Journal of Plant Development
Vol. **32**, Dec **2025**: 19-25
doi: 10.47743/jpd.2025.32.1.982
ISSN 2065-3158 print / e-ISSN 2066-9917
Available online: www.plant-journal.uaic.ro
Research Article

ANATOMICAL STRUCTURE OF THE PERENNIAL STEM IN DIFFERENT *ROSA* L. SPECIES

Lidia ADUMITRESEI[1*], Irina Neta GOSTIN[2]

[1] "Alexandru Ioan Cuza" University of Iași, "Anastasie Fătu" Botanical Garden, 7-9 Dumbrava Roșie, 700487 Iași – Romania.
[2] "Alexandru Ioan Cuza" University of Iași, Biology Department, 20 A Bulevardul Carol I, 700505 Iași – Romania.
[*] Corresponding author. E-mail: lidia.adumitresei@yahoo.com, ORCID ID: 0009-0000-9411-6597

Abstract: The observations comprise a comparative study of the anatomical structure of the perennial stem in the species *Rosa canina* L., *R. damascena* Mill., *R. rubiginosa* L., and R. *rugosa* Thunb., cultivated in the Botanical Garden of Iași.

Cambial activity gives rise to a relatively thin outer ring of secondary phloem and to several rings of secondary xylem, which indicate the age of the examined branch. Within the phloem ring, conducting elements (sieve tubes and companion cells), phloem parenchyma cells (some containing idioblasts), and irregularly dispersed libriform fibres with thickened, lignified walls can be distinguished, these fibres being more abundant in *R. rugosa*. In *R. rubiginosa*, the phloem fibres are arranged in two layers separated by phloem parenchyma. At the periphery of the phloem ring, in contact with the inner layers of the primary cortex, thick bands of sclerenchymatous fibres with very thick, heavily lignified walls (with a punctiform lumen) are visible, especially in *R. canina* and *R. damascena*.

Analysis of the secondary xylem shows its ring-porous aspect in all observed species. Each growth ring displays on its inner side vessels of larger diameter (representing the earlywood), separated by libriform fibres with strongly thickened, intensely lignified walls. On the outer side, each ring contains fewer vessels of smaller diameter, with libriform fibres predominating – a feature common to all examined species. In contact with the pith, remnants of the primary xylem can still be observed, completely lignified and protruding as small bundles into the pith.

All annual rings of secondary xylem are traversed by numerous multiseriate medullary rays, usually of uniform width or variable in *R. canina*. In all cases, the rays consist of radially elongated cells with relatively thin but lignified walls. In all species, the oldest ring of secondary xylem is thicker than the subsequent ones, in which large-diameter vessels predominate.

The pith exhibits a network-like appearance, in which very large, thin-walled parenchyma cells are separated by much smaller cells with moderately thickened but strongly lignified walls.

Key words: anatomy, earlywood, latewood, perennial stem, ring-porous species, *Rosa* spp.

Introduction

Species of the genus *Rosa* are shrubs and therefore possess a woody, aerial, multi-stemmed system with sympodial (anisotonic) branching, exhibiting an orthotropic growth direction in most cases [BUIA, 1956] and more rarely a plagiotropic one, with mesotonic branching [KAUSSMANN & SCHIEWER, 1989]. This is complemented by a basitonic branching pattern that appears with variable periodicity, depending on a series of extrinsic factors such as climatically favourable years and an intrinsic hormonal signal that triggers the rejuvenation of the shrub through the formation of new shoots that replace the old ones [ADUMITRESEI, 2011].

Received: 30 October 2025 / *Revised*: 28 November 2025 / *Accepted*: 3 December 2025

The stem originates in the hypocotyl axis and the gemmule of the seed embryo. After seed germination and throughout the plant's life, the stem grows in length and thickness through the activity of the initial (primary) meristematic tissues and, more importantly, through the activity of the lateral meristems – the cambium and phellogen – of secondary origin, which appear later. These secondary meristems act as two generative zones, giving rise to the definitive secondary tissues [TOMA & GOSTIN, 2000].

In exceptional cases, the stem may reach remarkable dimensions; the largest recorded specimen is a *R. canina* from the Cathedral of Hildesheim, with stem circumferences of 7-8 m and a crown projection of 13 m, its age exceeding 300 years [HEGI, 1923].

The oldest stem was 13-year-old [MESTRE & al. 2014], most of them though stems within a *Rosa* shrub are 4-5 years old. For this reason, we analysed their anatomical structure, especially since such studies are scarce in the specialized literature [PASHINA, 2021] maybe because many of the rose species are difficult to cut using classical methods. The majority of anatomical investigations focus on the primary and secondary structure of the annual shoot, both in classical works [PARMENTIER, 1898; FAHN, 1968; METCALFE & CHALK, 1988] and in modern studies, addressing all aspects from the histological structure of the stem [TOMA & al. 1997; ADUMITRESEI & TĂNĂSESCU, 2005; TÜRKER & al. 2005], to that of the leaf [ADUMITRESEI & al. 2005; ADUMITRESEI & al. 2006; AL-DOSKEY, 2023; ADUMITRESEI & al. 2024], fruit and seed [STARIKOVA, 1973; CHURIKOVA & BARYKINA, 2005], and appendages [ZHOU & al. 2021; ADUMITRESEI & al. 2024].

Materials and methods

Plant material

A comparative study of the anatomical structure of the perennial stem was conducted on *Rosa canina* L., *R. damascena* Mill., *R. rubiginosa* L., and *R. rugosa* Thunb., all cultivated in the Botanical Garden of Iași. Stem fragments were collected from mature shrubs and used for anatomical analysis.

Sample preparation

The samples, consisting of small stem segments, were rinsed several times with distilled water to remove residual ethyl alcohol from preservation. The material was subsequently kept in clean water for a minimum of 2 hours to ensure full rehydration.

Sectioning procedure

Stem fragments approximately 1.5 cm in length were sectioned using a Slee cryotome equipped with a Peltier-based freezing system. The material was frozen at -26 to -28 °C until completely embedded in ice. Transverse sections were obtained at thicknesses of 30-40 µm.

Staining

Histological staining was performed with iodine green for 30 seconds. Sections were then rinsed with ethyl alcohol to remove excess stain, followed by a final rinse in distilled water.

Microscopy and Imaging

Prepared sections were examined using an Olympus BX41 light microscope. Photomicrographs were captured with an Olympus C330 digital camera.

Results and discussion

The secondary structure is especially evident at the level of the central cylinder [TOMA & al. 1997]. The vascular bundles (xylem-phloem bundles) persist within the secondary structure, arranged in a ring and separated by typically narrow medullary rays typical of many *Rosaceae* [FLORIA, 1998; NIJSSE & PUT, 2007; NIKITIN & PANKOVA, 1982].

The phloem contains sieve tubes, companion cells, and few phloem parenchyma cells. The xylem shows vessels arranged in radial rows, separated by cellulosic parenchyma cells toward the pith and by lignified parenchyma and libriform fibres toward the phloem. At the periphery of each vascular bundle, adjacent to the phloem, there is a thick band of sclerenchymatous fibres with very thick but only weakly lignified walls, similar to those of the libriform elements. The vessels typically exhibit simple perforations. According to METCALFE & CHALK (1988), some rose species may show ring-porous or semi-ring-porous xylem, as well as spiral thickenings on the vessels. The paratracheal (vasicentric) parenchyma cells are palisade-like and elongated, with thin walls; their radial walls contain numerous simple, elliptical pits.

In *Rosa*, the multiseriate rays are partly homogeneous, composed of nearly square cells, and partly heterogeneous of the Kribs II type (marked heterogeneity), consisting of square to rectangular cells. Typically, *Rosa* presents 3-5 rays per mm. Growth rings were described by various authors [COSTER, 1927; METCALFE & CHALK, 1988; SCHOCH & al. 2004] as being present, but without distinctive features.

The pith is fully developed even during the herbaceous stages of the annual shoot. It is composed of cells of varying size – large and small – alternating to form a network-like structure, as reported for all species studied by various authors [ADUMITRESEI, 2011; DELINSCHI & al. 2009; LOTOVA & TIMONIN, 1999].

Macroscopically, in transverse section, the “wood” structure of shrubs resembles that of trees; however, clear distinctions between heartwood and sapwood are not evident. Microscopically, it is composed of cellulose, hemicellulose, or lignin fibres that provide mechanical strength and rigidity, and of vascular tissue arranged in a specific configuration through which crude and elaborated sap circulate. These tissues are often associated with parenchyma cells with thickened walls, which contribute to both support and nutrient transport [GLEMEZIU & SUCIU, 1959; ZHANG, 1992].

The following characteristics were observed in transverse sections of the perennial stem and are described below.

***Rosa damascena* Mill.**

Cambial activity gives rise to a relatively thin outer ring of secondary phloem and a number of secondary xylem rings, which indicate the age of the analysed branch.

Within the phloem ring, conducting elements (sieve tubes and companion cells), phloem parenchyma cells (some containing idioblasts), and irregularly dispersed libriform fibers with thickened, lignified walls can be distinguished. At the periphery of the phloem ring, in contact with the inner layers of the primary cortex, thick bands of sclerenchymatous fibres with very thick, heavily lignified walls (with a punctiform lumen) are visible.

Analysis of the secondary xylem shows that it is ring-porous. Each growth ring exhibits vessels of larger diameter on the inner side (representing earlywood), separated by libriform fibers with strongly thickened, intensely lignified walls. On the outer side, each ring contains fewer and smaller vessels, with libriform fibres predominating (Plate II, Figures 7-9).

In a transverse section of a 4-year-old branch, the oldest ring, located adjacent to the pith, contains the smallest-diameter vessels. In this ring, early and latewood are difficult to distinguish, the main difference being the large proportion of libriform fibres in the latewood.

In contact with the pith, remnants of primary xylem are still visible, fully lignified and protruding as small bundles into the pith.

All annual rings of secondary xylem are traversed by numerous multi-seriate medullary rays, composed of radially elongated cells with relatively thin but lignified walls.

The pith exhibits a network-like structure in which very large parenchyma cells with extremely thin walls are interspersed with very small cells with moderately thickened, heavily lignified walls, many of which function as true hydrocysts [ADUMITRESEI & al. 2024] as described by CHURIKOVA & BARYKINA (2005).

***Rosa rubiginosa* L.**

Compared to the previous species (*R. damascena*), only the observed differences are highlighted. In *R. rubiginosa*, within the thickness of the secondary phloem ring, the libriform fibers are arranged in two layers forming tangential chains, separated by phloem parenchyma cells (Plate I, Figures 4-6).

Regarding the secondary xylem, it is noteworthy that the oldest ring, adjacent to the pith, is considerably thicker than the subsequent four rings. In these younger rings, earlywood and latewood are more easily distinguished due to the differences in the transverse size of the vessels, which are otherwise irregularly distributed within the fundamental mass of libriform fibres.

***Rosa canina* L.**

In the secondary phloem ring of *R. canina*, very few libriform fibres are present, while the periphloemic bands of sclerenchymatous elements are thicker (Plate I, Figures 1-3).

In this species too, the internal secondary wood ring is thicker than the others and has smaller diameter vessels throughout its thickness.

In fact, in all species studied, the oldest ring of secondary xylem is thicker than the following rings, in which large-diameter vessels predominate.

The medullary rays vary in width, ranging from biseriate to multiseriate [ADUMITRESEI & al. 2006] as opposed to SCHOCH & al. (2004).

***Rosa rugosa* Thunb.**

The structure of *R. rugosa* is more similar to that of *R. damascena*. Within the thickness of the secondary phloem ring, numerous libriform fibres are present, though irregularly dispersed (Plate II, Figures 10-12).

The borders between annual rings are much clearer, with wider multiseriate rays predominating compared to the other species. In this case as well, the first annual ring is considerably thicker; within it, early and late wood can still be distinguished due to the marked difference in vessel diameter.

Conclusions

The secondary xylem is ring-porous in all species studied.

The annual rings of secondary xylem are traversed predominantly by multiseriate medullary rays. *Rosa canina* is notable for also exhibiting biseriate medullary rays.

The distinction between earlywood and latewood is determined both by vessel diameter and by the amount of libriform fibres. Latewood contains vessels of smaller diameter and a correspondingly higher proportion of libriform fibres.

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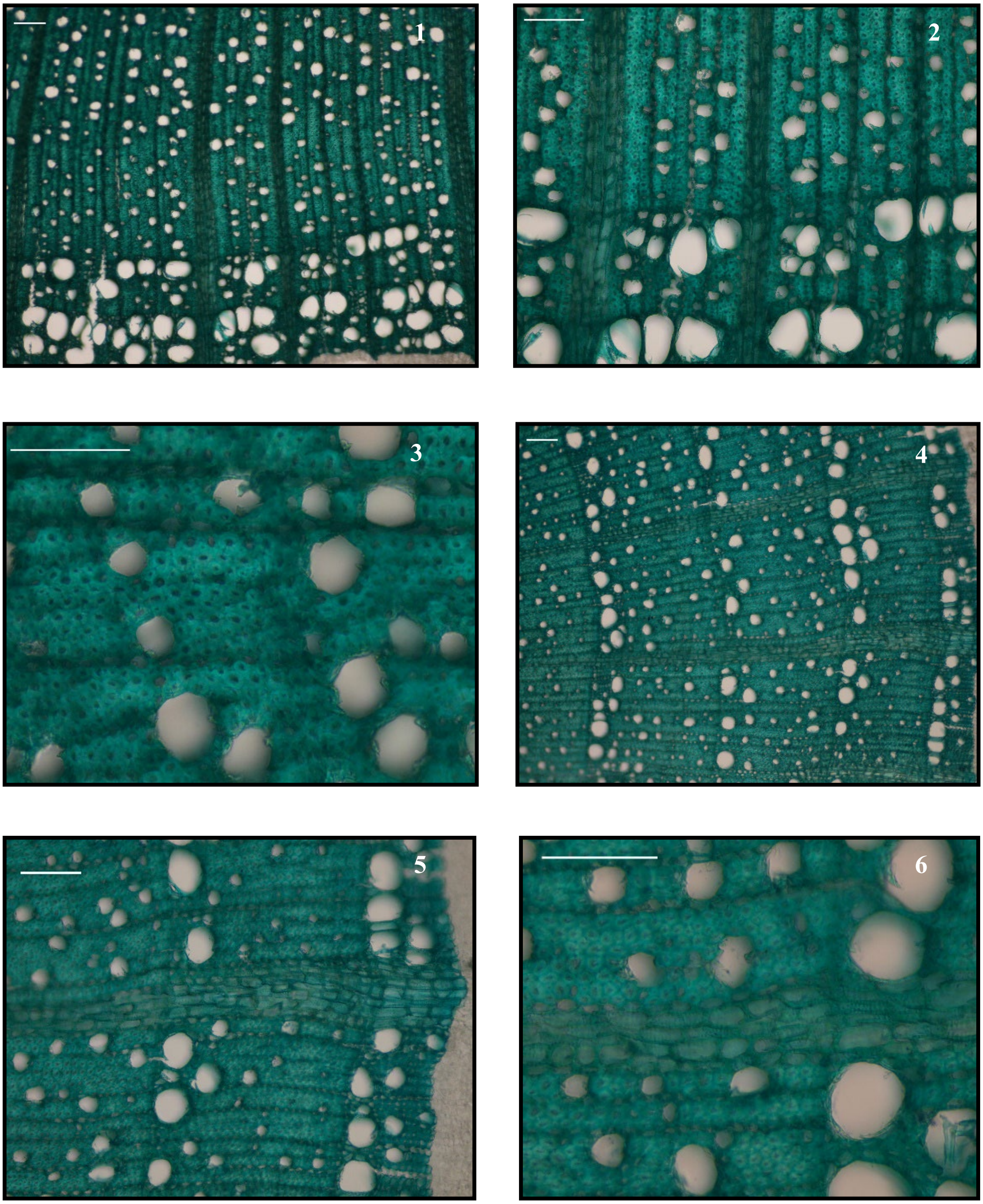
How to cite this article:

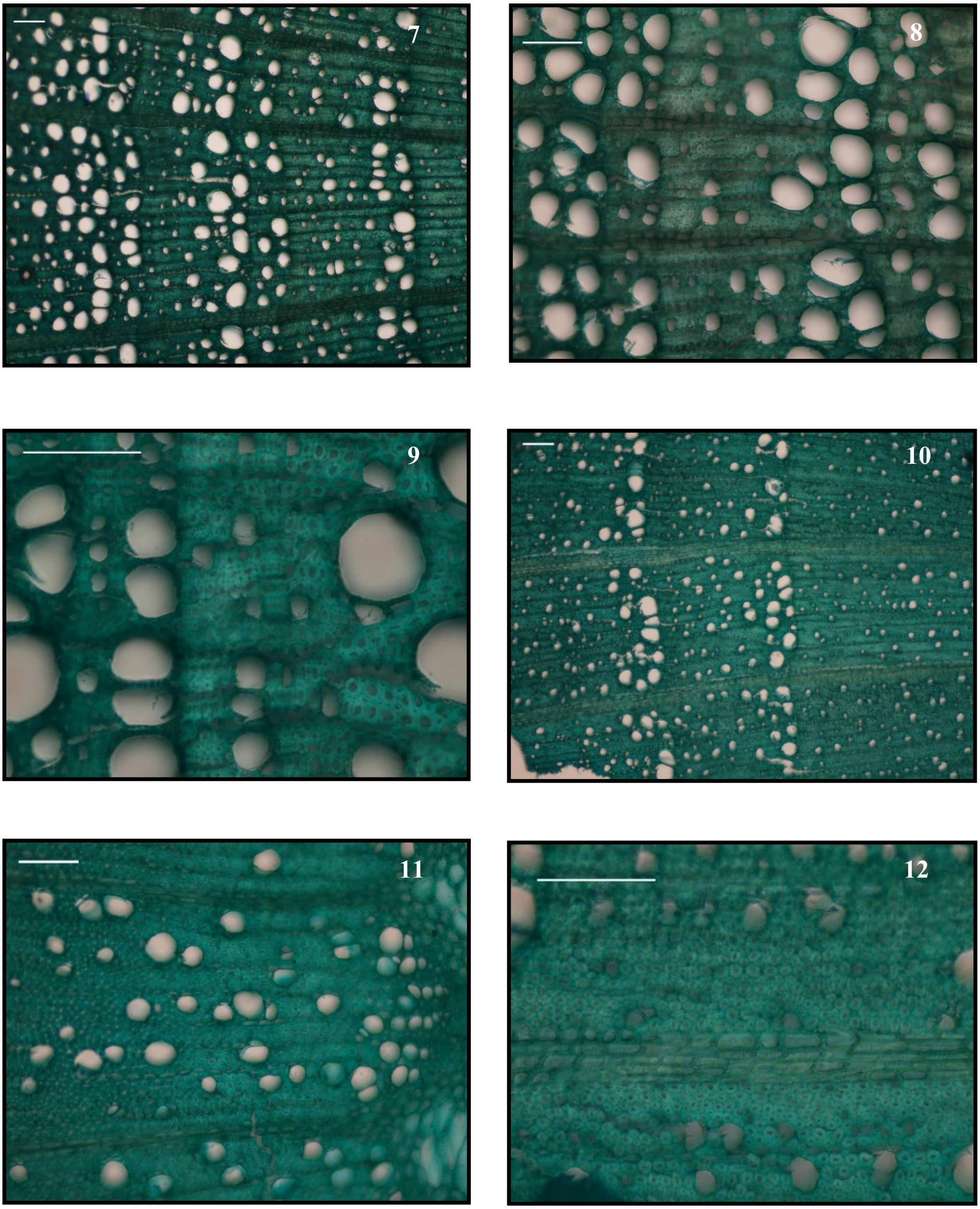
ADUMITRESEI L. & GOSTIN I. N. 2025. Anatomical structure of the perennial stem in different *Rosa* L. species. *J. Plant Develop*. **32**: 19-25. https://doi.org/10.47743/jpd.2025.32.1.982

Plate I



Figures 1-6. Anatomical aspects of the upper third of the stem in *Rosa* L. species – cross section through the stem of: 1-3 *Rosa canina*; 4-6 *Rosa rubiginosa* (scale = 100 µm)

Plate II



Figures 7-12. Anatomical aspects of the upper third of the stem in *Rosa* L. species – cross section through the stem of: 7-9 *Rosa damascena*; 10-12 *Rosa rugosa* (scale = 100 µm)