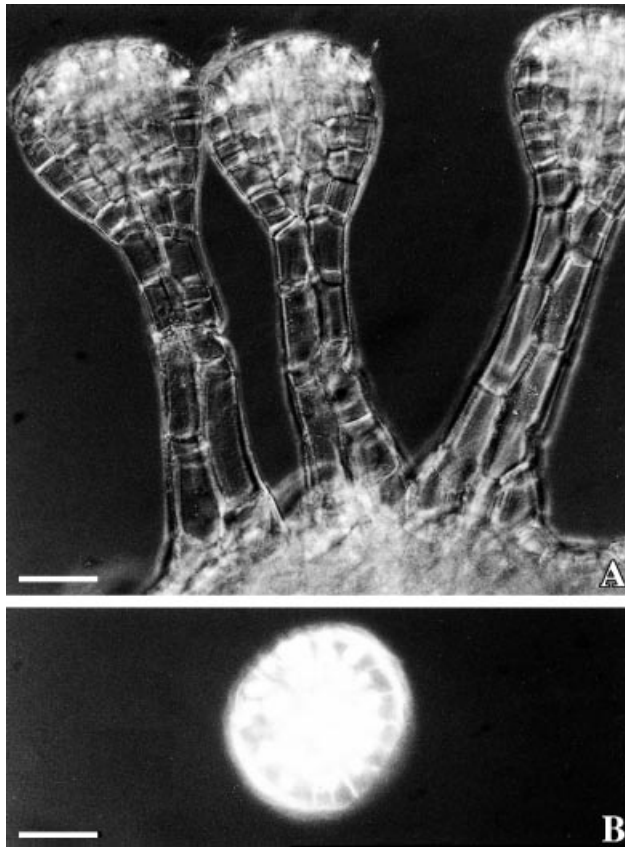


FIG. 4. LM micrographs of cleared tissue under polarized light show calcium oxalate crystals in the apical layer of the head cells of *Sigesbeckia jorullensis*. Bars = 50 μ m.

LAMMA technique

The secretion of the glandular hairs of three outer involucre bracts (covered with a plastic bag against dust) was collected on a copper grid for electron microscopy. Thirty droplets were analysed by means of a laser microprobe mass analyser (Lamma 500; Leybold-Heraeus, Cologne, Germany). The LAMMA instrument combines a light microscope for observation and exact localization of the sample as well as for focusing the beam of a pulsed laser into the specimen, and an MS (Heinen *et al.*, 1981; Heinrich, 1990). A minute volume of the sample is ionized by the high intensity laser. The resulting ions are analysed by time of flight MS, recording the complete mass spectrum of each laser shot.

RESULTS

Capitulum and involucre bracts

The capitula were small, in lax panicles or, rarely, solitary. The pauciflorous capitulum possessed five outer involucre bracts, which were much longer than the inner bracts and densely covered with long-stalked gland hairs, smaller glandular hairs and a few non-glandular hairs. The outer florets with short ligules were female, the inner tubular florets (without pappus) were hermaphroditic and subtended by receptacular scales (Fig. 1A). The outer involucre bracts

adhere to animals or humans due to the sticky material secreted by long-stalked hairs, causing the inner bracts and achenes to be torn out of the capitulum. The fruit dispersion unit is shown in Figs 1B and 3A. The outer involucre bracts were linear to linear-spathulate and possessed a large median vein and two lateral veins linked to smaller veins (Fig. 2A and B). The three main veins fused at the leaf tip leading to an epithem hydathode (Fig. 2B). Small veins, composed mainly of tracheids, terminated near the base of gland cells (Fig. 2D). The epidermis cells of outer involucre bracts contained flavonoids. The mesophyll of outer involucre bracts, composed of loose spongy parenchyma, contained numerous chloroplasts with large starch grains; after flowering the starch content diminished.

Long-stalked glandular hairs

Sigesbeckia jorullensis has two types of glandular hairs. The outer and inner involucre bracts were densely covered with long-stalked glandular hairs with multicellular heads (Figs 1, 2A, C and 3A, B, D, E). Most, but not all, of these hairs were established at an early stage of leaf differentiation, such that hairs of different developmental stages could be found on young involucre bracts. Some hairs were almost completely differentiated whereas others had passed through only a few cell divisions. Comparing a younger and an older capitulum of the same plant, a 2-mm long outer involucre bract had 40 ± 6 long-stalked hairs, whereas a 5 mm bract had 80 ± 15 (data are means \pm s.d. of five involucre bracts each). Besides well developed long-stalked hairs, some apparently mature hairs with fewer cells could be found (Fig. 1D).

Stalk

The stalk of fully developed glandular hairs consisted of 16–40 cells supporting the head of the gland. The cross-section of the base of the stalk sometimes showed more than 16 cells (Fig. 3C). It was not clear whether the two central cells of the basal region of the stalk derived from the subepidermal layer or not. The upper part of the stalk was composed of one (seldom) or four to six radially arranged cells (bi- to triseriate, Fig. 3D). Even in mature gland cells, the chloroplasts of stalk cells contained starch grains (not shown).

Head cells

Typically, a long-stalked hair in an early stage of development had one basal cell, two to four stalk cells and a head, initially composed of two cells, of which the apical head cell divided first. The head of a mature long-stalked glandular hair was $125 \pm 35 \mu$ m long, $117 \pm 30 \mu$ m (mean \pm s.d.; $n = 50$) in diameter and consisted of 60–120 cells arranged in two zones. The apical cell layer consisted of up to 24 cells, which differed fundamentally from the cells of the four to six sub-apical layers. The cells of the upper layer were yellowish, whereas those of the lower layers were green (Fig. 1C). Secretion started when the hairs were fully developed, and the product accumulated in the

TABLE 1. *Flavonoid aglycones in the secretion of Sigesbeckia jorullensis separated by TLC*

Substance zone	R _F -value	Colour after spraying with Naturstoffreagenz A	Abundance
1	0.88	Yellow	+++
2	0.74		Trace
3	0.62		Trace
4	0.49	Orange	++
5	0.40	Yellow	++
6	0.21		Trace
7	0.12	Orange	+++

subcuticular space of the glandular head formed by detachment of the cuticle from the walls of the apical cells (Fig. 1C). Small droplets of secondary compounds could be observed on the intact cuticle of the subcuticular space (Fig. 1C). In older hairs, or in young hairs treated with reagents, the cuticle was ruptured above the base of the apical cells releasing a lipophilic material. This attachment of the cuticle could be demonstrated in cleared hairs, prepared by incubation for 2 months in glycerol-ethanol, followed by one night in chloral hydrate. The cuticle of the subcuticular chamber was visible as a remnant attached to the apex of the hair, whereas the cuticle of the lower part of the hair was firmly fixed at the border between the upper tier and the chlorophyll-containing lower head cells (Fig. 2C). SEM micrographs also showed the upper zone of the hair where the cuticle was detached, forming a subcuticular chamber for the secreted material (Fig. 3B and D). The ESEM-mode facilitated observation of unprepared specimens without significant loss of secretion (Fig. 3E). In LM, the secreted material was first hyaline, later yellowish-brown and sticky. Calcium oxalate crystals were found exclusively in the upper layer of head cells (Fig. 4).

When subjected to UV light, the apical head cells revealed an intense blue autofluorescence, whereas abundant chloroplasts fluorescing red (chlorophyll autofluorescence) were seen in the lower layers of the head; only a few chloroplasts were found in stalk cells (Fig. 1E). The material secreted into the subcuticular space contained lipophilic substances as tested with Nile blue (not shown), Sudan black B (Fig. 1F) and Sudan III (Fig. 1G). The apical cells stained differently to the cells of the lower layers with Sudan black and Sudan III (Fig. 1F and G).

The fluorochromes for flavonoid detection, aluminium trichloride and Naturstoffreagenz A induced a yellow or green secondary fluorescence in head cells and secreted exudate (Fig. 1H and I). The central part of most cells did not fluoresce because nuclei surrounded by plastids occupy this space, as can be observed under high magnification (Fig. 1I). In addition, head cells containing chloroplasts stained positively for tannins using ferric chloride and the nitroso reaction (not shown).

Short-stalked glandular trichomes

Beside long-stalked multicellular hairs, smaller glandular trichomes occurred on the inner involucre bracts (Fig. 3A)

and on the outer ones amongst long-stalked hairs (Fig. 3F); they produced secondary compounds. Comparing the abundance of the two glandular trichome types on three inner and three outer involucre bracts, the ratio between long- and short-stalked hairs was 1 : 1.45 on outer involucre bracts and 1 : 0.16 on inner involucre bracts. The fully developed biseriate, short-stalked glandular trichome consisted in most cases of ten cells: two basal cells, two stalk cells and six cells forming the glandular head (Fig. 3F). The head cells contained calcium oxalate crystals. The head cells and the material secreted into the subcuticular space stained positively for lipids.

Nature of the secreted material

The secreted material was soluble in methanol (30 s), 2-propanol, ethyl acetate, chloroform, and only partly soluble in *n*-hexane. TLC on polyamide separated four main and three minor bands, which turned yellow or orange after spraying with Naturstoffreagenz A (Table 1).

Essential oil composition

The essential oil of *S. jorullensis* was viscous and bright yellow, and its qualitative and quantitative composition did not vary during the vegetative period. One hundred and four volatile components were detected, of which 52 (54.9 %, w/w) could be unambiguously identified (Table 2). A further 33 compounds representing 43.4 % of the sample were partially characterized on the basis of their mass spectral data and their retention indices. Fifty compounds, representing 73.7 % (w/w) of the essential oil, were characterized as terpenes. Main constituents of the 27 sesquiterpenes (35.4 %) were the hydrocarbons germacrene-D (13.6 %), α -farnesene (4.9 %), β -caryophyllene (4.1 %) and *trans*- β -farnesene (4.0 %). Almost all of the 19 diterpenes (together 38.1 %) were oxygenated compounds. The predominant components were epimanoil oxide (7.4 %) and diterpenes with retention indices of 2480 (12.4 %) and 2031 (8.6 %). Only four monoterpenes (together 0.2 %) were detected. Additional substance classes in the essential oil included *n*-alkanes (0.8 %), alkanals (0.5 %), ketones (0.2 %), alcohols (0.2 %), fatty acids (6.6 %) and esters thereof (5.1 %).

Cations in the lipophilic material

The mean of 30 LAMMA spectra of the lipophilic material of glands located in the outer involucre bracts demonstrated calcium to be the dominant cation (Fig. 5). The second most abundant ion was potassium, followed by sodium, magnesium, manganese and iron.

DISCUSSION

Species of *Sigesbeckia* and *Carpesium* are the only members of the Asteraceae known to produce adhesive exudates which assist in epizoic fruit propagation, although mucilaginous glandular trichomes on seeds are found in various plant families (Werker, 2000). In contrast to such trichomes

TABLE 2. Constituents of the essential oil of *Sigesbeckia jorullensis*

Components	Retention index	%
Monoterpenes		
Sabinene	970	tr
Myrcene	991	0-1
Limonene	1026	tr
Thymyl methyl ether	1234	0-1
Sesquiterpenes		
Geijerene (= geyrene)	1139	0-1
Bicycloelemene	1336	0-3
α -Copaene	1375	0-6
Sesquiterpene hydrocarbon (<i>m/z</i> : 161, 105, 91, 120, 119, 81, 93, . . . 204)	1388	0-1
β -Elemene	1391	0-2
β -Caryophyllene	1418	4-1
β -Cubebene	1428	0-4
<i>trans</i> - α -Bergamotene	1435	0-6
Sesquiterpene hydrocarbon (<i>m/z</i> : 161, 91, 105, 79, 93, 81, 41, . . . 204)	1443	0-1
α -Humulene	1452	1-2
<i>trans</i> - β -Farnesene	1458	4-0
Sesquiterpene hydrocarbon (<i>m/z</i> : 161, 105, 119, 93, 91, 79, 81, . . . 204)	1477	0-1
Germacrene-D	1482	13-6
Sesquiterpene hydrocarbon (<i>m/z</i> : 69, 93, 41, 161, 133, 79, 55, . . . 204)	1485	0-5
Bicyclogermacrene	1496	1-4
α -Muurolene	1499	0-1
α -Farnesene	1509	4-9
γ -Cadinene	1514	0-3
δ -Cadinene	1523	0-7
1- <i>endo</i> -Bourbonanol	1574	0-1
Oxygenated sesquiterpene (<i>m/z</i> : 117, 43, 99, 70, 83, 85, . . . 187)	1582	0-4
Oxygenated sesquiterpene (<i>m/z</i> : 93, 43, 79, 80, 121, 91, . . . 187)	1620	0-1
Oxygenated sesquiterpene (<i>m/z</i> : 95, 121, 43, 109, 71, 79, 119, . . . 204)	1642	0-4
Oxygenated sesquiterpene (<i>m/z</i> : 161, 119, 121, 105, 95, 79, 43, . . . 204)	1646	0-1
<i>epi</i> - α -Muurolol (= T-Muurolol)	1654	0-6
Oxygenated sesquiterpene (<i>m/z</i> : 67, 81, 79, 41, 95, 55, . . . 189)	1662	0-2
Oxygenated sesquiterpene (<i>m/z</i> : 79, 67, 80, 108, 93, 41, . . . 203)	1667	0-5
Diterpenes		
8,13-Epoxy-15,16-dinorlab-12-ene	1881	0-4
(<i>E,E</i>)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	1918	0-1
Epimanoyl oxide	2014	7-4
Oxygenated diterpene (<i>m/z</i> : 69, 81, 93, 41, 107, 95, . . . 290)	2031	8-6
Oxygenated diterpene (<i>m/z</i> : 91, 117, 145, 105, 131, 79, . . . 275)	2039	0-3
Oxygenated diterpene (<i>m/z</i> : 128, 115, 129, 91, 143, 77, . . . 275)	2054	0-1
Phytol	2112	0-2
Oxygenated diterpene (<i>m/z</i> : 120, 43, 93, 138, 121, 81, . . . 275)	2139	0-1
Oxygenated diterpene (<i>m/z</i> : 69, 81, 41, 93, 95, 67, 107, . . . 306)	2169	0-4
Oxygenated diterpene (<i>m/z</i> : 245, 43, 137, 81, 95, 69, . . . 279)	2177	0-2
Oxygenated diterpene (<i>m/z</i> : 69, 109, 41, 81, 137, 43, . . . 276)	2185	0-1
Oxygenated diterpene (<i>m/z</i> : 245, 43, 137, 81, 69, 95, . . . 291)	2227	0-8
Oxygenated diterpene (<i>m/z</i> : 245, 43, 137, 81, 69, 95, . . . 291)	2242	0-8
Oxygenated diterpene (<i>m/z</i> : 91, 81, 79, 105, 93, 123, . . . 286)	2258	0-2
Oxygenated diterpene (<i>m/z</i> : 257, 55, 137, 81, 69, 95, . . . 291)	2264	1-4
Oxygenated diterpene (<i>m/z</i> : 177, 81, 43, 95, 291, 55, . . . 291)	2318	0-1
Oxygenated diterpene (<i>m/z</i> : 245, 137, 43, 81, 69, 95, . . . 309)	2480	12-4
Oxygenated diterpene (<i>m/z</i> : 245, 43, 137, 81, 69, 95, . . . 309)	2501	3-1
Oxygenated diterpene (<i>m/z</i> : 69, 81, 41, 93, 71, 95, 43, . . . 290)	2406	1-6
Alkanes		
Heptacosane	2699	0-6
Nonacosane	2899	0-3
Aldehydes, ketones		
<i>trans</i> -2-Hexenal	846	0-1
2-Heptanone	890	0-2
<i>n</i> -Octanal	1003	0-1
<i>n</i> -Nonanal	1103	0-1
<i>n</i> -Decanal	1205	0-1
<i>n</i> -Dodecanal	1408	0-1
Alcohols		
<i>n</i> -Octanol	1073	tr
1-Dodecanol	1474	0-1
1-Tetradecanol	1675	0-1

TABLE 2. Continued

Components	Retention index	%
Acids		
Octanoic acid	1190	4.9
Nonanoic acid	1276	0.7
Decanoic acid	1370	0.8
Dodecanoic acid	1563	0.1
Hexadecanoic acid	1962	0.1
Esters		
Hexanoic acid 2-methylpropyl ester	1152	0.3
3-Methylbutyl hexanoate	1253	0.3
2-Methylpropyl octanoate	1348	0.1
Hexanoic acid hexyl ester	1386	0.3
Docosanoic acid methyl ester	2531	1.0
Docosanoic acid ethyl ester	2596	0.5
Tricosanoic acid methyl ester	2631	0.1
Tetracosanoic acid, methyl ester	2732	2.0
Tetracosanoic acid, ethyl ester	2797	0.6
Pentadecanoic acid, methyl ester	2832	tr
Hexacosanoic acid, methyl ester	2934	0.1
Unknowns		
<i>m/z</i> : 43, 69, 129, . . . 157	1161	1.2
<i>m/z</i> : 82, 67, 57, 55, 41, 127, . . . 161	1578	0.1
<i>m/z</i> : 127, 57, 128, 41, 55, 69, . . . 213	1703	0.1
<i>m/z</i> : 180, 165, 43, 181, 91, 166, . . . 250	1707	0.2
<i>m/z</i> : 69, 91, 145, 131, 41, 117, . . . 230	1916	0.1
<i>m/z</i> : 91, 117, 145, 105, 77, 131, . . . 230	1977	9.0
<i>m/z</i> : 91, 123, 119, 125, 77, 41, . . . 257	2036	0.2
<i>m/z</i> : 245, 43, 137, 69, 81, 95, . . . 263	2496	0.3

Retention indices were calculated from gas chromatographic separations on a non-polar stationary phase (column, 50 m CP Sil 5 CB).
tr, trace (< 0.05 %).

Besides the 85 compounds listed above, 19 minor unidentified components (together 1.7 %, w/w) could be detected with GC.

on seeds, the glandular hairs of *Sigesbeckia jorullensis* produce a sticky lipophilic exudate that adheres to animals even without previous wetting. Whereas the achenes of *Carpesium* species bear glands producing the exudate, the achenes of *S. jorullensis* are glabrous and the glands are located in the outer and inner involucre bracts. The diaspores of *Sigesbeckia* consist of involucre bracts and achenes that are torn out of the capitulum together when dispersed.

The long-stalked hairs of *S. jorullensis* are multicellular, bi- to triseriate and are trichomes, being derived mostly from epidermal cells. Hairs similar to the short-stalked trichomes of *S. jorullensis* have been observed in other members of the Asteraceae, e.g. *Artemisia campestris* ssp. *maritima* (Ascensão and Pais, 1987). Some anatomical features of the ten-celled glandular trichomes of *A. campestris* ssp. *maritima* resemble closely those of the long-stalked hairs of *S. jorullensis*, e.g. the arrangement of cells with chloro- and leucoplasts.

Many essential oil-producing glandular tissues are non-photosynthetic (Fahn, 1979). This is the case for the apical row of head cells of *Artemisia campestris* (Ascensão and Pais, 1987) and *S. jorullensis*, which contain only leucoplasts, whereas the lower tiers of cells contain many chloroplasts. The chloroplast-bearing cells of *S. jorullensis* contain tannins and flavonoids, as demonstrated by histochemical tests and, since there was no evidence for the occurrence of terpenoids in these cells, the apical layer

of cells is likely to be the main site of terpenoid production. The photosynthetic cells, comprising the greatest part of the glandular head, might be important for the synthesis of terpene precursors, using photosynthetic products generated locally or delivered from other parts of the plant.

It is noteworthy that small veins only are present close to the stalk of the glandular hairs, and that they are composed mostly of tracheids. The tracheids supply the glands with water and ions, with Ca^{2+} being the dominant cation. Although the secretion of *S. jorullensis* is calcium-rich, as demonstrated by LAMMA, the supply of Ca^{2+} is so great that excess Ca^{2+} has to be stored in one vacuole of each head cell. This is the only location in the entire outer involucre bracts and their trichomes where calcium oxalate crystals can be found. Calcium oxalate crystals have also been identified in lipid-secreting trichomes of *Inula viscosa* (Werker and Fahn, 1981), *Artemisia campestris* ssp. *maritima* (Ascensão and Pais, 1987) and in chlorophyll-free head cells of long-stalked hairs of *Nicotiana tabacum* and *N. sylvestris* (Meyberg *et al.*, 1991).

Terpenoids have many different functions in plants. Some volatile terpenoids may attract pollinating insects to flowers, while others may protect the plant and its reproductive structures from destruction by herbivores and other pathogens (Kelsey *et al.*, 1984; Harborne, 1993). Monoterpenes dominate the essential oils of conifers (Kubeczka and Schultze, 1987), Rutaceae species (Heinrich and Schultze, 1985) and the Lamiaceae (Werker *et al.*, 1985; Lawrence,

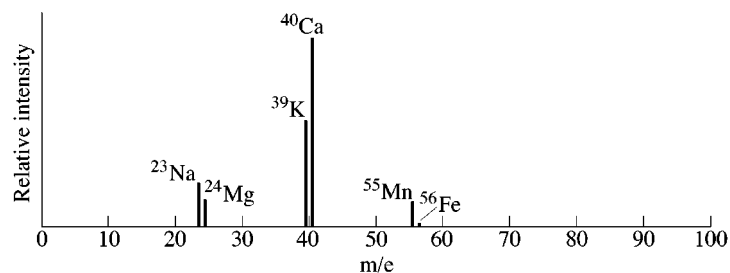


FIG. 5. Mean Lamma spectrum of 30 analyses of the cations found in the secretion of gland hairs from three outer involucre bracts of *Sigesbeckia jorullensis*.

1992); and sesquiterpene lactones, in association with mono- and sesquiterpenes, are characteristic constituents of the essential oils and resins of the Asteraceae (Spring, 2000). In *S. jorullensis*, sesquiterpenes and other terpenes of higher molecular mass are the main components of the sticky oil. In addition to the 27 sesquiterpenes, 18 compounds of the essential oil were found to be oxygenated diterpenes, which probably contribute much of the stickiness.

Because the adhesive strength of the secretion is so high that involucre bracts and achenes are torn out of the capitulum together, one might expect to find small insects trapped by the gland cells of the bracts. However, this was not observed in this study. It is possible that some volatile constituents of the secretion are repellent to insects, as reported for many other terpenoids (Harrewijn *et al.*, 1995). Compounds such as sesquiterpene lactones with antiherbivory properties are common secondary products in members of the Asteraceae, including *Sigesbeckia* species (Baruah *et al.*, 1979, 1980; Zdero *et al.*, 1991) and closely related genera (Quijano *et al.*, 1997; Bardon *et al.*, 2001). In this study, sesquiterpene lactones were not unambiguously detected, although some of the unidentified compounds listed in Table 2 may have been sesquiterpene lactones. However, these compounds are usually isolated from plant material by extraction with organic solvents rather than by steam distillation, and analysed and identified by methods such as LC-MS and LC-NMR which were not applied in this study.

Cheniclet and Carde (1985) proposed that the abundance of leucoplasts correlates with the amount of monoterpenes in the essential oils of various plant species. Although cells of the apical layer of the glandular head contain numerous leucoplasts, the essential oil of *Sigesbeckia* contains only a small proportion of monoterpenes. Thus, the observations by Cheniclet and Carde (1985) cannot be generalized to all essential oil-producing plants. In higher plants, the terpene precursor isopentenyl diphosphate (IPP) is synthesized in two different pathways (Lichtenthaler, 1999; Eisenreich *et al.*, 2001), and the site of the recently established 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway is in plastids, where IPP is subsequently metabolized to isoprene, mono-, di- and tetraterpenes (carotenoids). The classical acetate/mevalonate pathway of IPP synthesis proceeds in the cytosol and generates sesqui-, tri- and

polyterpenes, although partial export of IPP from plastids into the cytoplasm can also occur (Lichtenthaler, 2000). From the present results of light and scanning electron microscopy, it is not possible to identify the mechanism of terpene biosynthesis employed in *Sigesbeckia*.

Many terpenoid-synthesizing plants often produce free flavonoid aglycones, and most of these have been reported from the asteraceae (Wollenweber and Dietz, 1981). Light microscopic observations, the chromatographic behaviour at TLC on polyamide and the light absorption properties of the separated compounds indicate the presence of flavonoid aglycones in the secretion of *S. jorullensis*.

ACKNOWLEDGEMENTS

This paper is dedicated to Professor Dr O. Härtel on the occasion of his 90th birthday. The work was supported by the Jubiläumsfonds of the Österreichische Nationalbank (Project 5324, Heinrich).

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