


## ANATOMICAL CONSIDERATIONS ON THE ANNUAL SHOOT IN SOME WILD ROSES

Lidia ADUMITRESEI<sup>1</sup> , Camelia Mariana IFRIM<sup>1</sup> , Irina Neta GOSTIN<sup>2</sup> 

<sup>1</sup>“Alexandru Ioan Cuza” University of Iași, “Anastacie Fătu” Botanical Garden,  
Dumbrava Roșie Street no. 7-9, 700487 Iași – Romania.

<sup>2</sup>“Alexandru Ioan Cuza” University of Iași, Biology Department,  
Carol I no. 20 A, 700505 Iași – Romania.

\* Corresponding author. E-mail: lidia.adumitresei@yahoo.com, ORCID: 0009-0000-9411-6597

**Abstract:** The observations aim a comparative study of the anatomical structure of the stem and leaf, during anthesis, in some *Rosa* species and varieties (sports), in order to complete the data from the specialized literature because the morphological information is more numerous than the anatomical ones. Moreover, sometimes they are even inconsistent, as in the case of the glands/glandular trichomes on the leaves and stem.

A series of similarities are highlighted, which can be considered conservative characteristics of the *Rosa* genus, as for instance: the rapid transition to the secondary structure, the disposition of the vascular bundles (of collateral type) on a circle, the absence of the phelloderm's activity in the first year, the presence of the crystal idioblasts and the bifacial structure of the leaf. Also, a series of characteristics of each species is highlighted: disposition of the secondary xylem vessels, quantity of libriform fibres, the nature of the walls of the cells from the medullar rays, the thickness of the walls of the sclerenchyma fibres and the presence of the crystal idioblasts in various tissues.

**Keywords:** anatomy, annual shoot, leaf, *Rosa* species, stem.

### Introduction

The *Rosa* L. genus is a vast one, with over 200 spontaneous species in the Holarctic region, spread between 20 and 70 degrees Northern latitude [KRÜSSMANN, 1986].

Arctic and tropical areas are excluded from this region. This genus is heterogeneous, with its species adapted to various environmental conditions. Wild roses grow on all continents in the northern hemisphere, being widespread from the plains to the mountain ranges (even 4000 m – *R. morrisonensis* Hayata in Asia). A few species are only found on plains (*R. setigera* Michx., *R. foetida* Herrm., *R. sempervirens* L.) or flood prone areas (*R. clinophylla* Thory, *R. palustris* Marshall), and only *R. persica* Michx ex Juss. grows in cold desert. They present morphological, anatomical and physiological adjustments [ADUMITRESEI, 2011] related with their diverse ecology.

Ten wild species and two sports were included in the study. Five of these are native in Romania (*R. agrestis*, *R. gallica*, *R. glauca*, *R. rubiginosa* and *R. spinosissima*), while the others originate from Asia Minor (*R. damascena* and *R. foetida*) and East Asia (*R. multibracteata*, *R. multiflora* and *R. rugosa*). The two sports are included in collections due to their significance: *Rosa canina* 'Inermis' is an important rootstock, while *Rosa chinensis* 'Viridiflora' is remarkable for its flowers, which consist solely of bracts.

The reasons for including them in the study stem from the need to update and expand the information regarding this genus, especially considering that scientific morpho-anatomical data in classical literature is fragmented and rather brief, compared to the diversity and

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variability that characterize this genus. It mainly concerns point-based anatomical examples of the main vegetative or reproductive organs or morpho-anatomical aspects as a support for physiological processes. Thus, the studies on the anatomy of the shoot are better represented and refer to: the primary and the secondary structure of the annual shoot [PARMENTIER, 1898; METCALFE & CHALK, 1988; NIKITIN & PANKOVA, 1982; TOMA & al. 1997; TOMA & RUGINĂ, 1998; ADUMITRESEI & TĂNĂSESCU, 2005; ADUMITRESEI & al. 2006; SHAMSO & al. 2019; PASHINA, 2021], prickles' ontogenesis and their peculiarities [UPHOF & HUMMEL, 1962; FAHN, 1968; NAPP-ZINN, 1984], similarities between prickles and glands [KAUSSMANN & SCHIEWER, 1989], anatomical aspects and their taxonomic implication [PARMENTIER, 1898; LOTOVA & TIMONIN, 1999; FATEMI & al. 2008; NIJSSE & al. 2007; ADUMITRESEI & GOSTIN, 2016], anatomical aspects as a support of some physiological processes [HASHIDOKO & al. 2001; CAISSARD & al. 2006; ZAMFIRACHE & al. 2006; ADUMITRESEI & al. 2009; BURZO & TOMA, 2012; MENG & FAN, 2022; TORRE & al. 2023].

### **Material and methods**

The observations aim to provide a comparative study of the anatomical structure [TOMA & GOSTIN, 2000] of the stem and leaf, during anthesis, in *Rosa agrestis* Savi, *Rosa canina* L. 'Inermis', *Rosa chinensis* Jacq. 'Viridiflora', *Rosa damascena* Mill., *Rosa foetida* Herrm., *Rosa gallica* L., *Rosa glauca* Pourr., *Rosa multibracteata* Hemsl. et Wils., *Rosa multiflora* Thunb., *Rosa rubiginosa* L., *Rosa rugosa* Thunb. and *Rosa spinosissima* L., collected from the "Anastasiu Fătu" Botanical Garden of Iași [OSTACIUC, 1994; CAIRNS, 2000].

Shoot fragments were collected during the anthesis period and preserved in ethanol 70%. Sample sections were made with handling microtome using a razor blade. Their coloring was imparted using iodine green and alum carmine or ruthenium red based on a classical method from histo-anatomical studies of plants. Sections were fixed with glycerol gelatin.

Sections were made on the following levels: top of shoot (the middle part of stem, under vegetative cone) and leaves (through petiole at the stipule level, through rachis, on middle zone, through petiole on the first pair of leaflets, through lamina, at the median vein between secondary veins). Furthermore, some superficial sections had been used for epidermis description.

Microphotographs of anatomical sections were taken using Olympus, respectively Olympus BX 41 Microscope and a BH-2 JAPAN camera and an Olympus E 330 digital camera.

### **Results and discussion**

**The stem** generally has a circular contour in cross-section (e.g. *R. canina* 'Inermis', *R. glauca* and *R. rubiginosa*). The contour could be circular irregular, modified by prickles sometimes (as *R. spinosissima*, *R. rugosa* – Figures 6, 7), or elliptical (as *R. agrestis* and *R. chinensis* 'Viridiflora' – Figure 2).

At some taxa non glandular trichomes and glandular trichomes appear [WANG & al. 2019; ZHOU & al. 2021]. Non glandular trichomes appear only in juvenile stages at some species. Small glandular trichomes are present at *R. spinosissima*, even bigger at *R. agrestis*, *R. damascena*, *R. rugosa* and *R. rubiginosa*, they always have multicellular pedicel and it is pluriseriate, which consists of 4-6 or sometimes even 8 cell series. The glandular trichomes have

a terminal clavate part which is formed of polygonal cells with cellulosic walls; their number is variable, but always in the tens of cells.

In all cases, epidermal cells are covered by cuticle. It can be very thick with intercellular cuticle blades very deeply inside (for *R. multibracteata*, *R. rubiginosa* and *R. rugosa*), thick (*R. agrestis*, *R. chinensis* 'Viridiflora', *R. foetida*, *R. glauca*, *R. gallica* and *R. spinosissima*) or relatively thin (*R. canina* 'Inermis') (Figure 4).

Epidermal cells are either isodiametric (e. g. *R. chinensis* 'Viridiflora', *R. damascena*, *R. glauca*, *R. rubiginosa* and *R. rugosa*) or tangentially elongated (*R. foetida*, *R. gallica*, *R. spinosissima* and *R. multiflora*) (Figure 8). Regarding epidermal cells size, it is usually constant, an exception being the prickle area where epidermal cells are slightly radially elongated. The external wall of epidermal cells is thicker than the walls of other walls of the same cells. The external walls are very thick for *R. canina* 'Inermis', *R. glauca*, *R. multibracteata*, *R. multiflora* and *R. rugosa* (Figures 4, 5 and 6).

Stomata are situated either at level of epidermal cells in *R. canina* 'Inermis', or slightly above the level of epidermal cells at *R. multibracteata*. An exception is *R. glauca*, in which stomata can be observed both at the level of the epidermal cells or at a nearby height (either above or below that level) [ADUMITRESEI & TĂNĂSESCU, 2005]. The substomatic chamber can be reduced in *R. glauca*, with a medium size (in *R. canina* 'Inermis') [ADUMITRESEI & al. 2006], the suprastomatic chamber, relatively tall, can be observed in *R. damascena* as a result of the presence of tall annex cells.

Prickles are always present on the stem surface in variable numbers, more frequently in *R. spinosissima* and *R. rugosa*. They have different sizes and shapes depending on the species. In section, it is observed that the shape of the cells changes from isodiametric cortical cells (at the base) to radially elongated parenchymal cells on the flanks and towards the tip of the prickles. The older prickles get, do they become sclerified and lignified, becoming harder, and the epidermis on their flanks exfoliated (Figures 19, 20).

Fully developed prickles are based on a multilayered suberous tissue to which exterior the parenchyma is moderately sclerified and lignified, which in time leads to exfoliation.

In *Rosa rugosa*, in most of the stem circumference, in a hypodermic position, the phellogen differentiated, producing 6-8 layers of suber outwards and several layers of phellogen inwards. These anatomical features prove the fact that it is the most resistant species to low temperatures among the ones selected by this study.

The primary cortex is differentiated in an external collenchymatous area and an internal area cortical parenchyma of meatic type.

**The hypodermal collenchyma** is of the tangential type and is presented either as a continuous area with different thickness (in *R. agrestis*, *R. chinensis* 'Viridiflora', *R. foetida* and *R. rugosa*) or as collenchyma cords (larger or smaller) separated by some assimilating parenchyma islands (in *R. canina* 'Inermis', *R. damascena*, *R. gallica*, *R. multibracteata* and *R. multiflora*) or in an angular type as in *R. spinosissima* (Figure 7), which allows the shoot to grow in thickness. In *R. glauca* and *R. rubiginosa*, the entire hypodermic layer is collenchymatous [SHAMSO & al. 2019].

The number of cell layers included in the collenchyma structure is variable depending on taxa, but it is well represented in most cases. The cortex generally consists of 14-16 cell layers, from which 4-6 layers of external cells, in most species (*R. chinensis* 'Viridiflora'), are represented by collenchyma. The internal area of cortical parenchyma of the meatic type is composed of cells whose sizes are growing from beneath epidermis up to the central cylinder

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(Figures 3-6, 15, 18). In most taxa, cells are isodiametric, but tangentially elongated cells can be found, for instance, in *R. multibracteata* (Figure 15).

Cortex thickness is variable in each taxon, being thin in *R. rubiginosa* and very thick in *R. canina* 'Inermis', *R. damascena*, *R. foetida*, *R. glauca* and *R. spinosissima*.

**Central cylinder** consists of a variable number of collateral vascular bundles, with a circular arrangement, and pith. The central cylinder is usually relatively thick, situated on the upper limit in species such as *R. multibracteata*, *R. canina* 'Inermis', *R. glauca*, *R. rubiginosa* and *R. rugosa*.

The number of vascular bundles is variable, yet constant for each taxon: many (over 60) at *R. canina* 'Inermis', fewer (about 35-40) for *R. rubiginosa* and *R. glauca*; relatively few (about 25-30) in *R. agrestis*, *R. chinensis* 'Viridiflora' and *R. rugosa*, even less (about 20-22) in *R. multibracteata* and *R. chinensis* 'Viridiflora' (Figures 1-6).

The vascular bundles are arranged in a perfect circle (in *R. canina* 'Inermis', *R. damascena*, *R. gallica*, *R. multibracteata*, *R. multiflora*, *R. rugosa* and *R. rubiginosa*) (Figures 1, 5, 11, 17, 19), or they can be organized in a tortuous circle (e.g. *R. agrestis*, *R. glauca*, *R. rubiginosa* and *R. spinosissima*) (Figures 4, 6, 18). The general architecture of the central cylinder in *R. chinensis* 'Viridiflora' is rather interesting, for in this case the vascular bundles are no longer strictly arranged on a circle, but on several arcs that converge, so that the xylem is always oriented towards the pith. Each of the arcs presents two types of bundles, collateral ones and other formed by elements of phloem only. Between the bundles of the same arch, medullary rays are extremely narrow (one or two series of cells), whereas between arches of bundles, medullary rays are quite large (5 or 8 series of cells) (Figure 2). This atypical arrangement of the vascular bundles has also been observed in several horticultural varieties of *Rosa*, such as 'Laminuette', 'Luchian', 'Rose Gaujard', and 'Hurdal' [ADUMITRESEI & al. 2005; ADUMITRESEI & al. 2012; TOMA & TOMA, 2005; MONDER & al. 2021].

Generally, bundles' sizes vary within the same taxon; however, in the case of *R. agrestis*, *R. damascena*, *R. foetida*, *R. multibracteata*, and *R. multiflora* they can be approximately equal (Figures 15, 18, 19, 21, 23).

Concerning the structure of vascular bundles in the annual shoot, we highlight the following observations for investigated taxa: in the most analysed species cambium generates less phloem outward and more xylem inward (Figures 18, 19, 23, 25). The presence of cork cambium and its differentiation into 4-6 layers of phelloderm on the outer layer, with just a few layers of cork cambium towards the inner part, is noticeable only in *R. rugosa*, which is the most resistant to low temperatures among all the species included in the study (Figure 16).

The position of secondary xylem vessels is sometimes in radial and parallel rows (as in *R. canina* 'Inermis', *R. glauca*, *R. multiflora* and *R. rugosa*), in other cases it is irregular within the libriform mass (as in *R. agrestis*, *R. chinensis* 'Viridiflora', *R. gallica*, *R. rubiginosa*, *R. multibracteata* and *R. spinosissima*).

The amount of libriform in the secondary xylem is as follows: abundant (in *R. agrestis*, *R. damascena*, *R. gallica*, *R. multibracteata* and *R. spinosissima*) and reduced (in *R. canina* 'Inermis').

**Medullary rays** are relatively thin, often uniseriate, and generally very thin (*R. canina* 'Inermis', *R. damascena*, *R. foetida*, *R. multibracteata* and *R. rugosa*), with some bundles which form a continuous ring inside of the xylem, because here pith rays are slightly sclerified and lignified (in *R. agrestis*, *R. gallica* and *R. rubiginosa*) (Figures 21, 22). In most cases, medullary rays consist of 2-3 cell rows. As mentioned before, *R. chinensis* 'Viridiflora' has extremely large rays between arches (8 rows of cells), making it an exception within the *Rosaceae* family.

Regarding the composition of cell walls in medullary rays: at the phloem level, they are parenchymatic, cellulose, while at the xylem level they are moderately sclerified and lignified (in *R. glauca*, *R. damascena*, *R. multibracteata*, *R. multiflora* and *R. rugosa*); in some taxa, the cell walls of medullary rays from the xylem are slightly lignified and sclerified (in *R. agrestis*, *R. foetida* and *R. rubiginosa*) (Figures 18, 21, 22).

**Perivascular sclerenchyma** bundles are generally well represented at stem level. The differences are related to walls of sclerenchyma fibres, which can be: very thick and fully lignified with lumen cells that are almost punctiform (*R. agrestis*, *R. gallica*, *R. multibracteata*, *R. rubiginosa* and *R. rugosa*), strongly thickened, but partially lignified (*R. multiflora*), moderately thickened, but completely lignified, with a large lumen (*R. damascena*) or strongly thickened and partially lignified (*R. canina* 'Inermis' and *R. glauca*) (Figures 21, 22, 23, 25). In the perimedullary area, cells have lignified walls. Similar structures are mentioned by DELINSCHI (FLORIA) & al. (2009).

The configuration of the pith is similar in *Rosa* species and varieties. It is formed by very small cells or islands of cells, with moderately thickened and lignified walls, surrounded by extremely large cells with very thin, cellulose walls, often arranged in a rosette with respect to the small cells, suggesting a pattern (Figure 26).

**Crystal idioblasts** are found in various tissues, depending on cultivar or hybrids. Generally, they are relatively common, as in other *Rosaceae* species [METCALFE & CHALK, 1988; TOMA & al. 1983; FLORIA & DERID, 1998; FLORIA, 1998; LERSTEN & HORNER, 2006]. Some taxa predominantly contain druses while others are more likely to have prismatic crystals (Figures 16, 17, 22, 23, 24). Both forms can coexist in certain taxa, though one type may predominate in different tissues. For example, crystal idioblasts are present in hypodermic collenchyma layer contain either druses (*R. chinensis* 'Viridiflora', *R. glauca*, *R. rugosa*, *R. spinosissima* sometimes grouped together, *R. rubiginosa* relatively rare) or prismatic crystals (*R. foetida*).

Within of the cortical parenchyma, crystal idioblasts containing druses are present in *R. glauca* and *R. multiflora*. Some species, such as *R. chinensis* 'Viridiflora', *R. damascena*, *R. glauca* and *R. rubiginosa*, have only a few crystal idioblasts at the cortex level.

In the phloem parenchyma, we identified cells with druses in *R. glauca* and *R. multiflora* or both druses and fewer prismatic crystals in *R. rugosa*. *R. rubiginosa* contains fewer druses and more prismatic crystals. Species such as *R. damascena* and *R. multibracteata* are relatively poor in crystal idioblast cells at bark level.

In the perimedullary area and at the level of the medullary rays, crystal idioblast cells are more frequent in *R. damascena* (containing druses), *R. multibracteata* (containing druses and prismatic crystals).

Inside the pith there also are cristal idioblasts (containing druses) at *R. multibracteata*, *R. rugosa* and *R. glauca* (also appearing prismatic crystals). Crystal idioblasts are missing from the pith from *R. damascena* and *R. multiflora*.

"Hydrocytes" are present both in the pith (in *R. damascena*, *R. glauca*, *R. rubiginosa*, *R. multibracteata*) and in the perimedullary area (in *R. agrestis*, *R. damascena* and *R. gallica*). Sometimes, they are present on the periphery of periphloemic sclerenchyma (Figure 18). These structures, characterized by thickened and lignified walls with visible punctations, have been assimilated to "hydrocytes" [ANDRONACHE & al. 2006; ABDINAZAROV & al. 2017]. The term "hydrocytes" was described especially in Russian botanical literature as part of a system that ensures the flow of nutritive and biologically active substances in areas of active growing processes [CHURIKOVA & BARYKINA, 2005; CHURIKOVA & BARYKINA, 2015].

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In both wild roses and cultivated hybrids, the growth process is very fast; in spontaneous species and climbing roses, shoots go through the phenophases from bud to flowering in almost 3 weeks, reaching lengths of approximately 15-40 cm, and cultivated hybrids go through these phenophases in a month, reaching lengths ranging between 20 and 80 cm, sometimes even 160 cm. Also, in roses, the annual shoot has an intermediate “behavior” between herbaceous and woody plants in terms of growth, a fact supported both anatomically and physiologically. It is likely that this type of growth is correlated with the hydrocytic system that is present in the stem and the leaf, respectively in several types of tissues in both organs.

**The petiole, at the level of the stipule**, typically has a semicircular contour in cross-section, although it is rarely semielliptical. This shape is modified by two adaxial wings, represented by the stipule, which has an adaxial surface with a groove that can vary in width. Sometimes, the adaxial surface is almost flat (Figures 27, 29).

Epidermal cells are either isodiametric (*R. canina* 'Inermis', *R. glauca*, *R. damascena* and *R. multiflora*) or slightly tangentially elongated (*R. multibracteata*).

The cuticle can be thick (*R. chinensis* 'Viridiflora', *R. damascena*, *R. multiflora*, *R. multibracteata*, *R. rubiginosa* and *R. rugosa*) or thin (*R. canina* 'Inermis' and *R. glauca*).

Non glandular trichomes are present on both the petiole and the rachis surfaces in *R. agrestis*, *R. foetida* and *R. rugosa* species (some of them very long, others are shorter, fairly uniformly distributed). In *R. damascena*, non-glandular trichomes are of various sizes, primarily located on the adaxial face, though they are relatively rare. In *R. multiflora*, non-glandular trichomes are few, small, and mostly found in the adaxial groove (Figures 27, 29, 30, 31-33, 36). Generally, trichomes are most abundant on the bases of young leaves and gradually decrease in density toward the top. In all cases, non-glandular trichomes have very thick walls and an almost filiform lumen.

Glandular trichomes are present in all species, particularly on the stipule extremities, and are most frequent in *R. agrestis*, *R. damascena*, *R. foetida*, *R. multiflora*, *R. multibracteata*, *R. rubiginosa* and *R. rugosa*. In species such as *R. canina* 'Inermis', *R. glauca* and *R. gallica*, secretory glands are primarily located on the edge of the stipule and they tend to be scarce. Some taxa have glands both on the edge of stipules and on the rachis, the lower epidermis of leaflets or stipules (*R. agrestis*, *R. spinosissima*, *R. rubiginosa*, *R. rugosa*, more rare at *R. damascena*) (Figure 29). These glandular trichomes have a multicellular pluriseriate foot and clavate terminal head. In cross-section, the terminal head features a pluricellular core composed of isodiametric cells, surrounded by an external layer of elongated cells, the structure of which varies depending on the species (Figures 27-30, 31-33, 44).

In *R. rugosa* sepals there were observed [SULBORSKA & WERYSZKO-CHMIELEWSKA, 2014] similar structure called multicellular glandular trichomes, but it is specified that they have similar structures with colletes, already mentioned by FAHN (1968) and DALVI & al. (2024) in the *Rosa* genus. We found this type of emergence in *Rosa agrestis* on the rachis of the leaf (Figure 27).

**Hypodermic collenchyma**, of tangential type, is typically composed of 3-4 cell layers in most species, but is thinner in *R. damascena*. We observed angular collenchyma in *R. multibracteata* and *R. spinosissima* (only on the abaxial face of the petiole) which differs from the one found in the stem (Figures 35 and 43). In some cases, the collenchyma layers contain cells with druses (*R. spinosissima*), prismatic crystals (*R. rubiginosa* and *R. rugosa*) or both types (*R. glauca* and *R. multibracteata*). In *R. glauca* and *R. rubiginosa*, all the hypodermic layers are colenchymatous.

Cells with prismatic crystals are more frequent in *R. rugosa* and *R. multiflora*, and the ones with prismatic crystals are more common in *R. damascena* (Figure 38).

The number of vascular bundles varies from 3, in *R. chinensis* 'Viridiflora', *R. multiflora*, *R. gallica*, *R. rugosa* and *R. spinosissima* to 5-6 in *R. damascena*, *R. glauca*, *R. foetida*, *R. canina* 'Inermis' and *R. rubiginosa*. Sometimes, periphloemic sclerenchyma bundles are interrupted by medullary rays, which can be narrower (*R. multiflora* and *R. rubiginosa*) or larger (*R. damascena*). This suggests that multiple smaller bundles may merge to form a larger one.

The size of the bundles typically decreases, from the centre toward the periphery. In some cases, several bundles from the central area are approximately equal in size (*R. canina* 'Inermis').

The secondary structure at the level of vascular bundles is present in *R. multibracteata*. Primary and secondary structures at this level are found in *R. chinensis* 'Viridiflora', *R. damascena*, *R. foetida*, *R. rubiginosa* and *R. rugosa*, while only the primary structure appears in *R. canina* 'Inermis', *R. glauca* and *R. spinosissima*.

In all cases, vascular bundles have sclerenchyma at the periphery of the phloem forming thick sheaths (*R. canina* 'Inermis' and *R. glauca*), which can be interrupted by medullary rays more or less narrow (*R. multibracteata*, *R. multiflora* and *R. rubiginosa*).

Periphloemic sclerenchyma contains cells with very thick lignified walls, resulting in a reduced lumen, in most cases. However, in *R. multiflora*, cells with a larger lumen can be found, although they are completely lignified. In other cases, such as in *R. rubiginosa*, the cells have partially lignified walls but are still thick.

Stipules vary in width, being either wider or narrower, and are relatively thin. Structurally, the mesophyll consists of homogeneous parenchyma, composed of 2-4 layers of more or less elongated cells, with small air spaces in species with narrow stipules (*R. glauca*, *R. multibracteata*, *R. multiflora* and *R. spinosissima*). In species with wider stipules (*R. canina* 'Inermis' and *R. rugosa*), the mesophyll is differentiated into palisade and spongy tissue throughout the length of the stipule (*R. chinensis* 'Viridiflora') or only along the edge of the stipules (*R. agrestis*, *R. damascena*, *R. foetida* and *R. gallica*). In some cases, the homogeneous parenchyma is replaced by collenchyma, as observed in *R. canina* 'Inermis' and *R. multiflora*.

The non-glandular trichomes are present on the surface of the stipules in *R. agrestis*, *R. foetida*, *R. multiflora*, *R. rubiginosa*, *R. rugosa* and *R. spinosissima*. Glandular trichomes are found either only on the edges of the stipules in *R. agrestis*, *R. canina* 'Inermis', *R. chinensis* 'Viridiflora', *R. multibracteata*, *R. spinosissima*, *R. rubiginosa* and *R. rugosa* (Figure 28 and 30), or across their abaxial surface (*R. multiflora*).

**The rachis**, in its median position, has the same structure plan as the petiole. The cross-section contour is almost circular but modified by latero-axial crests, sometimes almost parallel, sometimes slightly divergent, which delimits a more or less wide groove, but usually shallow. The number of vascular bundles is maintained as in the petiole, though the central bundles may be grouped together to form a larger one. At this level, the structure is mainly of primary origin with some secondary development. (Figures 31, 34, 35, 39). Also, we can notice the presence of "hydrocytes", at both poles of the xylem and phloem. Hypodermic collenchyma is almost continuous in all investigated cases (Figure 41). The sclerenchyma is not continuous in periphloemic position; it is interrupted at intervals by groups of parenchymatic cells, sometimes containing "hydrocytes" between them, which are more frequent in *R. gallica* (Figures 40-42).

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Both the petiole at the stipule level and the rachis, including the midrib of the last leaflet display spines on the inner face. These spines are composed of rows of cells with strongly lignified, thick walls, especially in the outer layer. In species such as *R. canina* 'Inermis', *R. glauca*, and *R. gallica*, the spines are smaller and fewer in number.

The **petiole of the leaflets** has a similar structure to that of the midrib of the leaflet lamina with a notable presence of mechanical tissue surrounding the vascular bundles. In these cases, only collenchyma elements are present within the periphloemic area, alternating with sclerenchyma cells that have lignified walls. In species where sclerenchyma is present in the periphloemic areas, lignified parenchyma cells are found intermittently. For example, *R. chinensis* 'Viridiflora' and *R. foetida* exhibit sclerenchyma cells with thinner, more lignified walls. Both on the rachis and, especially, on the petiole of the leaflets, non-glandular trichomes and/or glandular trichomes are present, similar to those on stem or other leaf parts, often being more frequent at this level.

At the level of the leaflet lamina the midrib has a structure similar to that of the rachis and petiole, with the distinction that on this level conductive tissues form a single collateral bundle, which usually has a primary structure.

The periphloemic sclerenchyma fibres are absent in many species (*R. damascena*, *R. foetida*, *R. rubiginosa*), or they are present as a continuous ring, with cells having cellulosic walls in *R. multibracteata*, well developed in *R. spinosissima* and *R. multiflora*, or discontinuous in *R. rugosa*.

The upper epidermis has its external wall thickened and cutinized. The cuticle is very thick at *R. multibracteata* species. Notably, air space cavities with irregular contours are present on both flanks of the midrib in *R. rugosa*.

The palisade represents more than half of the mesophyll's surface in species such as *R. agrestis*, *R. gallica*, *R. glauca*, *R. multibracteata*, and *R. multiflora*.

The **leaflet's lamina** has a bifacial structure at all investigated taxa, the mesophyll is differentiated in palisade parenchyma under adaxial surface and spongy parenchyma under abaxial surface.

*Palisade parenchyma* is generally bistratified (Figures 45, 46, 54) consisting of tall cells. In some species, such as *R. agrestis*, *R. chinensis* 'Viridiflora', *R. gallica*, and *R. foetida*, it appears tristratified; in these cases, the inner layer is composed of much shorter cells (Figures 48, 49, 53, 58).

*Spongy parenchyma* consists of 3-5 layers of cells in most cases; the variation limits are from 2-3 cell layers (*R. chinensis* 'Viridiflora', *R. rugosa*) up to 3-4 layers of cells. Based on size and frequency of air spaces of spongy tissue, we identified two following situations: very lax (*R. chinensis* 'Viridiflora', *R. damascena* and *R. multibracteata*) (Figure 46) and relatively lax (*R. agrestis* and *R. multiflora*) (Figures 48, 49, 52).

Regarding the shape of spongy parenchyma cells, several patterns can be distinguished: *R. foetida* shows both round and elongated cells in the multilayered spongy tissue, *R. glauca* has small, tangentially elongated cells, *R. spinosissima* features a compact spongy parenchyma where the cells are almost vertically oriented, creating the appearance that the palisade tissue is continuous from one epidermis to another, and *R. glauca* which has narrow cells arranged into a horizontal disposition. The epidermal cells are larger in the superior epidermis than the inferior one. Larger cells were found in *R. chinensis* 'Viridiflora', *R. gallica*, *R. glauca*, yet the specie *R. gallica* shows considerably larger cells in the lower epidermis than in many other species.

The contour of epidermal cells is different from one taxon to another and, in some cases, from upper epidermis to lower epidermis, even at the same taxon: polygonal cells with



straight walls were identified inside of upper and lower epidermis (in *R. multiflora*, *R. rubiginosa* and *R. rugosa*); cells with an irregular shape with slightly wavy walls in both epidermis (*R. glauca* and *R. multibracteata*); polygonal cells with slightly wavy walls in upper epidermis, and slightly wavy in the lower epidermis with lateral walls almost sinuous (*R. canina* 'Inermis' and *R. damascena*). These results are in concordance with those observed in similar taxa originated from areas with different climate conditions [AL-DOSKEY, 2023]. Papilliform cells occur in the inferior epidermis in *R. rugosa*, as METCALFE & CHALK (1988) mentioned (Figure 56).

Notably, in *R. chinensis* 'Viridiflora', the upper epidermis, when viewed from above, consists of cells with flat lateral walls. Among these, there are isolated, slightly circular cells surrounded by the polygonal ones, forming a rosette-like pattern. The size of polygonal cells is variable, while the circular cells are uniform in size.

A particular feature occurs in *R. rugosa*, where a layer of isodiametric, colourless cells acts as a hypoderm between the palisade parenchyma and the upper epidermis. This layer results from the division of cells through the tangential walls of the initial epidermis. A similar structure is found on the lower surface of the lamina, many spongy cells are idioblasts, predominantly containing druses.

Additionally, tangential wall divisions are observed in some cells of the upper epidermis of *R. canina* 'Inermis', where two layers of unequally sized cells are formed in scattered spots.

The cells of the upper epidermis have thickened (*R. rubiginosa*) and cutinized (*R. chinensis* 'Viridiflora' and *R. multiflora*) external walls, with the exception of *R. canina* 'Inermis' and *R. glauca*, which have thinner, less cutinized walls. The two latter are more sensitive to attacks by phytopathogenic agents than other taxa that have thicker cuticles.

*Stomata* are located in the lower epidermis. They are by anomocytic type, a characteristic of the *Rosaceae* family. Stomata are numerous in *R. canina* 'Inermis', *R. damascena*, *R. multiflora* and *R. multibracteata* (Figures 59). The phenomenon of stomatal clustering within the axils of veins, characteristic for hybrids, occurs in species such as *R. damascena*, *R. multibracteata* and *R. multiflora*. Also, there is sometimes a tendency for stomatal cells to be positioned close together, separated by just a single epidermal cell (*R. canina* 'Inermis' and *R. damascena*). Typically, stomata are located at the same level of epidermal cells. In *R. damascena*, we also observed a suprastomatic chamber, a feature shared with *R. gallica*, where stomata are placed above the epidermal cells and form a tall substomatic chamber.

The second wall of division in epidermal cells, a characteristic feature of the *Rosaceae* family, sometimes appears at the level of the upper epidermis (*R. canina* 'Inermis', *R. damascena* and *R. rugosa*) or at level of the lower epidermis (*R. multibracteata* and *R. rugosa*).

*Crystal idioblast*, which are relatively frequent in the foliar lamina, as in other *Rosaceae* species, are present in various tissues. In the palisade parenchyma they occur as prismatic crystals and druses (*R. canina* 'Inermis', *R. damascena*, *R. gallica*, *R. multibracteata*, *R. multiflora* and *R. spinosissima*), or only as druses (*R. rugosa*). In spongy parenchyma they appear as prismatic crystals and druses (*R. canina* 'Inermis', *R. damascena* and *R. spinosissima*); in the phloem they are found in form of prismatic crystals and druses (*R. damascena*, *R. multibracteata*, *R. multiflora* and *R. spinosissima*); along to the main veins or their lateral veins many crystal idioblast cells with druses are present (*R. canina* 'Inermis' – under lower epidermis, *R. multiflora* – in both epidermis) or prismatic crystal (*R. damascena* and *R. multiflora*) (Figures 45, 47, 52, 54, 56, 57, 60).

## Conclusions

The general structural plan is conservative. Anatomical investigations reveal rapid development of secondary structure as characteristic of the taxa, with the primary structure maintained only beneath the shoot apical meristem. The transition to secondary structure is primarily due to intense cambium activity in most cases. Phellogen activity during anthesis is observed only in *R. rugosa*, where phelloderm and suber is produced in a hypodermic position.

The hypodermic collenchyma is most often of the tangential type. *R. spinosissima* is distinguished by angular collenchyma arranged in a continuous layer.

Vascular bundles are commonly arranged in a circular pattern, with exception of *R. chinensis* 'Viridiflora'. Secondary xylem is sometimes arranged in radial, parallel strings. Libriform fibres from the secondary xylem are more abundant in some species, but found in smaller quantities in most. Sheaths of periphloemic sclerenchyma are well-distributed, with varying wall thicknesses and lower lignification in some cases.

The leaf is bifacial, typically with bistratified palisade tissue, sometimes tristratified with a smaller inner layer. Spongy tissue consists of 3-5 layers, often thin. Stomata are numerous and often grouped in the axils of the veins, characteristic of hybrids. Crystal idioblast, including druses and prismatic crystals, are present in various tissues.

The pith consists of large, thin-walled, cellulosic cells surrounded by smaller, lignified cells in a network. Non-glandular trichomes are found on the petiole and rachis, covering the external layer or adaxial groove.

Glandular trichomes are more abundant at the edges of stipules.

The second division wall, typical of the Rosaceae family, is present at the epidermal cell level, occasionally in the upper or lower epidermis.

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## ANATOMICAL CONSIDERATIONS ON THE ANNUAL SHOOT IN SOME WILD ROSES

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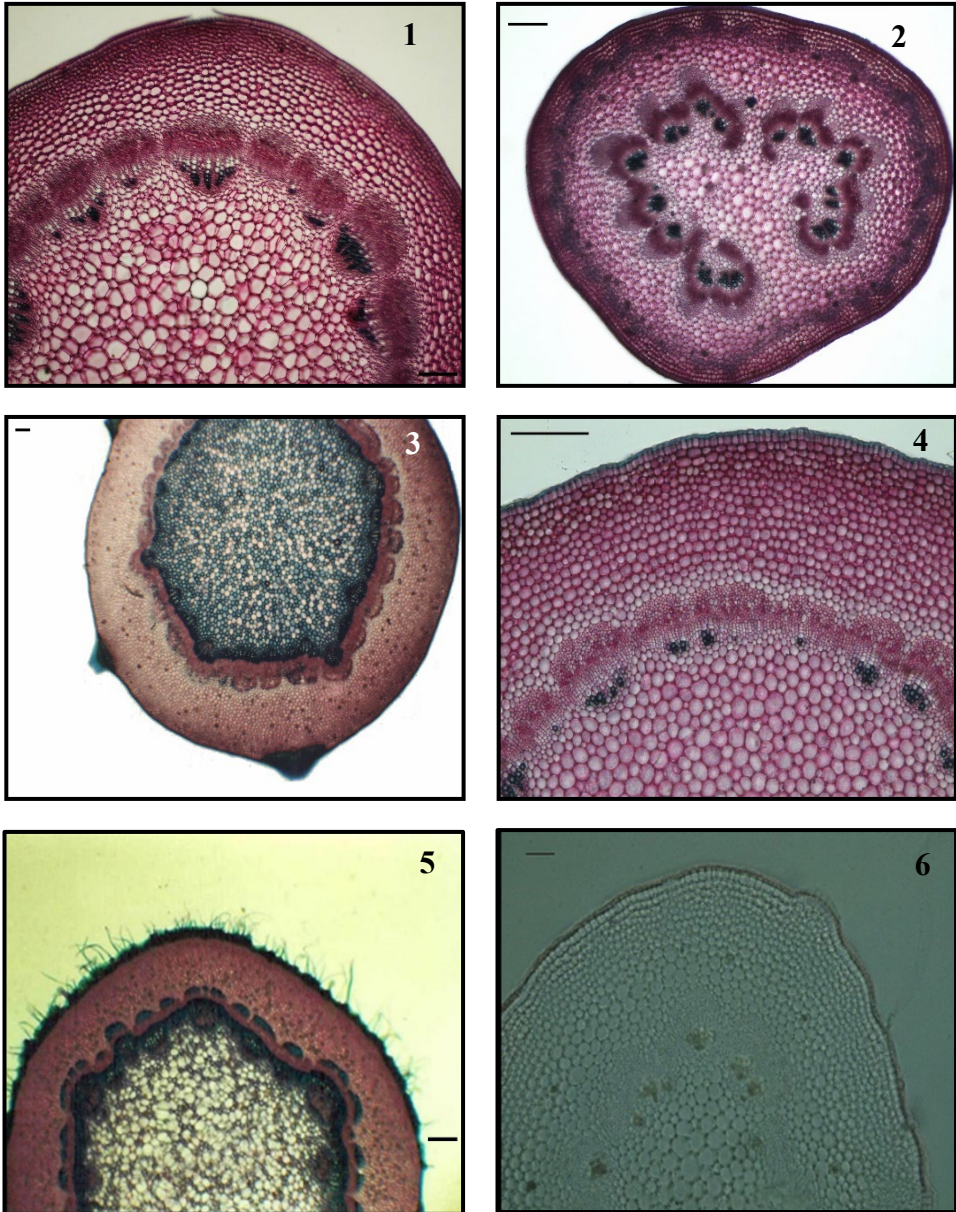
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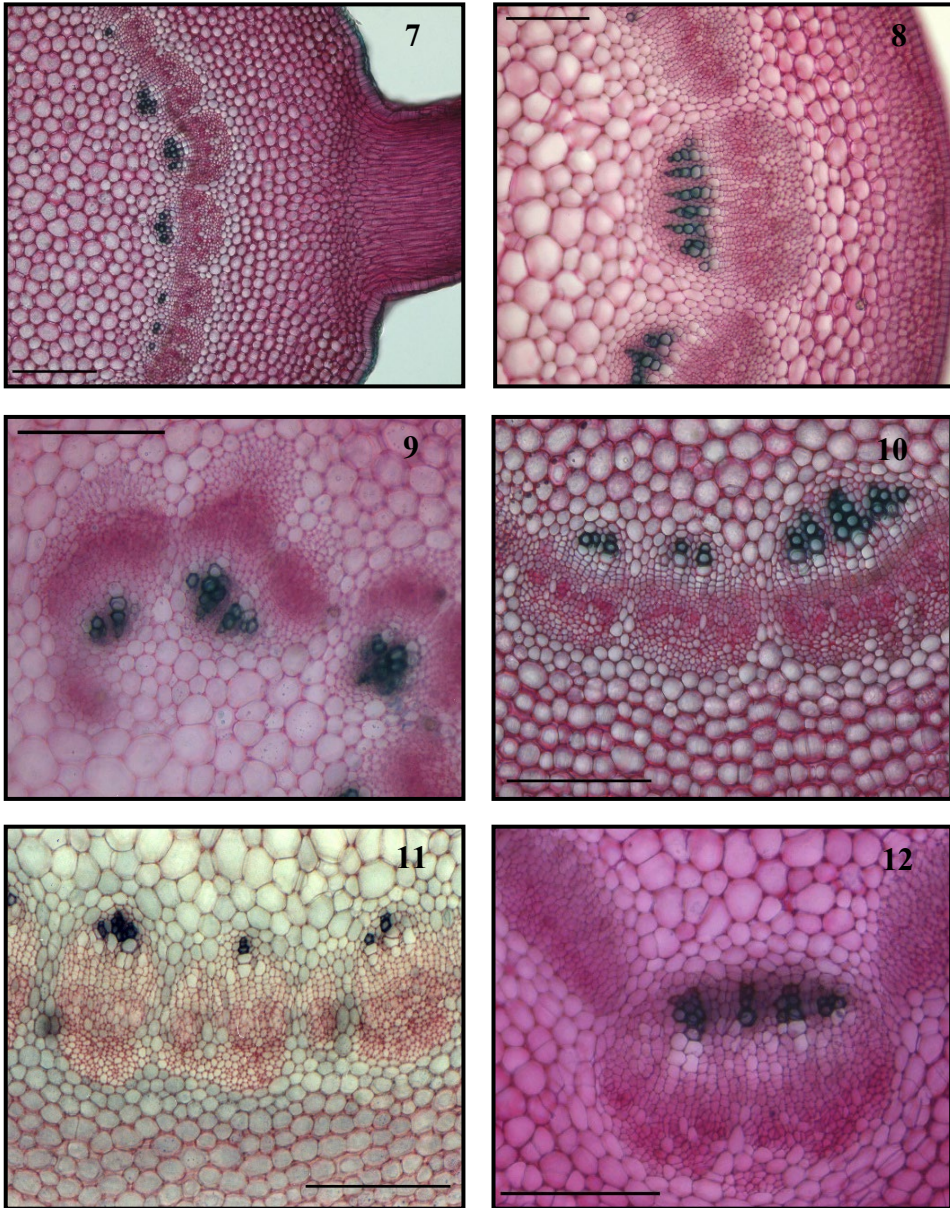
## Plate 1



**Figures 1-6. Anatomical aspects - upper third of the stem (scale = 100 μm):** 1. Cross section through the stem of *Rosa damascena*; 2. Cross section through the stem of de *Rosa chinensis* 'Viridiflora' (overview); 3. Cross section through the stem of *Rosa rubiginosa* (overview); 4. Cross section through the stem (overview); 5. Cross section through the stem of *Rosa rugosa* (overview); 6. *Rosa spinosissima* (overview).

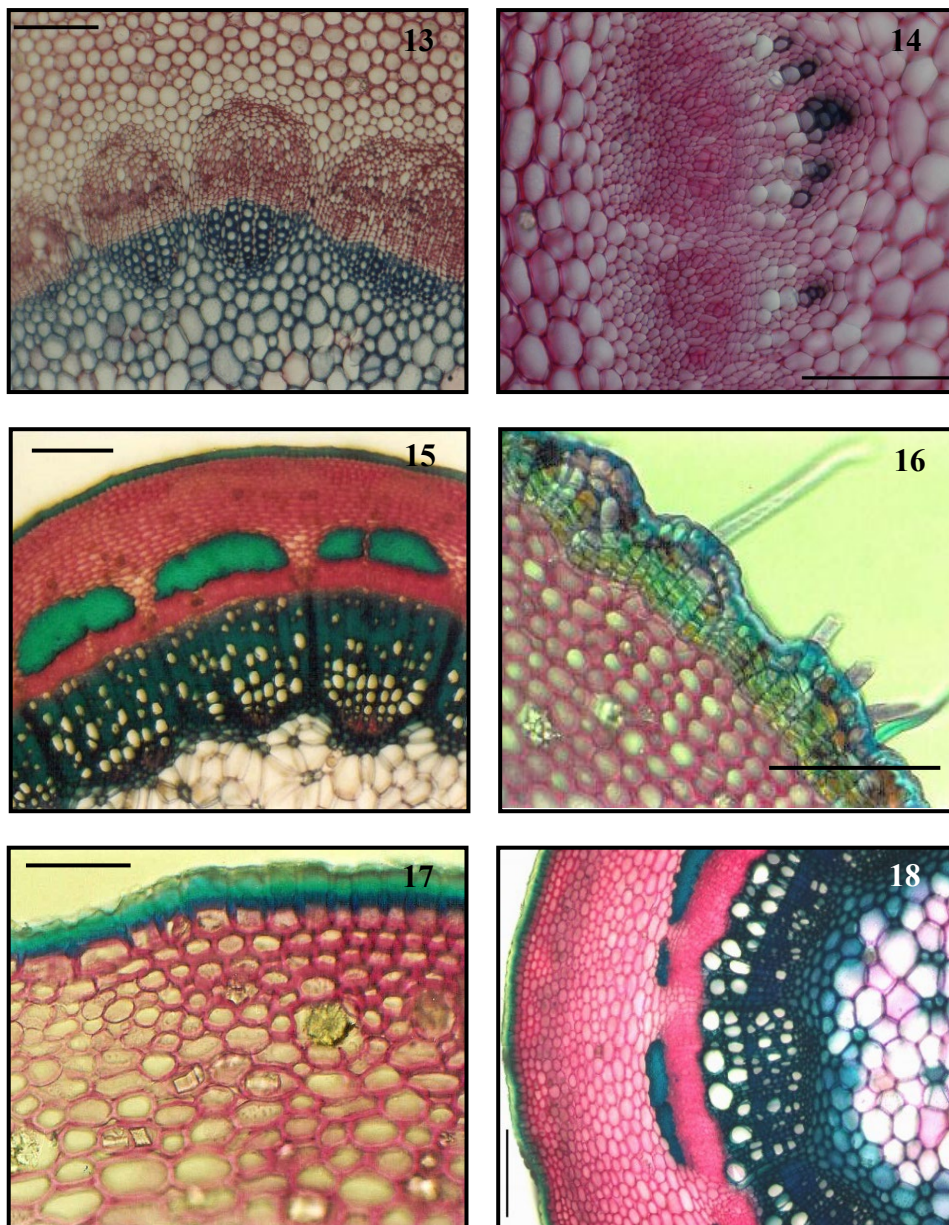


## Plate 2



**Figures 7-12. Anatomical aspects - upper third of the stem (scale = 100  $\mu$ m):** 7. Cross section through the stem of *Rosa foetida*; 8. Cross section through the stem of *Rosa damascena* – bark and central cylinder detail; 9. primary vascular bundles details at *Rosa chinensis* 'Viridiflora'; 10. primary vascular bundles details at *Rosa foetida*; 11. Cross section through the stem of *Rosa canina* 'Inermis' (cortex and central cylinder details); 12. Cross section through the stem of *Rosa agrestis* (cortex and central cylinder details).

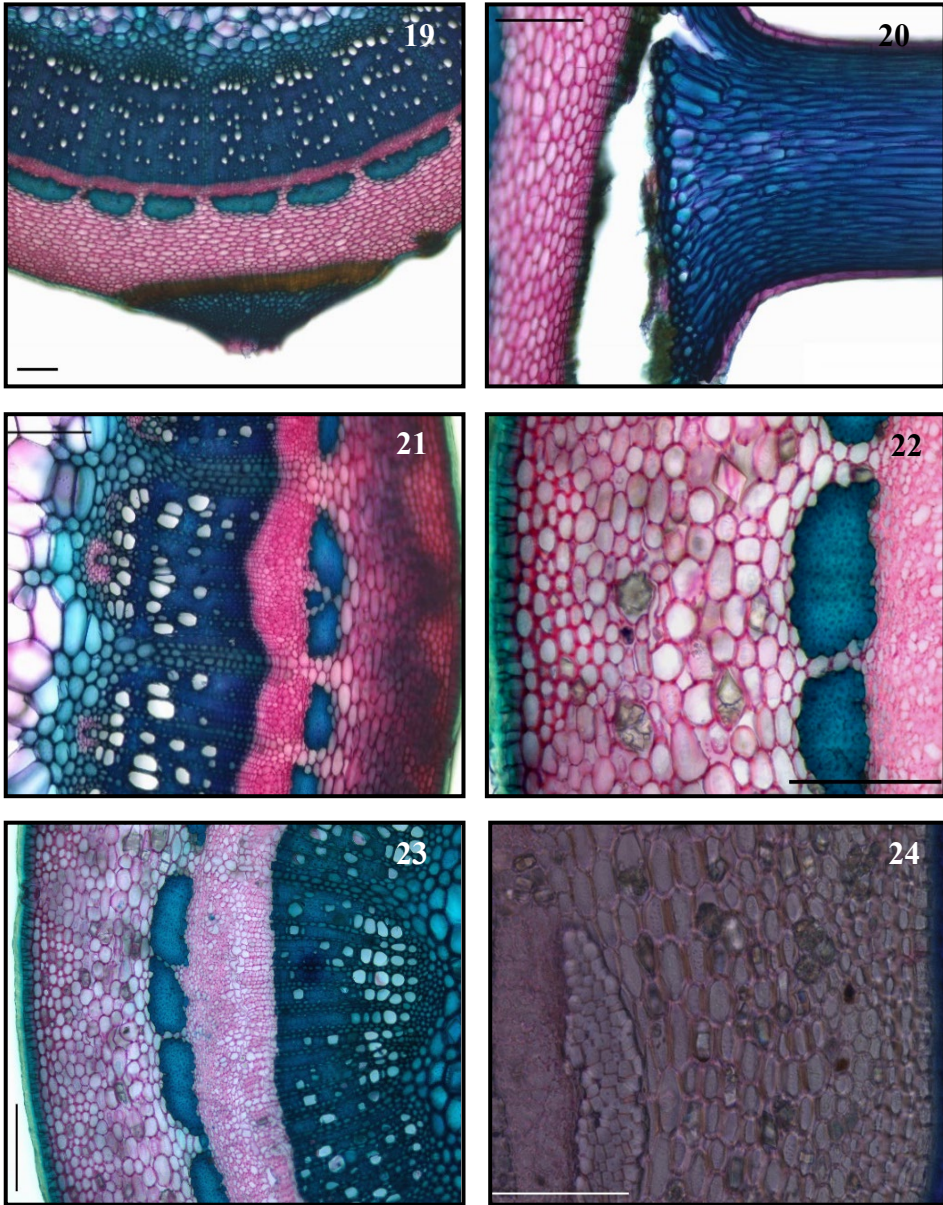
## Plate 3



**Figures 13-18. Anatomical aspects - upper third of the stem (scale = 100  $\mu$ m):** 13. the primary structure of the stem at *Rosa glauca*; 14. primary vascular bundles details at *Rosa damascena*; 15. *Rosa multibracteata* (overview); 16. *Rosa rugosa* (bark detail and cork cambium, one of few species where the phelloderm is active since the first year giving birth to cork cambium); 17. *Rosa rubiginosa* (detail of crystal idioblasts found in bark); 18. *Rosa agrestis* (details of bark, central cylinder and pith).



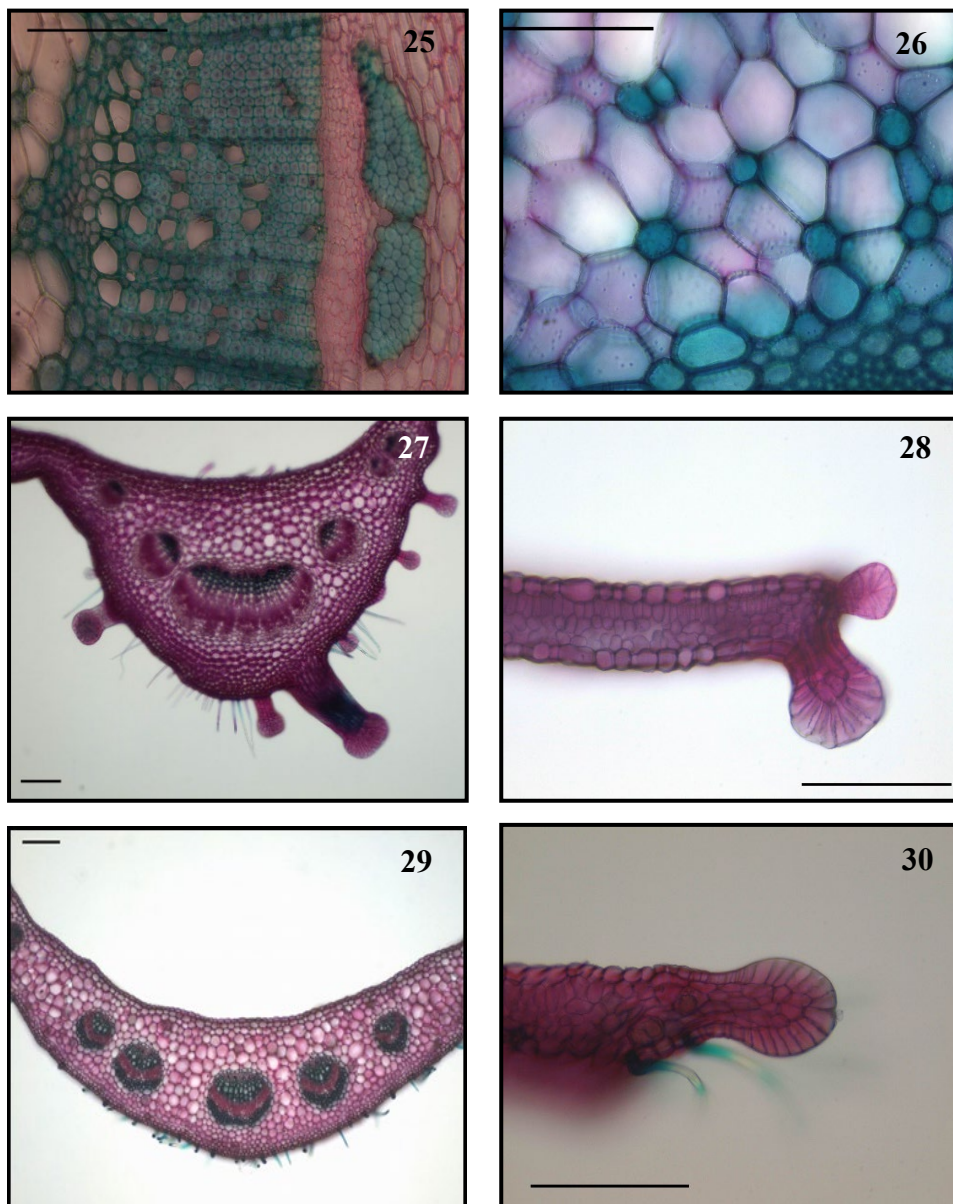
Plate 4



**Figures 19-24. Anatomical aspects - middle/upper third of the stem (scale = 100  $\mu\text{m}$ ):** 19-20. *Rosa foetida* (cortex and central cylinder details, spine that will detach from the stem, forming suber at its base); 21. *Rosa chinensis* 'Viridiflora' (cortex and central cylinder details); 22. *Rosa damascena* (cortex details); 23. *Rosa damascena* (cortex and central cylinder details); 24. *Rosa rubiginosa* (cortex detail with frequent crystal in cells).

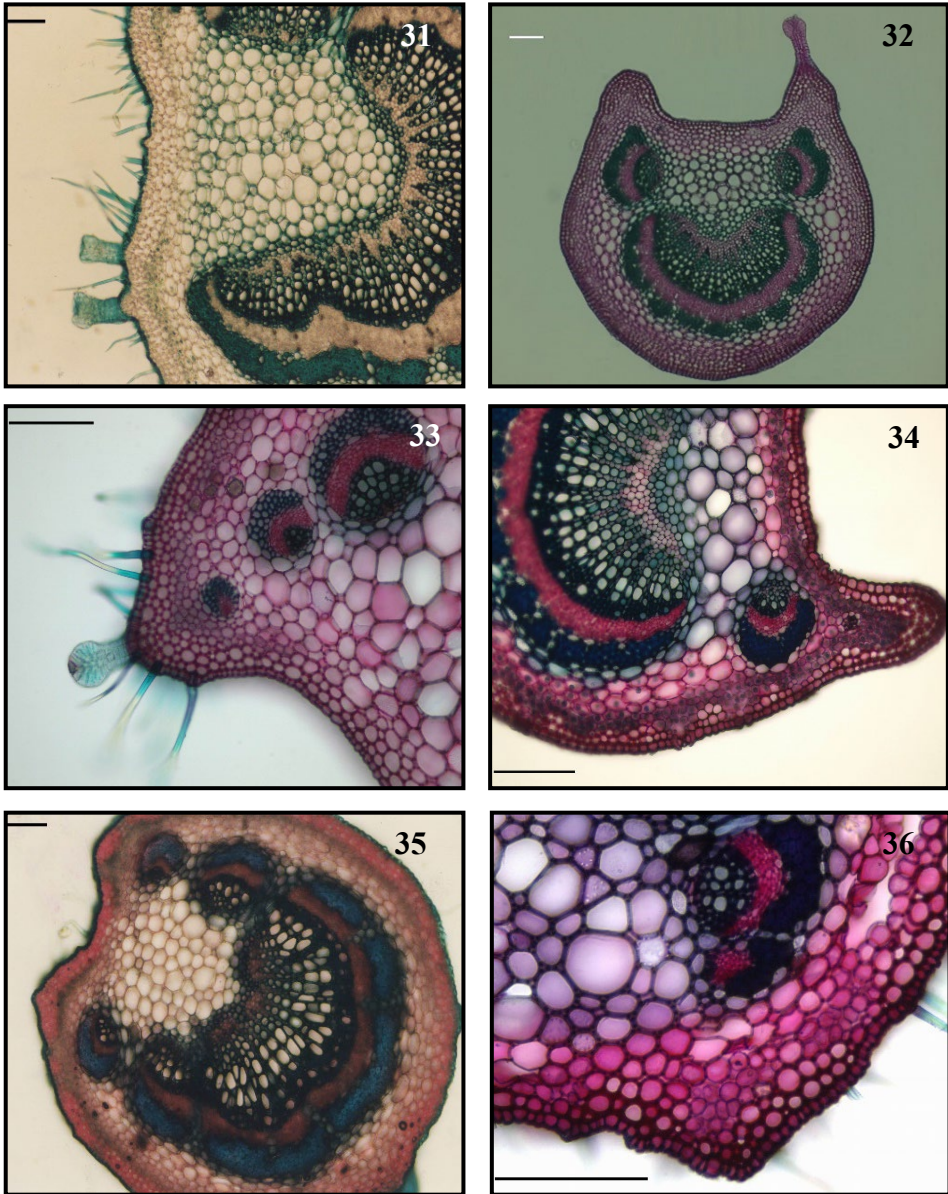


## Plate 5



**Figures 25-30. Anatomical aspects in the stem and leaf of *Rosa* L. – Cross section through the middle third of the stem (scale = 100  $\mu$ m): 25. *Rosa gallica* (cortex and central cylinder details); 26. *Rosa foetida* (pith pattern); 27-28. *Rosa agrestis* (tector trichomes and secretory glands on petiole and stipules); 29-30. *Rosa damascena* (tector trichomes and secretory glands on petiole and stipules).**

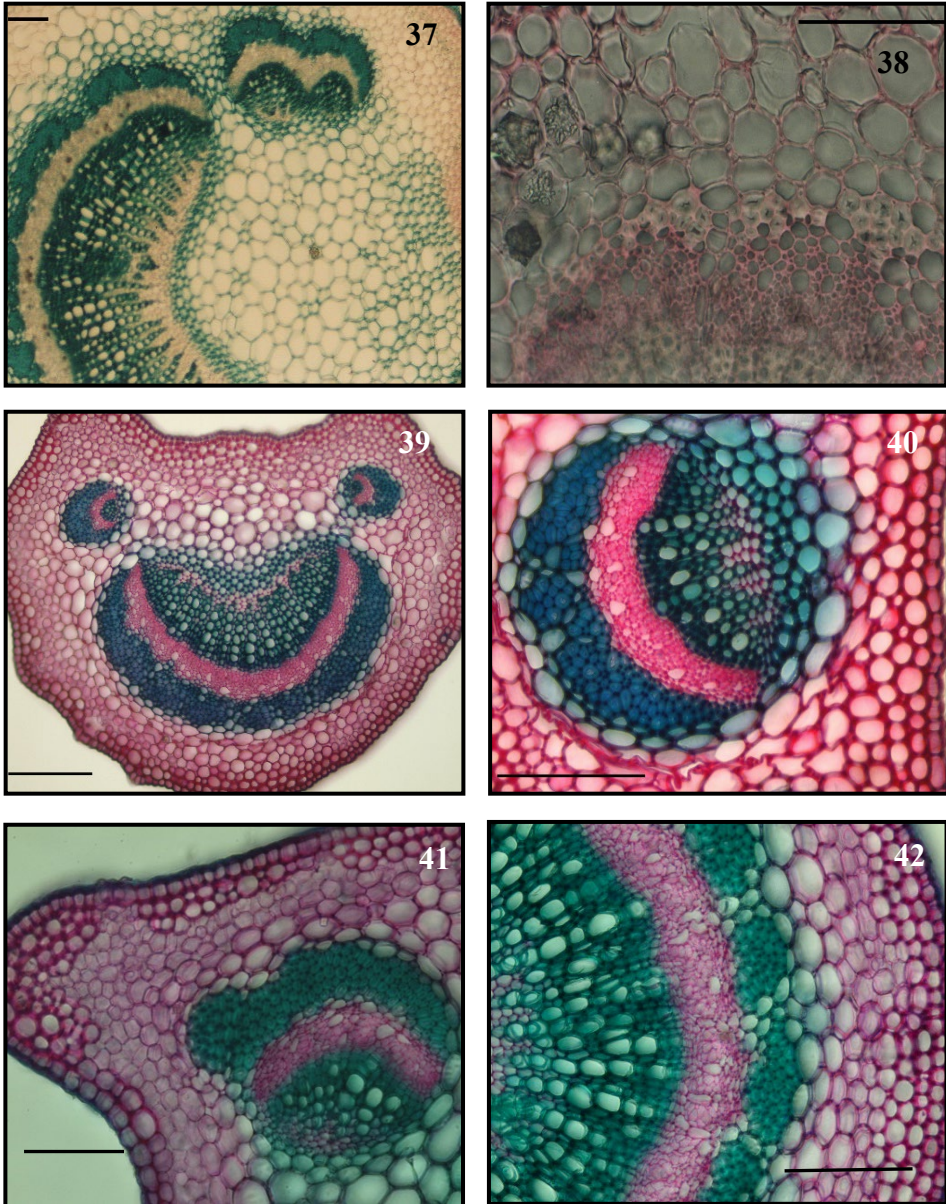
Plate 6



Figures 31-36. Anatomical aspects of the leaf of *Rosa* L. – Cross section through leaf's rachis (scale = 100  $\mu\text{m}$ ): 31-33. *Rosa damascena* (tector trichomes and secretory glands on rachis); 32. *Rosa gallica*; 34. *Rosa chinensis* 'Viridiflora'; 35. *Rosa multibracteata*; 36. *Rosa gallica*.

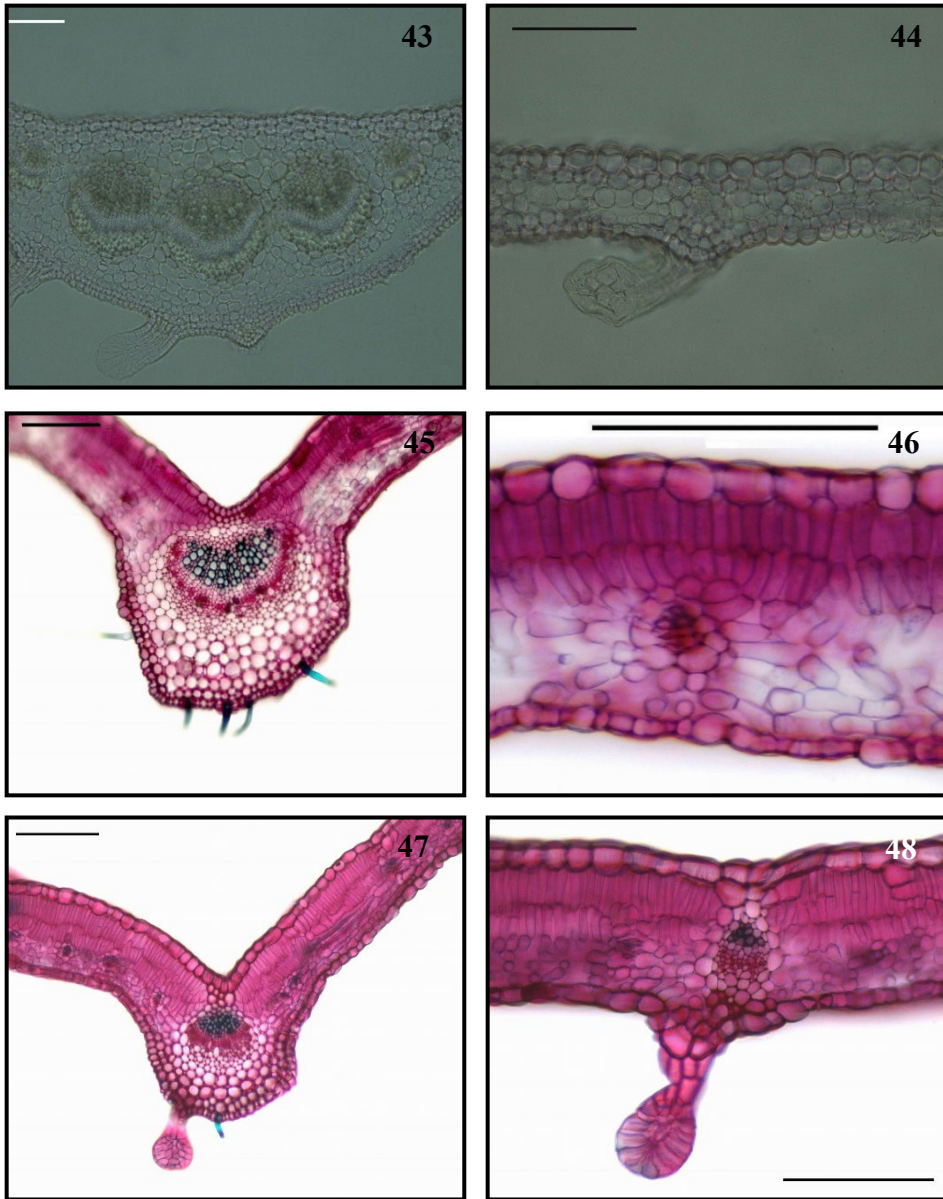


Plate 7



Figures 37-42. Anatomical aspects of the leaf of *Rosa* L. – Cross section through leaf's rachis (scale = 100  $\mu$ m): 37-38. *Rosa rugosa*; 39-40. *Rosa foetida*; 53-54) *Rosa gallica*.

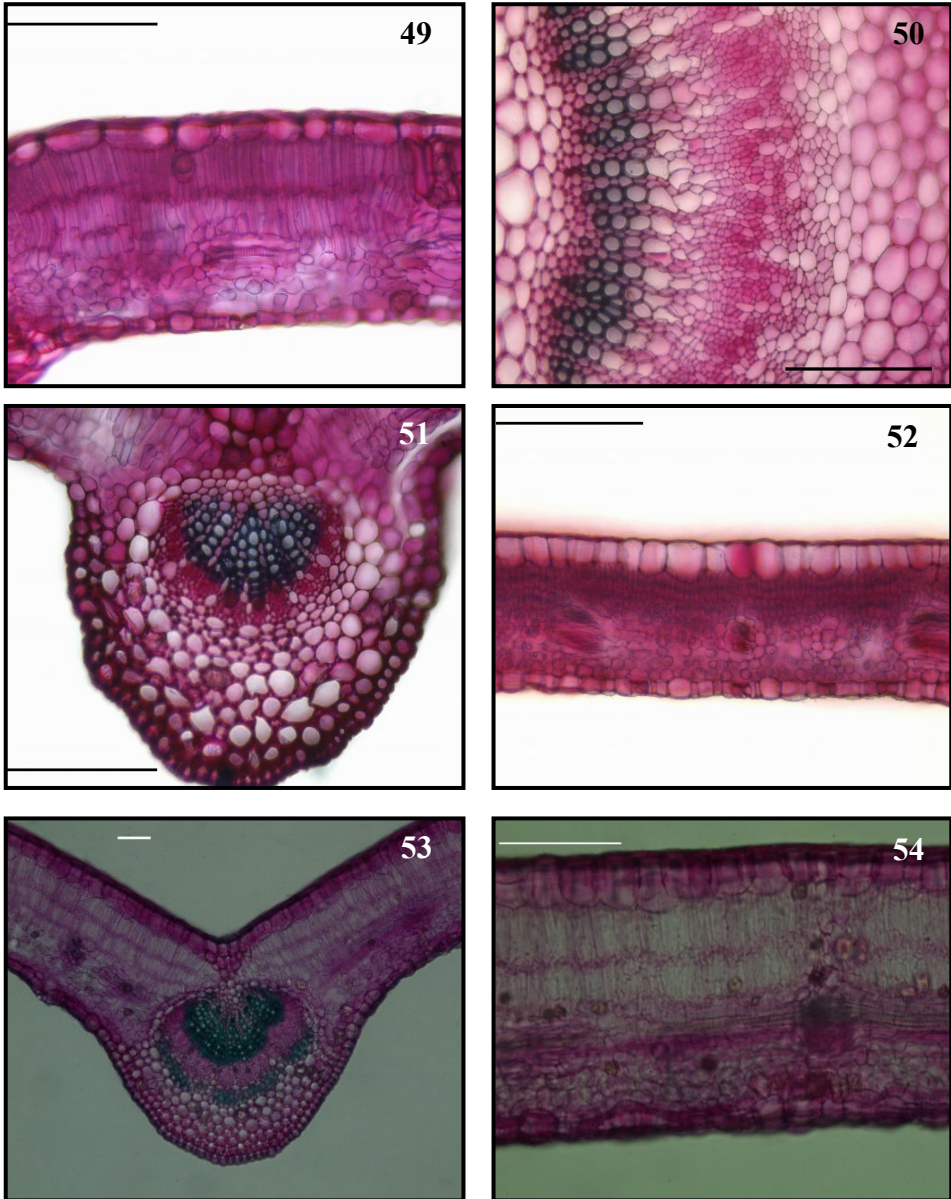
Plate 8



Figures 43-48. Anatomical aspects of the leaf of *Rosa L.* – Cross section through leaflet (scale = 100 μm): 43-44. *Rosa spinosissima*; 45-46. *Rosa damascena* (midrib and leaflet lamina); 47-48. *Rosa foetida* (leaflet's lamina).

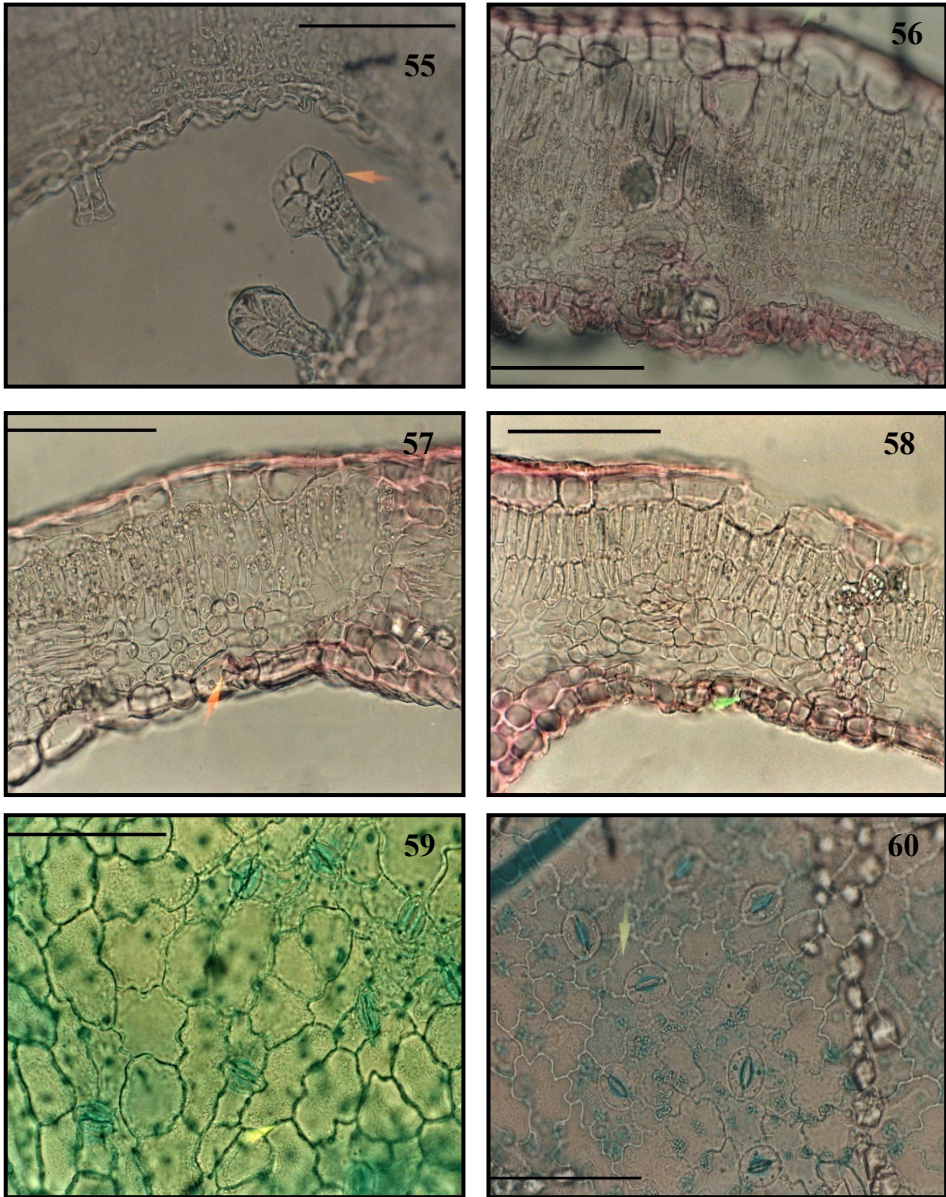


## Plate 9



Figures. 49-54. Anatomical aspects of the leaf of *Rosa* L. – Cross section through leaflet (scale = 100  $\mu$ m): 49-50. *Rosa agrestis*; 51-52. *Rosa chinensis* 'Viridiflora'; 53-54. *Rosa gallica*.

Plate 10



**Figures 55-60. Anatomical aspects of the leaf of *Rosa* L. – Cross section through leaflet (scale = 100  $\mu$ m): 55-56. *Rosa rugosa* (secretory glands on the abaxial face of the lamina and papilliform adaxial epidermis); 57. *Rosa canina* 'Inermis'; 58. *Rosa multibracteata* (detail of the midrib); surface view of abaxial epidermis: 59. *Rosa multibracteata*; 60. *Rosa damascena*.**