



REPRODUCTIVE BIOLOGY OF *RUSCUS HYPOGLOSSUM* L. IN SLOVAKIA

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Ruscus hypoglossum L. (Asparagaceae), a rare and endangered species of the Malé Karpaty Mts. in Slovakia, is an evergreen geophyte having rigid leaves with special bracts developed on them. The flowers are unisexual, and the plant is dioecious. Well-developed stamens and rudimentary pistils in different stages of development occur in male flowers. Remnants of degenerated anthers persist in female flowers, which have two ovules in the ovary. Male plants prevail in local populations; the average ratio of male to female plants is 5:1. Reproduction in *Ruscus hypoglossum* is generative and vegetative. Fruit (red berry) development lasts from May to October. The seeds are round, 6–8 mm in diameter, and albuminous. The endosperm is helobial, with a haustorial chalazal chamber. The endosperm cell walls in mature seeds are thickened and are composed of reserve cellulose. The mature embryo consists of one elongated cotyledon, a plumule, short hypocotyl and radicle. Seed dormancy lasts one year. Vegetative propagation is accomplished by rhizome fragmentation. The populations should be protected above all against direct destruction, shoot collecting, and grazing by animals, especially deer.

Key words: *Ruscus hypoglossum*, seed, embryo, reproduction.

INTRODUCTION

Ruscus hypoglossum L. is a rare and threatened species of the Slovak flora. It is listed as a critically endangered (CR) species on the national red list (Marhold and Hindák, 1998; Halada and Feráková, 1999). This species is usually linked to the family Liliaceae (Marhold and Hindák, 1998), but some authors have classified it in the closely related family Asparagaceae, subfamily Ruscoideae (Komar, 1985; Satarova, 1990). Davis (1966) introduced the separate family Ruscaceae.

Ruscus hypoglossum L. is a perennial, dioecious, evergreen rhizomatous geophyte. The assimilation organs have interesting morphology, and there are different opinions about their origin. We believe that their origin is from leaf (Schlitter, 1960; Markgraf, 1974), against the view that they originate from flattened stems – the phylodia or phyllocladia presented by other authors (Čelakovský, 1893; Kausmann, 1955; Sachs et al., 1993). The bracts are located in the middle of the upper surface of leaves, and flowers are developed in their axils. The species is adapted to life in semi-shade, but the vigorous rhizome and rigid leaf structure en-

ables it to survive during transitional periods of drought and also at localities with rapidly changing light conditions.

Ruscus hypoglossum L. is a Submediterranean species, distributed in Southeast Europe and the eastern part of Central Europe (Yeo, 1968): from Italy in the west to northern Turkey in the east, with isolated stations in Crimea. The species reaches the northern limit of its geographical range in Slovakia. Its local populations occur only in the Malé Karpaty Mts. in southwest Slovakia. Halada (1994) registered 13 sites in the southeast part of this mountain range, between Bratislava and Častá. The species grows mainly in oak-hornbeam forest of the association *Carici pilosae-Carpinetum* R. et Z. Neuhl. 1964, and rarely in beech forests. It is distributed exclusively on acid rock, mainly granites. The soils are deep, humus-rich cambisols (Halada, 1994). Murín and Májovský (1974) determined the chromosome number $2n = 40$ and their detailed morphological structure using material from the Malé Karpaty Mts.

In this paper, we focus on its reproduction, continuing previous studies of this species in Slovakia (Halada, 1985, 1994).

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MATERIALS AND METHODS

The morphological and sexual structure of eight local populations was studied during population-biological study of the species in the Malé Karpaty Mts. (Halada, 1985). Two local populations (Bratislava, Zoo; Modra, Harmónia) were studied in detail. The population structure, phenology (flowering and seed development) and population dynamics were observed. Individual shoots were marked in the field for this purpose. The shoots were measured and divided into categories according to their sex, morphology and ontogenetic phase. The local population in Modra-Harmónia was studied on a 10 × 10 m permanent plot, starting in 1988.

For embryological analysis, flowers and fruits were collected at one-month intervals at localities in Bratislava and Harmónia. Specimens were fixed in FAA (70% ethyl alcohol, 40% formaldehyde, 99% acetic acid, 18:1:1), dehydrated in a tertiary butyl alcohol series, and embedded in Histoplast S (Serva, Heidelberg, Germany). Sections 10 µm thick were stained with Heidenhain's hematoxylin or PAS (periodic acid, Schiff) reaction.

Seed dormancy was studied by planting fruits or seeds isolated from fruits in autumn or winter to flowerpots with forest soil. The pots were partly sunk in soil in areas bordering the localities of *Ruscus hypoglossum* populations.

RESULTS AND DISCUSSION

LIFE CYCLE - PHENOLOGY

After germination, juvenile shoots having only two leaves without bracts are established in late April/early May (Fig. 1a). In the following years, juvenile shoots with more leaves but without bracts and flowers grow from rhizomes in the springtime. The life span of shoots is 3–5 years, and because new shoots are established every year from the same rhizome, they grow in groups. The duration of the juvenile or pre-maturity phase varies, and probably depends on site and climate conditions.

The generative shoots are established only during the next ontogenetic phase, which is the generative phase. Generative shoots have more leaves (3–16), the bracts are on the upper side of the leaves, and flowers develop in their axils each year. The growth of every shoot finishes in the year of its establishment; further longitudinal growth or regeneration does not occur during the following years. Older shoots gradually stop blooming and become senescent. Each generative shoot lives 3–5 years, then dries out. The life span of individual plants is not known. Golubeva (1975) states that the generative phase of *Ruscus ponticus* lasts more than 50 years. The number of shoots in individual ontogenetic phases of *Ruscus hypoglossum* local populations, as found in our observations, is shown in Table 1.

The habitus of male and female plants differs in some features. Male plants usually have numerous erect shoots. Female individuals have fewer shoots. These shoots are procumbent, and the leaves are broader and less acuminate.

FLOWER BIOLOGY AND STRUCTURE OF GENERATIVE ORGANS

Ruscus hypoglossum is a diclinous, dioecious species. Study of the sexual structure of populations indicates that male individuals dominate in local populations. The ratio of male to female individuals averages 5:1 and varies from 15:1 to 2.4:1.

At the time of anthesis, well-developed stamens and degenerated pistils occur in male flowers (Fig. 1b). In female flowers there are well-developed pistils and remnants of anthers degenerating already in early developmental phases of the flower bud (Fig. 1c). Three to six flower buds are developed in each bract axil on the upper part of the leaf. They develop and flower successively. The flower stalk is 5–8 mm long. Six light green sepals form two rings. The outer sepals are 3–5 mm long, and the inner ones 2.5–3.5 mm long.

The filaments of the three stamens in the male flowers coalesce to one cylindrical tube, violet in color. The anthers are situated at the top of the tube (Fig. 1b) and the pistil is reduced. The anther walls, typical of the whole family Asparagaceae (Satarova, 1990), are composed of exothecium, endothecium, middle layer and secretory tapetum. At pollen maturity the tapetum is already degenerated. The two-celled pollen grains (diameter 35 µm) are sporomorphs of intermediate size (Futák et al., 1966).

The cylindrical tube of the female flower is narrow, dark violet, and tapering upward. The upper part of the style with stigma extends beyond the top of the flower tube. The rudimentary anthers are situated on the top of the tube, below the stigma (Fig. 1c). The ovary is developed in the center of the female flower tube.

In the unilocular ovary, two hemianatropous or anatropous ovules are situated on the placenta (Fig. 1d). The seed develops usually from one of the ovules, but sometimes two seeds can occur in the fruit. The ovules are crassinucellate and bitegmic. At anthesis, the egg apparatus, the polar nuclei or secondary nucleus, and three degenerating antipodals are present in the widened embryo sac. The hypostase is developed in the chalazal region of the ovule.

ANTHESIS AND POLLINATION

Flower bud development begins in the axils of bracts during summer and continues during autumn, but they remain closed during winter. Some buds flower already in autumn, but they do not form fruits. Anthesis of female flowers from which fruits may develop is pro-

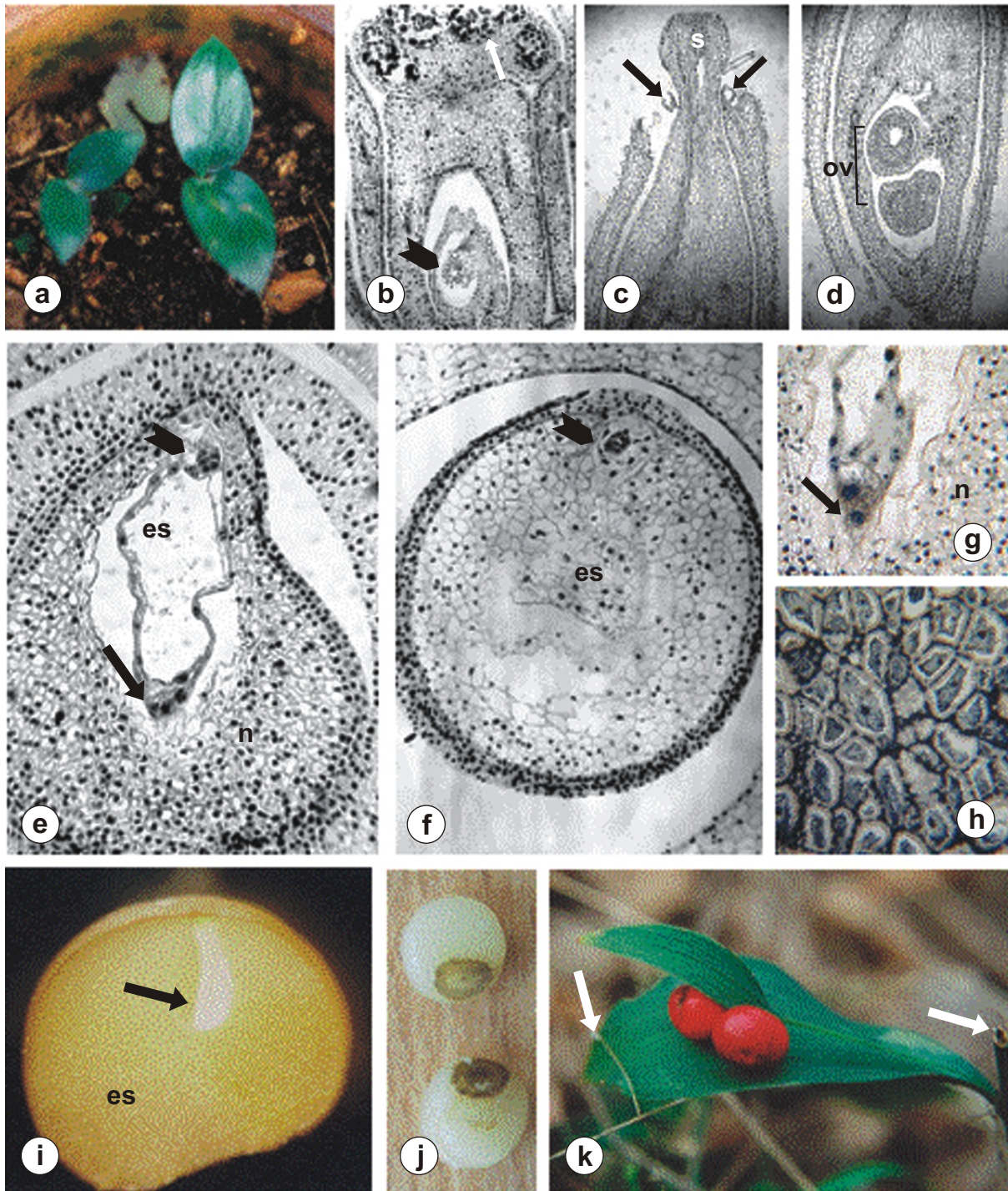


Fig. 1. (a) Juvenile plants in pots, (b) Male flower with anthers (arrow) and degenerating ovary (arrowhead). $\times 40$, (c) Top of female flower with degenerated anthers (arrows) and stigma (s). $\times 40$, (d) Female flower with two ovules (ov) in ovary. $\times 40$. (e) Young seed with embryo (arrowhead), endosperm (es) and chalazal chamber (arrow). n - nucellus. $\times 220$, (f) Seed with globular embryo (arrowhead) and cellularizing endosperm (es). $\times 220$, (g) Polyploid nuclei in chalazal haustorial chamber of endosperm (arrow). n - nucellus. $\times 220$, (h) Maturing endosperm with thick cell walls. $\times 340$, (i) Embryo (arrow) in endosperm (es) of mature seed. $\times 10$, (j) Seeds of *R. hypoglossum*. $\times 2.5$, (k) Female plants with red fruits, stem and leaf damaged by grazing (arrows).

TABLE 1. Proportion of shoots [%] in different ontogenetic phases in eight local populations

Site	1	2	3	4	5	6	7	8
Juvenile	38.4	46.3	62.9	32.2	36.1	44.1	42.1	21.7
Premature	3.9	4.0	1.5	4.0	8.4	4.1	6.3	4.7
Generative	29.6	18.7	10.6	28.9	27.1	28.3	30.2	48.2
Senile and damaged	28.1	41.7	25.0	34.9	28.4	23.4	20.6	25.3
Total number of shoots	595	175	132	152	155	145	126	170

Sites: 1 – Bratislava, ZOO; 2 – Rača, Veľká Baňa; 3 – Rača, Fanglovske údolie; 4 – Sv. Jur, Gaštanica; 5 – Sv. Jur, Biely Kameň; 6 – Sv. Jur, Neštich; 7 – Modra, Široké; 8 – Modra, Harmónia. Site locations can be found in Halada (1985, 1994).

longed from March to May the following year. Entomophily is the prevalent mode of *R. hypoglossum* pollination, but wind pollination cannot be ruled out (Daumann and Synek, 1968).

SEED AND FRUIT DEVELOPMENT

The helobial endosperm develops in the embryo sac after fertilization. This type of endosperm is also typical for other species of the genus *Ruscus* and some other species of the family Asparagaceae (Komar, 1985; Satarova, 1990). After the first division in the primary endosperm cell, the cell wall separates the chalazal and apical chambers of the endosperm (Fig. 1e). In the apical part, the development of free-nuclear endosperm follows. It later cellularizes (Fig. 1f). In the chalazal chamber, free and highly polyploid nuclei develop (Fig. 1g). The wall labyrinth on the outer wall of this pluricellular chamber is successively formed and the whole chamber acquires haustorial character. Finally the endosperm fills up the major part of the albuminous seed. The endosperm cell walls, composed mainly of cellulose, become thick by the time of maturation (Fig. 1i). In the genus *Ruscus*, the storage cellulose from endosperm walls is consumed during seed germination. In the endosperm cells, lipids and proteins are also present as storage substances (Komar, 1985).

The surface of the seed is formed of a seed coat composed of compressed cells and cuticula remaining from the inner and outer integuments, covered with the thickened outer epidermis of the outer integument and its cuticular layer. The structure of the seed coat is similar to that of *Ruscus hypophyllum* L. described by Komar (1985).

The first globular embryos occur in the small green fruits in May, but in some other fruits the same phenomenon was observed one month later, in June. This lag is connected with the gradual, successive flowering and fertilization, which is prolonged to at least 2 months.

In the middle of September there are already asymmetrical embryos typical of monocotyledons, in the seeds. Embryo development is completed in October together with fruit maturation. The embryo with

the radicle, plumule and one prolonged cotyledon represents only a relatively small part of the albuminous seed (Fig. 1i).

The fruit is a red berry which usually hangs on the fruit stalk till winter, sometimes even till spring. The berry is globular or slightly oblong (1–2 cm in diameter) with one or two seeds (Fig. 1j). Shoots with only one fruit are most frequent (53.8%). The fruits are most often located on the second leaf from the base of the shoot. The seeds are round, 6–8 mm in diameter (Fig. 1k), and have distinct tissue remnants in the area of the cicatrice.

The pericarp is bordered by inner epidermis and outer epidermis that is covered with distinct cuticle. The cells of the subepidermal layer are larger than those of the outer epidermis, are tangentially prolonged, and have granular cell content. The vascular bundles are branched in the layer of large and thin-walled cells of the inner pericarp.

Fruits occur only in populations composed of male and female plants. We did not observe fruits on isolated plants located far from the population. Exceptionally we observed the development of fruits on male plants in the population. Probably this was elicited by special environmental conditions, as is known in other species with separate sexual organs (e.g., *Zea mays*).

SEED GERMINATION AND VEGETATIVE REPRODUCTION

The dormancy of *Ruscus hypoglossum* seeds lasts one year. Seeds maturing in autumn did not germinate in our experiments in the spring of the next year, but only one year later (Fig. 1a). Removal of the pericarp before planting did not shorten seed dormancy.

According to our observations, vegetative reproduction by division of branched rhizomes was frequent in the observed localities. Separate plants also originated from parts of experimentally divided rhizomes. Our observations are in accordance with those of Pilát (1953), who considered both generative and vegetative reproduction of *Ruscus hypoglossum* to be common.

THREAT

The rarity and small size of local populations are the main factors of threat. Human impacts and damage by animals represent other types of threats. One threat from humans is collection of shoots or whole plants (especially female ones) for decorative purposes during the autumn. Because of its evergreen character, the species is attractive as a food source for animals, especially during winter and spring months (Fig. 1i). Significant differences in shoot numbers in autumn and spring indicate the relatively heavy damage caused by animal grazing. The extent of this disturbance is seen in Table 1, where the numbers in the "senescent and damaged" category reach from 20.6 to 41.7% in individual local populations. This category consists mainly of shoots damaged by grazing, since senescent shoots are rarely detected. The underground part of the plant survives grazing, so grazing does not directly result in the death of plants, but their vitality and number of shoots are decreased.

Regular forest management (forest clearing) restricts the reproduction of *Ruscus hypoglossum* as well. Light and moisture conditions are changed dramatically after felling of trees. In spite of that, it looks as though *Ruscus hypoglossum* has been able to adapt to new conditions quite successfully and to persist at the site. Conditions suitable for population growth and reproduction are restored soon after reforestation.

Because of the plants' resistance to the mentioned types of disturbance (rigid structure of aboveground and belowground organs; a certain level of generative and vegetative reproduction), the majority of local populations seem relatively stable, while not expanding. We recorded fluctuations rather than distinct trends. This can be documented by the successive numbers of shoots recorded annually at the 100 m² permanent plot in 1988–1997: 159, 229, 234, 164, 147, 139, no data, 248, 269, 344. The shoots were counted each year in autumn (October or November): the 1994 record is missing.

To preserve *Ruscus hypoglossum*, at the least it is necessary to avoid building activity at all localities of its populations, and to prevent direct collection of plants by educating the public and enforcing its protected status.

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