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Morphology and anatomy of blackberry pyrenes (*Rubus* L., Rosaceae) Elementary studies of the European representatives of the genus *Rubus* L.

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ABSTRACT

This article presents the results of studies on the pyrenes of selected European brambles belonging to different subgenera and sections of the genus *Rubus*. Differences between the pyrenes of particular species are mainly visible in their shape, size and the outer endocarp sculpture. Numerous SEM photographs revealed that the differentiation of the endocarp surface is due to variations in a thin layer of transition sclereids developing between the outer endocarp and a mesocarp parenchyma. The endocarp structure of the genus *Rubus* can be used in determining some species belonging to different subgenera, even if generally its significance in the taxonomy of the European *Rubus* is limited.

1. Introduction

The pyrenes of *Rubus* species, often erroneously called seeds or stones, have been studied by palaeobotanists and archaeologists more often than botanists. They have frequently been found as fossils in the Tertiary Flora of Western Siberia (Dorofeev, 1963) and from the late Cenozoic Flora in Japan (Kokawa, 1966). Fossils of *Rubus* drupelets have also been found in Poland in the Miocene and Pliocene Flora (Szafer, 1961) and in the Early Middle Ages (Baas, 1936; Klichowska, 1960, 1972; Lechnicki, 1956).

The morphology of the blackberry fruit has so far been the subject of only a few scientific studies. Macroscopic studies of drupelets of *Rubus* fruits were carried out on some Japanese species in Japan by Satomi and Naruhashi (1971). The pyrenes of selected Central and East European species were studied by Bojansky and Fargasova (2007). In both cases, the general morphology of the drupelets was presented in the form of drawings. The morphological structure of blackberry pyrenes has rarely been described, not only in standard floras but also in classical taxonomic studies of the genus.

The literature concerning the anatomy of *Rubus* pyrenes is also meager. The pioneer and the most important investigations in this field were made by Reeve (1954a, b). The author studied the fruit of one species only, the American blackberry *Rubus strigosus* Michx, which is closely related to *Rubus idaeus* L., and is often regarded as a variety or subspecies. The author analysed the structure and morphology of *Rubus* endocarp during ontogenesis of the fruit and introduced the principle terminology of fruit anatomy and morphology, which is still up-to-date.

The blackberry fruit is composed of more or less numerous drupelets situated on the convex torus. The singular drupelet consists of a thin exocarp, a fleshy mesocarp and a hard, lignified endocarp called the pyrene. (Fig. 1 A and B,). According to Reeve (l. c.), the endocarp of *R. strigosus* is stratified and consists of two layers of elongated thick-walled sclereids: the inner layer consisting of sclereids that form transversal rings bounding the ovary and oriented perpendicularly to the longer axis of the pyrene, and the outer layer, in which sclereid cells run at a right angle to the inner layer of sclereid cells, parallel to the longer axis of the pyrene (Fig. 1C).

In Reeve's detailed studies of *Rubus* endocarp during ontogenesis (l. c.), special attention was paid to the narrow zone in the outer layer of the endocarp. She called this peripheral layer, containing several rows of cells next to the mesocarp parenchyma, the "transition zone" and the cells creating it the "transition sclereid cells". In terms of the shape and wall thickness, transition sclereid cells are intermediate between the mesocarp parenchyma cells and the endocarp sclereids lying beneath this zone.

The aim of this study was to establish whether the data presented by Reeve, concerning the structure of the *R. strigosus* endocarp, are representative for the whole genus *c* and to check which of the endocarp features can be used in the taxonomy of European brambles.

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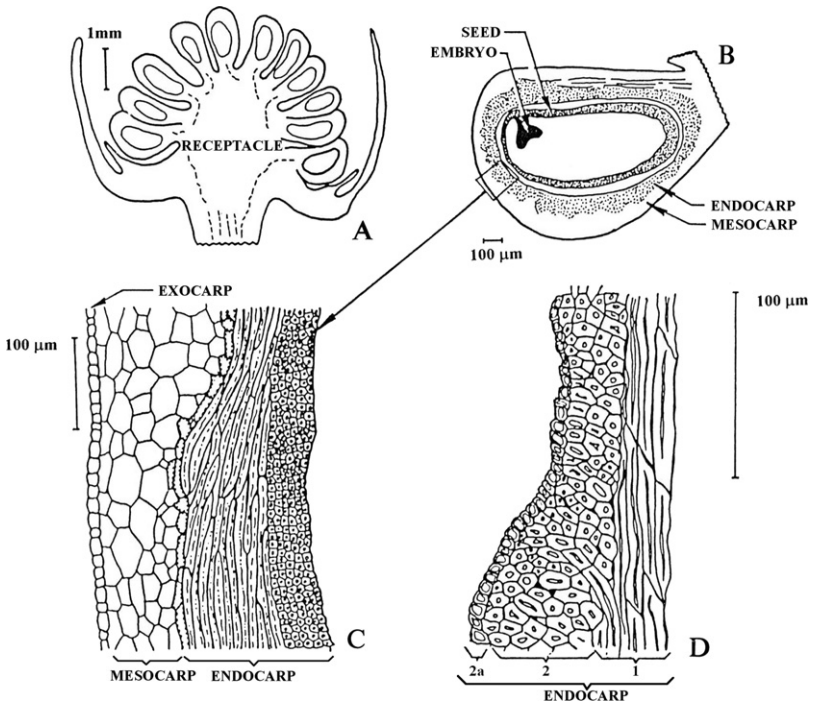

Fig. 1. The structure of *Rubus* compound fruit (unripe stage of development). (A): transection of a drupelet; (B): single drupelet; (C): longitudinal section of a drupelet; and (D): transverse section of the endocarp of *Rubus caesius*: (1) inner endocarp, (2) outer endocarp, and (2a) transition layer. A, B, C (Esau, 1977; Reeve, 1954), and D (original).

2. Material and Methods

Pyrenes of selected European *Rubus* species, representative of four subgenera, were analysed: *Rubus chamaemorus* L. (subgenus *Chamaerubus* O. Kuntze), *Rubus arcticus* L. and *Rubus saxatilis* L. (subgenus *Cylactis* (Raf.) Focke), *R. idaeus* L. (subgenus *Idaeobatus* (Focke) Focke) and *Rubus canescens* DC., *Rubus caesius* L., *Rubus gracilis* J. Presl and C. Presl, *Rubus ulmifolius* Schott (subgenus *Rubus*).

Mature drupelets were collected from comparable parts of fruiting stems. They were macerated in water with citric acid and dehydrated in absolute ethanol (99.8%), then dried at 60 °C. Endocarp cross-sections of all examined species were made with a razor and a microtome in the middle of each pyrene. Longitudinal sections of *R. chamaemorus* drupelets were also made. Prior to sectioning, the drupelets were stored for a few days in a glycerin–alcohol solution to ensure satisfactory embedding and microtoming. The shape and size of the pyrenes were determined from 40 to 50 sections. The shape of the drupelets was estimated from the side view.

Both whole drupelets and sectioned ones were observed using a Hitachi S-3000 N Scanning Electron Microscope, following coating using the Sputter Coater S 150 B Edwards according to standardized methods. The SEM photographs of the endocarp surface were studied at comparable magnification.

3. Results and Discussion

The pyrenes of the studied species are 2–4 mm long. The smallest ones are those of *R. idaeus* (Fig. 5A and B) and *R. arcticus* (Fig. 2A), at approximately 2.1 mm long, 0.65 mm wide and 0.5–0.7 mm thick. The largest among the examined species are the pyrenes of *R. chamaemorus* (Fig. 2A) and *R. saxatilis* (Fig. 4A and B) with an endocarp approximately 4.0 mm long, 1.8 mm wide and 1.4 mm thick. The endocarp varies in shape. It is usually laterally compressed, often flattened or slightly concave on the adaxial side and rounded on its abaxial side (Fig. 1). In side view they are ± ovate (*R. chamaemorus*, Fig. 2A), elliptical (*R. ulmifolius*, Fig. 9A), and reniform (*R. arcticus*, Fig. 3A, *R. caesius*, Fig. 10A, and *R. idaeus*, Fig. 5A) or depressed ovate and are usually more or less oblique. The shape of the pyrenes ± varies within the same species.

The pyrene surface (seen with the naked eye) in *Rubus* species is either smooth or hollowed. It is ± smooth in *R. chamaemorus* and *R. arcticus* (Figs. 2A, 3A and B) and ± distinctly hollowed out in other species. The hollows are shallow in *R. caesius* (Fig. 10A and B) and deep in *R. gracilis* (Fig. 8A,B and D), and *R. canescens* (Fig. 6A and B). The bottom of shallow hollows is usually flat or rounded and clearly visible; in deep hollows it is often very narrow and barely visible. The hollows are separated from each other by narrow or wide roller-shaped muri. Together, the hollows and muri give the pyrenes of *Rubus* their characteristic appearance.

The pyrenes are wound by anastomosing vascular bundles that are slightly immersed in the endocarp tissue and form a characteristic reticulate pattern. In species with a hollowed endocarp the vascular bundles run on the ridges of the muri. When the vascular bundles are removed, narrow grooves – vascular traces – are visible on the endocarp surface.

Histological studies of the sectioned endocarp of the European *Rubus* species and their surface using SEM proved that the endocarp structure of all analysed species is generally similar and does not differ substantially from the endocarp structure of *R. strigosus* that was described in detail by Reeve (1954a).

As in *R. strigosus*, the endocarp of all examined species has a stratified structure, however, their inner and outer layers are not always oriented perpendicularly to each other but sometimes diagonally. It is usually clearly visible in places distant from the central part of the pyrene.