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Morphological and phytochemical analysis of *Bornmuellera dieckii* *(Brassicaceae)*

Abstract

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Macro- and micromorphological characters of the S. Yugoslav endemic, *Bornmuellera dieckii,* were analysed. The medifixed, tuberculate trichomes occur in two types, being thick-walled and sessile on leaves but slender and stalked on the stem. Trichome morphology is compared with that in allied genera. The dimensions of leaves and siliculae are much more variable than was originally described, and trichomes are scattered over both leaf surfaces, not only abaxially. In a chemical survey of fatty acids from C_{14} to C_{22} (saturated and unsaturated) were found in seeds, and long-chain *n*-alkanes (C_{29} and C_{31}) were identified in the waxes covering stem and leaves.

Introduction

The genus *Bornmuellera* Hausskn. is a Balkan endemic with three species: *B. dieckii* Degen in S. Yugoslavia, *B. baldaccii* (Degen) Heywood in N.W. Greece and C. Albania, and *B. tymphaea* (Hausskn.) Hausskn. in N. Greece (Heywood 1964).

Bornmuellera dieckii is a perennial herb or small shrub that grows on serpentine rocks in a few localities (Malo Borče, Borovi vrh). It is described as having leaves that are oblong-spathulate, glabrous above and with sparse medifixed hairs beneath, a branched inflorescence, white flowers, filaments bearing a tooth-like appendage at the base, and a silicula measuring 4 mm in diameter, ovoid, somewhat compressed (Degen 1900, Heywood 1964, Nikolić 1972).

The biology and chemistry of this species are poorly known. In this paper, the results of a macro- and micromorphological study of this species and of a chemical analysis of some of its compounds are presented.

Material and methods

Samples of *Bornmuellera dieckii* were collected in summer 1992 at Malo Borče (Brezovica, Yugoslavia). One part of the dried material was used for morphological, a second part for chemical analysis. Samples of *Alyssum markgrafii* O. E. Schulz were collected in Brdjani, near G. Milanovac (Yugoslavia). For other species, previously collected herbarium specimens were analysed.

For scanning electron microscopy (SEM), at least three samples of leaves and mature seeds were analysed. The samples were mounted on stubs and coated with c. 30 nm of gold-palladium (85 : 15) in a Jeol JEE 4B vacuum evaporator, then examined under a Jeol JSM T.35 scanning electron microscope.

Total lipids were extracted as described previously (Marin & al. 1989). Gas chromatographic (GC) analysis of methyl esters of fatty acids was performed in a Varian 3400 gas chromatograph with flame ionization detector (FID). H_2 served as the carrier gas, and a Supwax capillary column (60 m long) was used. The temperature of the injector was set at 250°C, that of the detector at 300°C. Peak surfaces were measured by means of an electron integrator Varian DS 604.

Leaf waxes were extracted by petroleum ether. The alkanes were analysed by GC, using a DB-1 capillary column (4 m long). The temperature programme was set at 50-250°C/15°C/min. The identification of components was achieved by co-chromatography with authentic markers.

Results

The variation of the following morphological characters was analysed: length and width of basal leaves, number of trichomes per mm^2 on both adaxial and abaxial leaf surfaces (at the base, middle and tip of the leaves), length of trichomes, length and width of the silicula, length and width of the seed. The length of basal leaves ranged from 10 to 45 mm (x = 21.7 ± 7.1 mm), their width from 1.9 to 8.4 mm (x = 3.9 ± 1.2 mm). Trichomes were scattered over both leaf surfaces, their mean density being lower on the adaxial surface (9 ± 4.7 mm^{-2} at the tip, 7.1 ± 4.5 mm^{-2} in the middle, 4.6 ± 2.8 mm^{-2} at the base; Fig. 1) than on the abaxial surface (18.6 ± 8.3 mm^{-2} at the tip, 16 ± 6.9 mm^{-2} in the middle, 13.4 ± 5.6 mm^{-2} at the base; Fig. 2). The diameter of the medifixed, two-armed trichomes was 0.6 ± 0.09 mm. Silicula width ranged from 2.1 to 4.0 mm (x = 2.99 ± 0.37 mm) and its length from 2.9 to 5.5 mm (x = 4.6 ± 0.5 mm), seed width from 1.4 to 2.1 mm (x = 1.8 ± 0.1 mm), and their length from 2.2 to 3.45 mm (x = 2.7 ± 0.2 mm).

Microcharacters of leaf and stem trichomes, and of seeds, were studied by SEM. Seed sculpturing showed an irregular pattern of undulating ridges (Fig. 3A-B). On vegetative parts two types of trichomes were found: on both leaf surfaces they were medifixed, tuberculate, thick walled, and sessile (Fig. 3C-D), whereas on the stem they were also medifixed and tuberculate, but more slender and borne on short stalks (Fig. 3E-F).

In a chemical survey, the fatty acid composition of total seed lipids and the alkanes from leaves were analysed. The dominant fatty acids in seed oils were palmitic (16 : 0), stearic (18 : 0), and erucic (22 : 1) acid, minor constituents being behenic (22 : 0), arachidic (20 : 0), and myristic (14 : 0) acid, in the following proportion:

palmitic acid	48.29	behenic acid	5.18
stearic acid	24.06	arachidic acid	2.02
erucic acid	19.26	myristic acid	1.19

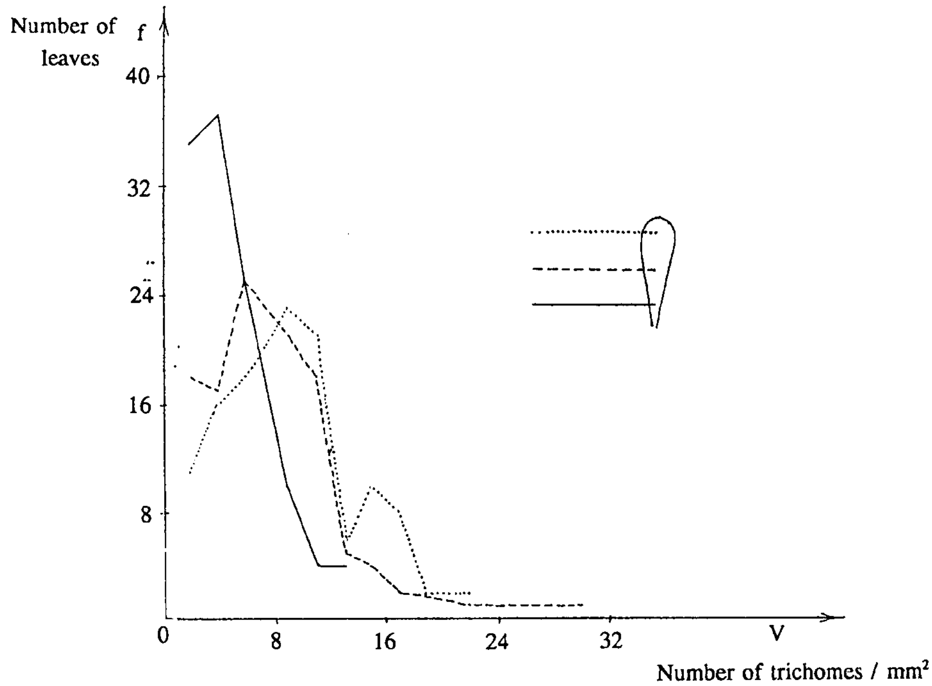

Fig. 1. Trichome numbers on the upper leaf surface in *Bornmuellera dieckii* (tip, middle, base).

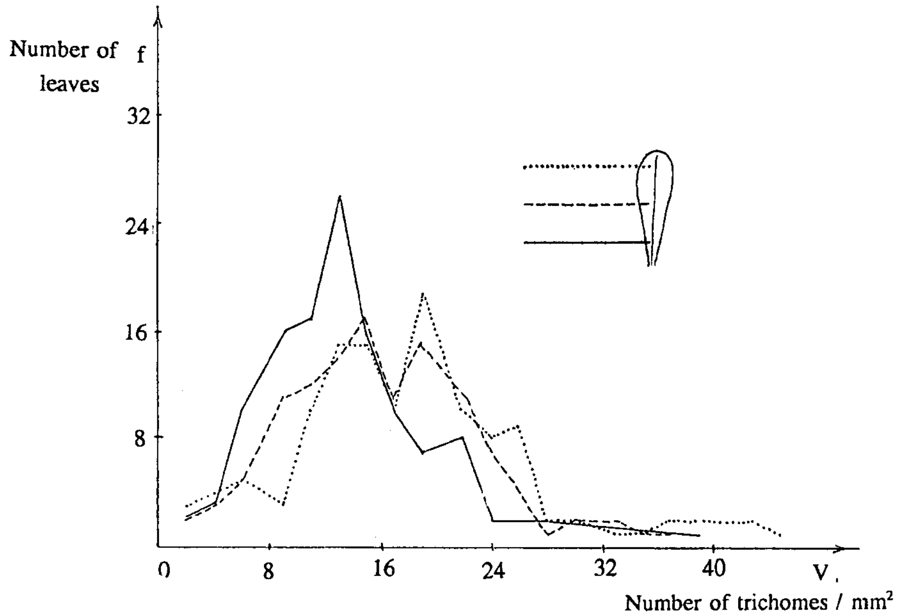

Fig. 2. Trichome numbers on the lower leaf surface in *Bornmuellera dieckii* (tip, middle, base).

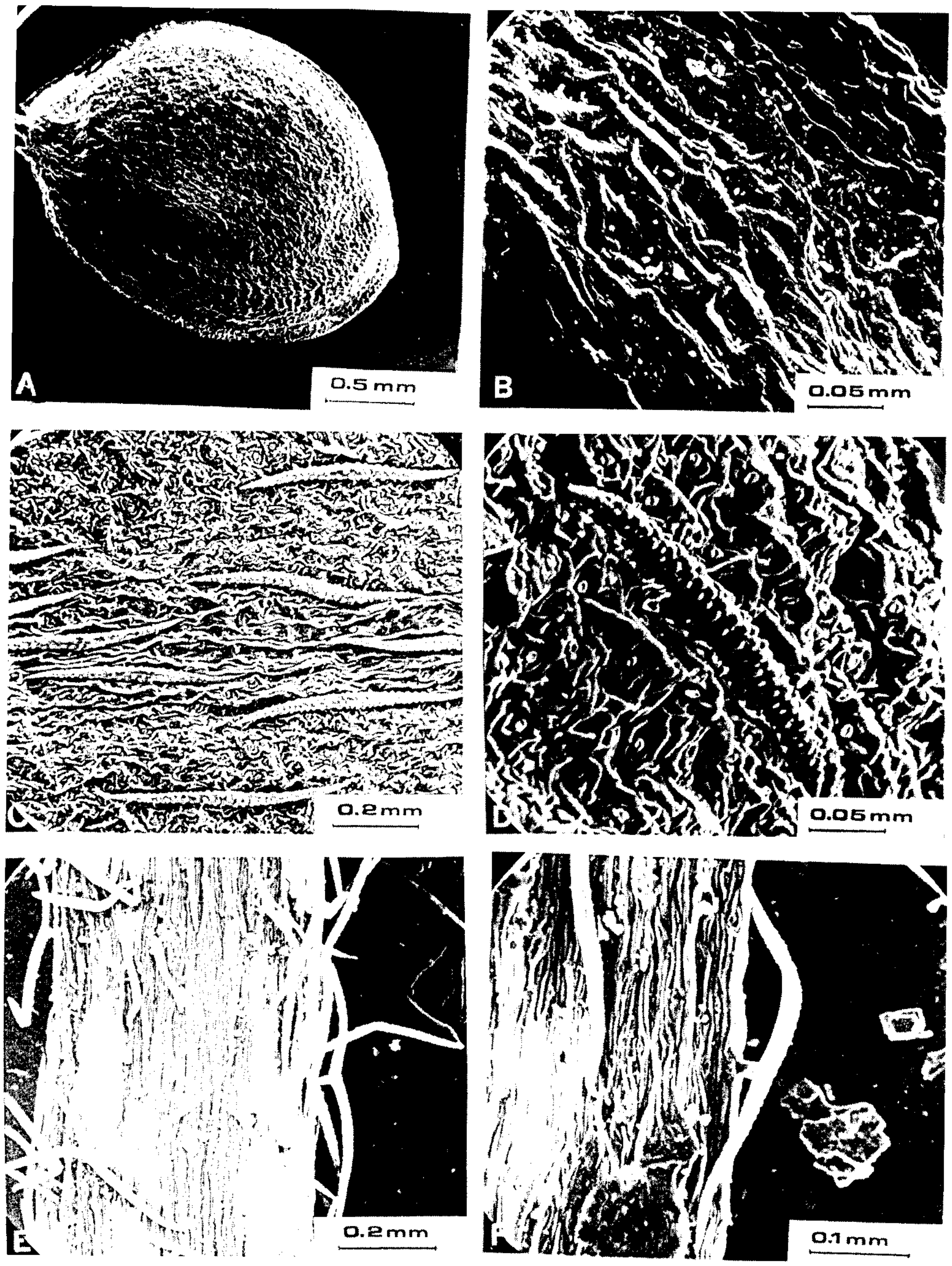

Fig. 3. Scanning electron micrographs of *Bornmuellera dieckii*. – A-B, seed surface; C-D, leaf indumentum of medifixed, sessile, robust trichomes; E-F, indumentum of medifixed, stalked, slender trichomes.

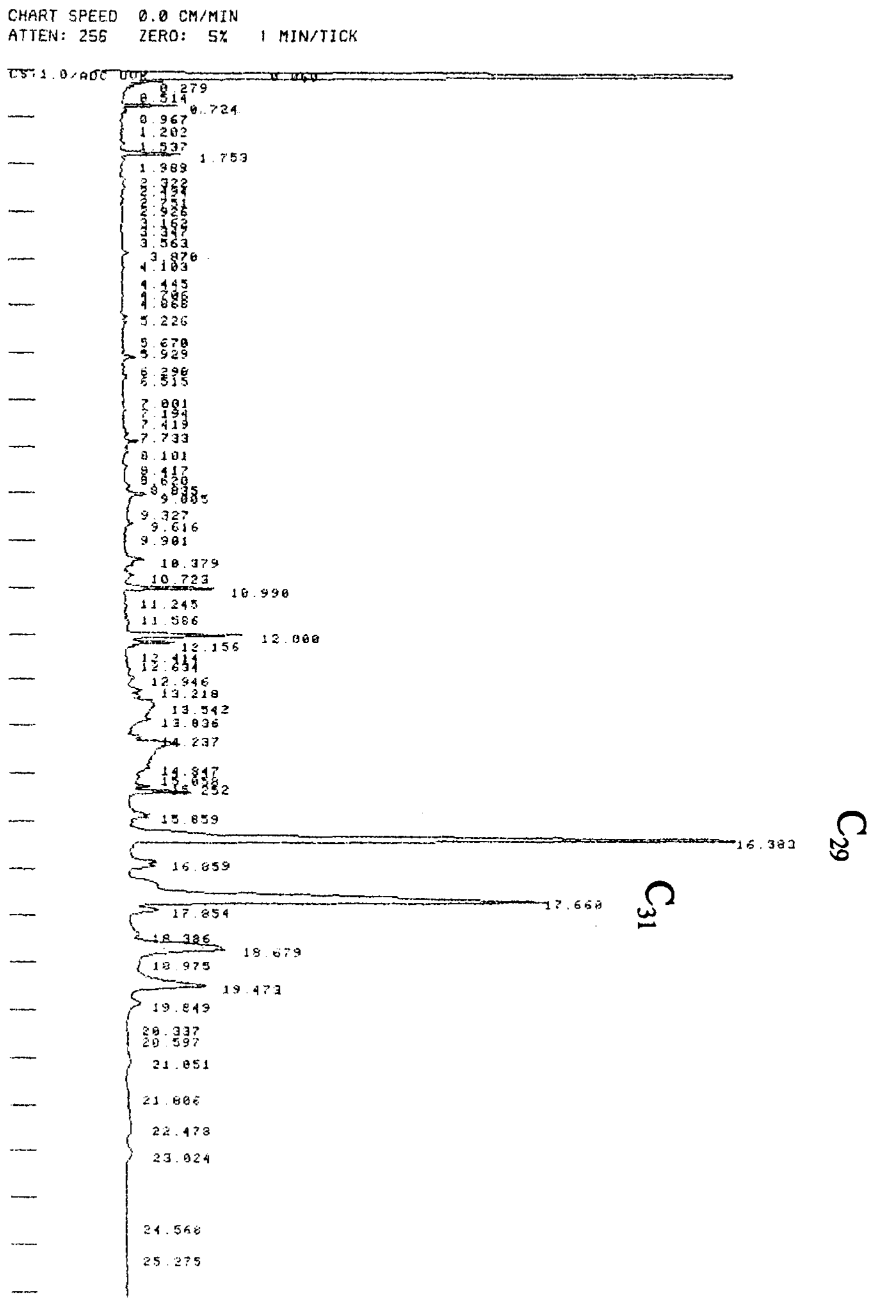

Fig. 4. Gas chromatogram of alkanes from the leaf wax of *Bornmuellera dieckii.*

In the alkane analysis of leaf waxes, two major constituents were identified: *n*-nonacosane ($C_{29}H_{60}$) and *n*-hentriacontane ($C_{31}H_{64}$). The content in C_{29} was 0.84 mg, and of C_{31} 0.79 mg, per gram of dry leaf material (Fig. 4).

Discussion

The variation of width and length of leaves as well as silicula length has a much wider range than was given in Degen's original diagnosis. Leaf length is 1-4.5 cm (2-3 cm according to Degen), width 1.9-8.4 mm (4-7 mm in Degen), silicula length 2.9-5.5 mm (4 mm in Degen).

In contrast to Degen's diagnosis the leaves are not glabrous above, but have trichomes on both surfaces. The average number of trichomes on the abaxial surface was about 16 per mm^2, and about 7 per mm^2 on the adaxial surface.

Two types of medifixed, tuberculate trichomes were found in *Bornmuellera dieckii.* On the leaves they are thick-walled and sessile, while on the stem they are slender and borne on a thin stalk.

The genus *Bornmuellera* is morphologically very similar to *Lobularia* Desv., both genera having medifixed trichomes. However, in *Bornmuellera* the ovary and silicula are completely glabrous and the filaments have a short tooth at the base. In *Lobularia* the ovary and silicula are pubescent (the latter sometimes ± glabrescent) and the filaments lack appendages (Ball 1968).

Bornmuellera differs clearly from all other related genera (*Alyssum* L., *Alyssoides* Mill., *Berteroa* DC., *Ptilotrichum* C. A. Mey.) by the morphology of its trichomes. While *Bornmuellera* trichomes are medifixed, in the other genera they are dendritic or stellate. Results of our analysis of *Alyssum markgrafii* (in preparation) will complete the data from an extensive investigation of this genus by Ančev (1991).

Stellate and dendritic trichomes probably originated from simple or branched trichomes (Ančev 1991). *Bornmuellera dieckii* and the two other *Bornmuellera* species bear simple, medifixed trichomes. *Bornmuellera* and *Lobularia,* two small genera that have the same type of medifixed trichomes, may represent separate lines of evolution.

The indumentum of *Alyssum* species has mainly a protective function, which depends on the density of the trichomes and their position in space. The species with a denser indumentum have thick trichomes in several layers (Ančev 1991). It has been demonstrated that in *Fibigia* Medik., another genus related to *Bornmuellera,* the leaf indumentum of very dense dendritic trichomes plays the role of a light filter and thermal insulator, preventing excessive transpiration (Damjanović & Stevanović 1991). Trichomes may also protect plants from phytophagous insects (Ančev 1991). In contrast to other related genera, in which trichomes are fairly dense, the leaves of *B. dieckii* have but scattered trichomes that cannot be viewed as providing a strong defence against phytophagous insects. One may then ask the question, which kind of defence do the three *Bornmuellera* species have? Is it a chemical constituent, or some other device?

Since the indumentum, which may play an important role for a plant's gas and temperature regimes, is poorly developed in *Bornmuellera dieckii,* we have analysed the alkanes of leaf waxes which are also known for their protective role. Alkanes have water repellent properties and provide a means of controlling the water balance in leaves and stems (Harborne & Turner 1984). We could identify two major constituents in *B. dieckii*, $C_{29}H_{60}$ (*n*-nonacosane) and $C_{31}H_{64}$ (*n*-hentriacontane), both of which are common in flowering plants. The alkane composition in the related genus *Alyssum (A. markgrafii)* was found to be similar: again, C_{29} and C_{31} were the major components; the content in C_{29} was 0.73 mg per gram of dry weight (about the same as in *B. dieckii*), while the C_{31} content was 0.43 mg/g (significantly less than in *B. dieckii*).

Our analysis of the fatty acid composition of seed lipids of *Bornmuellera dieckii* shows the characteristic presence of erucic acid, a compound that is widely distributed in the *Brassicaceae* (Harborne & Turner 1984). Erucic acid is known to be harmful to mammals if ingested in sufficient amounts. We do not dispose of data on the possible toxicity of erucic acid for other animals, insects in particular. Still, the presence of this acid in the seeds of *B. dieckii* may be interpreted as an additional defence mechanism.

Acknowledgements

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