



ANATOMICAL AND CHEMICAL ADAPTATION OF *SPARTIUM JUNCEUM* L. IN ARID HABITAT

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A number of anatomical adaptations to an arid habitat can be noted in the microscopic structure of *Spartium junceum* L. vegetative organs. The xerophytic adaptation of the leaf is seen in its short life and the transformation of its inner structure, with palisade parenchyma dominating. The top part of the stem has taken over the leaf function, while sclerenchyma fiber and conductive elements occupy most of its secondary units. A peculiar feature of the primary structure of the root is its incompletely developed endodermis, and the secondary structure points to its storage and mechanical roles. GC/MS analyses of the essential oils showed that they consist mainly of monoterpene hydrocarbons with α -thujene as the main compound. The essential oils also play a xeromorphic role in this plant species.

Key words: *Spartium junceum* L., xeromorphic adaptation, stem, leaf, root, essential oil, α -thujene.

INTRODUCTION

Spanish broom (*Spartium junceum* L.) grows naturally on rocky ground over steep slopes near the sea. Besides its xeromorphic adaptations, it has also adapted to higher salt concentrations in the soil. As an undergrowth species of a rather specific habitat, dating as far back as the post-glacial period, it spreads easily in closed ecosystems and is widely represented in the Mediterranean regions of Europe (Pignatti, 1982). Due to its invasive nature there are a variety of phenotypes oriented towards stem photosynthesis. Stems are often the primary photosynthetic organs in desert species (Gibson, 1983; Nilsen et al., 1989).

It is exploitable as a medicinal plant because of its content of alkaloid sparteine, which is isolated from the stem and has been shown to have an analeptic effect on the muscular system (Cave, 1995). The glucoside scoparin, which represents most of the plant's diuretic activity, has been iso-

lated from broom flowers (Wagner, 1988). A new oleanene-type saponin with potent anti-ulcerogenic activity, spartitrioside, has also been isolated from the flowers (Yesilada and Takaishi, 1999).

The vegetative organs of the Spanish broom show an ability to adapt to arid habitats. This study reports anatomical features of the leaf, stem and root resulting from adaptation to environmental conditions, as well as the significance of the flower's essential oil.

MATERIALS AND METHODS

PLANT MATERIAL

Spartium junceum L. plant material was gathered in April and May 2000 and 2001 in the littoral part of Kozjak Mt. near the city of Split. Voucher specimens are deposited in the Department of Biology of the University of Split, Croatia.

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ANATOMY

Slides were made from transversal and longitudinal sections of material preserved in 50% ethanol. The presence of fats (cuticle) was revealed by red staining of the lipophilic stain Sudan III [0.1% solution in 96% ethanol and glycerol (1:2)]. To show lignin in cell walls, Safranin (1 g in 50 ml ethanol and 50 ml water) was used, giving a red stain in positive reactions.

An Opton Axioskop MC 63A light microscope was used for all microscopic observations. Statistics included calculation of arithmetic means (number of stomata per 1 mm² leaves and stem) of 100 specimens.

ISOLATION OF ESSENTIAL OILS

Fresh buds and flowers were used for analysis of essential oil composition; 100 g plant material was subjected to hydrodistillation for 3 h using a modified Unger-type apparatus. The essential oils obtained were dried over anhydrous sodium sulphate and 2 µl of each was used for GC/MS analyses.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY

The volatile compounds were analyzed with a Hewlett-Packard GC/MS system (GC 5890 Series II, MSD 5971A).

A fused silica HP-20 M polyethylene glycol column (50 m × 0.2 mm, 0.2 µm film thicknesses) was coupled directly to the mass spectrometer. The carrier gas was helium (1 ml/min). The program used was 4 min isothermal 70°C, then 70–180°C at a rate of 4°C/min, then held isothermal for 10 min. The injection port temperature was 250°C. The sample components were ionized in the EI mode (70eV).

IDENTIFICATION OF COMPONENTS

The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C₈-C₂₂ *n*-alkanes (Van Den Dool and Kratz, 1963). The individual constituents were identified by their

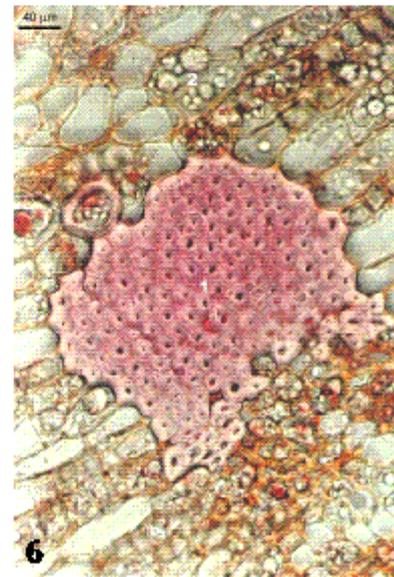
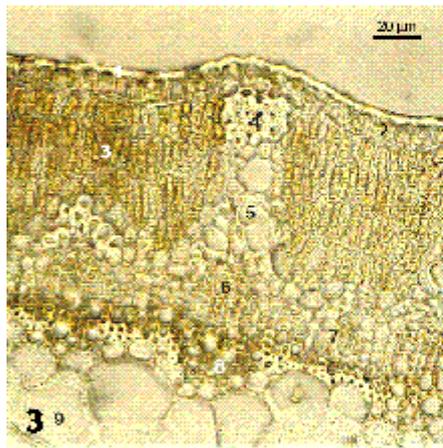
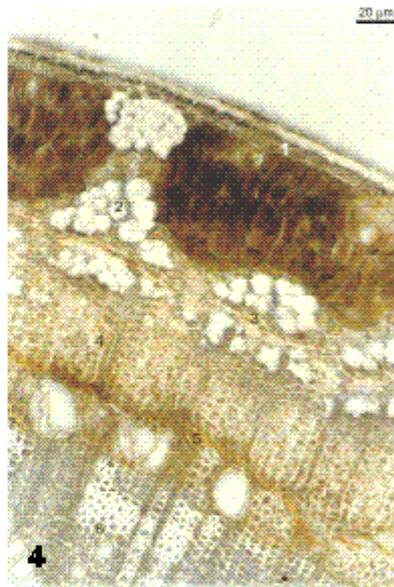
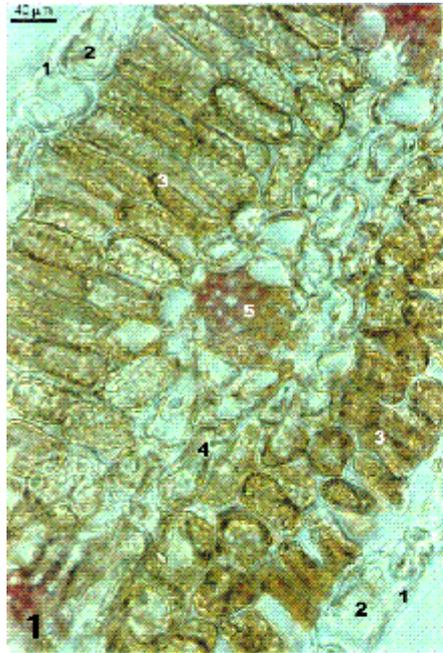
identical retention indices with reference to compounds known from literature data (Adams, 1995) and also by comparing their mass spectra either with known compounds or with the Wiley mass spectral database.

RESULTS AND DISCUSSION

Most morphological characteristics of Spanish broom (*S. junceum*) indicate its xerophytic character. The stem allows for profiled positioning of the vegetative parts so that the effects of overheating are reduced. The plant loses its tiny leaves before the beginning of summer, thus reducing transpiration and creating conditions for better endurance of drought stress. The grass-shaped stem is one of the adaptations helping to reduce the total exposed area of the plant, whereas the root is well developed and ramified. All these morphological characteristics mean that this is a typical euxerophytic plant. Notwithstanding the comparatively short life of the leaf, the number of its xeromorphic anatomical features should be noted: an equifacial type of leaf, incomplete separation between the palisade and spongy parenchyma, a thick cuticle with a wax layer, the number of stomata and their proximity, and conspicuous scleromorphic veins.

The leaf has bifacial structure in terms of its xylem and phloem orientation within veins, while the arrangement of the palisade parenchyma and stomata is related to its unifaciality (Fig. 1). The palisades are arranged next to the upper and lower epidermis, while the spongy parenchyma with some intercellular tissue is limited to the area around the veins. This is a typical xerophytic adaptation, because high irradiance and the slowing of water circulation due to drought results in stronger development of palisade parenchyma (Fahn, 1990). Recessed xerophytic stomata of amarylidic type (Fig. 2) are arranged on both sides of the leaf, while a thick cuticular layer and delicate filament hairs prevail on the surface. The leaf of Spanish broom is not leathery and contains no sclereides (unlike many xerophytes), and thus its functioning is limited to a

Fig. 1. Cross section of leaf lamina stained with Safranin. 1 – cuticle; 2 – epidermis; 3 – palisade parenchyma; 4 – spongy parenchyma; 5 – vascular system. **Fig. 2.** Stomatal apparatus. 1 – guard cells; 2 – central pore; 3 – subsidiary cells; 4 – substomatal chamber. **Fig. 3.** Cross section of primary stem. 1 – cuticle; 2 – epidermis; 3 – palisade parenchyma; 4 – sclerenchyma cells; 5 – parenchyma cells; 6 – phloem; 7 – cambium; 8 – xylem; 9 – pith. **Fig. 4.** Cross section of secondary stem. 1 – epidermis; 2 – primary cortex; 3 – phloem; 4 – secondary cortex; 5 – cambium; 6 – secondary xylem. **Fig. 5.** Cross section of young root stained with Safranin. 1 – epidermis; 2 – cortex; 3 – xylem; 4 – phloem; 5 – sclerenchyma cells; 6 – endodermis. **Fig. 6.** Cross section detail of mature root stained with Safranin. 1 – sclerenchyma cells; 2 – parenchyma with starch grain.



short period of time. After it falls off in early spring, its function is taken over by the stem. It is well known that aquatic multicellular algae and the earliest terrestrial vascular plants (about 400 million years ago) performed photosynthesis exclusively with stem tissue (Stewart and Rothwell, 1993), but there is no question that photosynthesis in stems is an ancient characteristic of plants dating back to the origin of land plants (Nilsen, 1995). Nowadays, stem photosynthesis is found at hot dry sites with high irradiance (Gibson, 1983). Besides assimilation parenchyma (multilayer palisades), groups of sclerenchyma fibers appear (Fig. 3). These anatomical features are characteristic of the photosynthetic stem (Bowes, 1996). Recessed stomata are almost regularly found above the groups of palisade cells. The average number of stomata per 1 mm² of stem is almost the same as that of the leaf, but the number of stomata per surface unit of stem varies more than in the leaf (Tab. 1). Epidermal tissues may be one or several cell layers thick, and the stomata may have a variety of spatial orientations (Gibson, 1983).

The domination of conductive elements, especially tracheides, is obvious in the transition from the primary to secondary structure of the plant (Figs. 3, 4). Such structures make photosynthesis possible within the green grass-shaped stems in primary structure and in the transition from primary to secondary structure. No assimilation takes place in the secondary structure part of the plant, but the predominance of palisade layer cells filled with starch granules is evident (Fig. 3). The plant gets its strength from sclerenchyma fibers pervading the whole length of the stem, arranged among softer tissues.

Spanish broom has a very long and ramified root with a normally diarchic radial vein (Fig. 5). In the primary root of the investigated plant, which grows under extreme desert conditions, the number of cortical layers is reduced (Fig. 5). An advantage of this feature may be that it shortens the distance between the soil and the stele. Another peculiarity of the primary structure of the root is the almost parenchymal endodermis, with only a slight thickening of the entire cell wall. This type of very wide endodermis enhances selective transport into the stele. Such a structural adaptation is characteristic for xerophytes and halophytes (Fahn and Cutler, 1992).

Semicircularly arranged sclerenchyma groups provide the strength in the core itself (Fig. 5). The secondary structure of the root can be related to the secondary structure of the stem. Specific features

TABLE 1. Statistical values of stomata number per 1 mm²

	\bar{X}	S ²	S	V
Abaxial side of leaves	40.2	69.2	8.3	20.00
Adaxial side of leaves	39.6	49.4	7.0	17.76
Primary stem	39.6	167.4	12.9	36.68

\bar{X} – Arithmetic mean

S² – Variance

S – Standard deviation

V – Coefficient of variance (V = S × 100/X)

TABLE 2. Percentage composition of *Spartium junceum* essential oils

Compound	Flower buds	Flowers
Hydrocarbons		
α-Thujene	27.4	28.9
α-Pinene	–	0.3
Sabinene	6.5	–
Myrcene	9.2	2.7
α-Terpinene	12.4	–
γ-Terpinene	16.4	12.9
p-Cymene	19.6	13.8
Oxygen-containing compounds		
1-octen-3-ol	6.5	–
Nerolidol ^a	–	6.4
Dimethyl 1-methyl-1H-indole-4,6-dicarboxylate ^b	–	15.8
Carvacrol	0.7	–
Unknown	–	11.6

^aCorrect isomer was not identified

^bTentatively identified from mass spectra (MS) only.

MW = 247; m/z: 247(100), 57(25), 249(21), 175(7), 161(7), 147(5), 128(3)

are noted in the arrangement of mechanical elements towards the core: lignified trachea walls, and the appearance of groups of sclerenchyma fibers within starch-filled cortex parenchyma (Fig. 6). The morphological and anatomical structure of Spanish broom is most similar to the species *Retama raetam* (Forsskal) Webb et Berth., but the latter never develops leaves, and horizontal roots in addition to the usual vertical root ensure better circulation of water (Fahn, 1990).

Spanish broom begins to flower in early spring (April, May) as the ambient temperature rises. Flowers intensively discharge essential oils, creating an oily film around the mother plant; this is one of its xerophytic means of protection. The presence of oil in broom-like shrubs makes them transpire much less, thus generally reducing the temperature of the plant's micro-environment. Moreover, stem assimilation in broom is more inhibited in summer than in spring, due to the low moisture and low

nitrogen content in tissue (Nilsen and Karpa, 1994). The appearance of dry fruit in the period of highest temperature signals the end of the vegetative growth phase.

The flower scent, that is, the release of specific volatile components, is most intense during the day. Essential oil is isolated from fresh plant material. This work reports comparative GC/MS analyses of *S. junceum* essential oils collected at two stages of flower development: flower buds and fully opened flowers. The yield of these essential oils was very low (~0.005%). The chemical composition of the oils isolated from flower buds and fully opened flowers of this species is shown in Table 2. Eight compounds were identified in flower bud oil and seven compounds in flower oil, accounting for 98.7 and 92.4% of the total oil composition, respectively. One compound in the oil from flower buds remained unidentified.

The *S. junceum* oils were characterized by a high degree of hydrocarbons, especially monoterpene hydrocarbons. The major compounds in both oils were α -thujene (27.4% and 28.9%), *p*-cymene (19.6% and 13.8%) and γ -terpinene (16.4% and 12.9%). Other important compounds in the oil from flower buds were α -terpinene (12.4%) and myrcene (9.2%). The essential oils of this plant seem very different from known monoterpene-emitting plant species (Owen et al., 1997). Emission of these components is regulated by many environmental variables such as temperature, light, humidity (Dement et al., 1975) and also the physiological status of the vegetation (Owen et al., 1997). These oils contained only small amounts of oxygenated compounds. The flower bud oil contained two oxygenated compounds: 1-octen-3-ol (6.5%) and carvacrol (0.7%). The oil from flowers contained three oxygenated compounds but one of them remained unidentified (11.6%) and the other one was identified only tentatively on the basis of the mass spectra (15.8%).

Thick cuticle and discharged essential oil have similar xeromorphic functions as an adaptation to arid habitats. Essential oil protects the plant from

losing water through evaporation and also protects it by diffracting light.

REFERENCES

- ADAMS RP. 1995. *Identification of essential oil components by gas chromatography and mass spectroscopy*. Allured, Carol Stream IL.
- BOWES BG. 1996. *Plant structure*. Manson Publishing Ltd., London.
- CAVE A. 1995. *Pharmacognosy, phytochemistry, medicinal plants*. Lavoisier Publishing Inc., Paris.
- DEMENT WA, TYSON BJ, and MOONEY HA. 1975. Mechanism of monoterpene volatilization in *Salvia mellifera*. *Phytochemistry* 14: 2555–2557.
- FAHN A. 1990. *Plant anatomy*, 4th edition. Pergamon Press Ltd., Oxford.
- FAHN A, and CUTLER DF. 1992. *Xerophytes*. Gebrüder Borntraeger, Berlin-Stuttgart.
- GIBSON A. 1983. Anatomy of photosynthetic old stems of nonsucculent dicotyledons from North American deserts. *Botanical Gazette* 144: 347–362.
- NILSEN ET. 1995. *Stem photosynthesis: extent, patterns, and role in plant carbon economy*. Academic Press, San Diego.
- NILSEN ET, and KARPA D. 1994. Seasonal acclimation of stem photosynthesis in two invasive, naturalized legume species from coastal habitats of California. *Photosynthetica* 30: 77–90.
- NILSEN ET, MEINZER FC, and RUNDEL PW. 1989. Stem photosynthesis in *Psoralea arguta* (smoke tree) in the Sonoran Desert of California. *Oecologia* 79: 193–197.
- OWEN S, BOISSARD C, STREET RA, DUCKHAM SC, CSIKY O, and HEWITT CN. 1997. Screening of 18 Mediterranean plant species for volatile organic compound emissions. *Atmospheric Environment* 31: 101–117.
- PIGNATTI S. 1982. *Flora d' Italia*, vol I. Edagricole, Bologna.
- STEWART W, and ROTHWELL GW. 1993. *Paleobiology and the evolution of plants*. Cambridge University Press, New York.
- VAN DEN DOOL H, and KRATZ PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography* 11: 463–471.
- WAGNER H. 1988. *Pharmazeutische Biologie*. Gustav Fischer Verlag, Stuttgart, New York.
- YESILADA E, and TAKAISHI Y. 1999. A saponin with anti-ulcerogenic effect from the flowers of *Spartium junceum*. *Phytochemistry* 51: 903–908.