Petioles of terrestrial plants

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Contents

1. Introduction

The principal structure of a “normal” dicotyledonous plant consists of roots, stems, leaves and the flowering system. The anatomical structure of trees has previously been analyzed to various degrees. Features of the xylem in stems were defined by Wheeler et al. (1989) and Crivellaro & Schweingruber (2015). The xylem and its function have been for many decades in the foreground of scientific research (e.g. Evert 2006). However, other plant parts have not received much attention. Holdheide (1951) and Esau (1969) described the bark of a few species of the temperate zone. The major goal of most wood anatomical studies used to be the identification of woody species (e.g. Schweingruber 1990) or barks (Holdheide 1951). Doležal et al. (2018) related the xylem and the bark of herbs and shrubs from high altitudes to environmental conditions. Occasionally, twigs of various species or petioles have been studied (Schweingruber 1976, Zibulski & Schweingruber 2018). Troll (1937, 1939, 1943) presented a general macroscopic overview of a few monocots and dicots and some gymnosperms. In addition, he studied the longitudinal and radial growth of petioles of trees and herbs as well as the distribution of the conductive system.

The anatomical analysis of petioles of most previous studies concentrated on the taxonomy of trees. The most comprehensive and integrative anatomical work was undertaken by Metcalfe and Chalk (1957), studying the vascular system of leaves and stems, mainly in relation to taxonomy. The taxonomic significance of petioles in trees from individual families are in the focus of studies of many authors. Noraini et al. (2016) analyzed 16 species of Dipterocarpaceae in Malaysia, Song & Hong (2012) some genera of Oleaceae, Patil & Patil (2012) 19 species within the Acanthaceae, Jung & Hong (2005) some Thymelaeaceae in Korea, Mitra & Maity (2014) species of Indian Sterculiaceae, Akçin et al. (2011) some Lamiaceae and Ratikanta et al. (2016) 36 species in Mexico. All authors conclude that the petiole anatomy contains important taxonomic indicators. The taxonomic significance of petioles in herbs of Brassicaceae was demonstrated from northern Africa by El. Rabiai (2015) and of Asteraceae from Nigeria by Akinnubi et al. (2013). Both studies show that solitary vascular bundles dominate in herb petioles.

Comparisons of leaf and petiole anatomy were undertaken by Tadavi & Bhadane (2014) in 43 Indian species of Euphorbiaceae, Ecevit-Genc & Yildrim (2018) evaluated *Viburnum opulus* (Adoxaceae) and Kelij et al. (2018) analyzed *Vitis vinifera* (Vitaceae). Structural variations are especially large within the Euphorbiaceae and small in the Acanthaceae (Patil & Patil 2012). Variations within petioles were demonstrated on *Populus* by Leach (1924).

Levionnois et al. (2018) studied in detail the anatomy of juvenile and adult leaves and petioles in *Cecropia obtusa* (Urticacea), highlighting the pith as the main driver of petiole surfaces and vessel area, frequency and size being the main determinants of hydraulic requirements. Xylem and phloem studies of petioles on two grapevine cultivars by Hochberg et al. (2015) demonstrated that the anatomy of the conductive area is related to site conditions and different cultivars.

Troll (1938) and Levionnois et al. (2018) recognized the cambial activity in petioles. Napp-Zinn (1974) extensively summarized the results of previous studies and discussed different petiole parts such as epidermis, stomata, chlorenchyma and ducts in various species throughout the plant system.

1. 1. Aims of the study

The present study shows the anatomical variability of petioles in a large number of species in different taxonomic units and growth forms from the temperate and Mediterranean zones. Four major questions are raised:

a) What are the anatomical structures of the funnel-like petioles of a large taxonomic spectrum (Spermatophyta and Pteridophyta) and a large number of angiosperms?
   The present study concentrates on petioles of selected trees of conifers, Cycadaceae, dicotyledonous trees, shrubs and dicotyledonous and monocotyledonous herbs and on the leaf basis of ferns. The focus is on the form and structure of the vascular system, the sclerenchyma and parenchyma of the cortex, and crystals.
b) How do vessel diameters and conductive areas vary within a leaf and between the petiole and the shoot?
c) How are petioles structurally different from other shoots in the flowering system?

d) What is the relationship between the leaf surface and the water-conductive area in the petiole and the stem (twig)?

2. Material and methods

Analyzed were a total of 198 species from temperate and Mediterranean regions, and some Coniferopsida from the tropics (Tab. 1). For 14 individuals sections were obtained from the middle and basal parts of the petiole (Tab. 2) from the middle of the petiole and the shoot base (Tab. 3), surface area of single leaf to the xylem of the corresponding petiole (Tab. 4), and surface areas from all leaves to the xylem of the corresponding twig (Tab. 5).

All parts were sectioned using a sledge microtome, double stained with Safranin/Astrablue and finally embedded in Canada Balsam. The method is described in detail by Gärtner & Schweingruber (2013).

3. Macroscopic characteristics

The macroscopic structure is illustrated in the monographic part. The microscopic structure of all species is related to a macroscopic picture of the leaf and its petiole.

Four parts of the petiole can be distinguished:

- The petiole is the continuation of a net of leaf veins.
- The petiole differs from the leaf in the absence of lateral parenchymatic tissues.
- The length of petioles varies between species from 1 mm to more than 20 cm.
- The petiole is macroscopically mainly characterized by circular or oval shape, with ribbed epidermis.

The basic green part of all petioles, the transition zone towards the abscission layer, is thicker than the middle of the petiole but hardly longer than 2–3 mm. Radial growth occurs in the petiole distal area, adjacent to the basal portion of leaf lamina, which defines the leaf position. The proximal section near the abscission zone contains a lot of parenchymatic tissue which reacts to changes in turgor pressure and brings the leaf in an optimal position towards the light (Troll 1939).

4. Microscopic characteristics

Petioles of all plants are microscopically structured into an epidermis with a cuticle, a cortex with sclerenchyma and crystals, a phloem, a xylem and a pith. Some species feature nectaries at the base of the leaf.

The structure of the xylem/phloem zone varies. Isolated vascular bundles are principally collateral open, including their following xylem/phloem structures (Fig. 1a). Characteristic is the lateral merging of vascular bundles and the presence of a cambium. Therefore, petioles belong to the group of dicotyledonous stems with secondary growth.

Monocotyledons have collateral closed vascular bundles (Fig. 1b). Vessels are present in all monocotyledonous and dicotyledonous plants (Figs. 1a and b).

Open collateral vascular bundles with tracheids are characteristic for Conifers (Fig. 1c).

All ferns contain tracheids in concentric vascular bundles with the xylem in the center (Fig. 1d). Xylem/phloem complexes in ferns are surrounded by an endodermis (Fig. 1e).

5. Principal structure

5. 1. Principal microscopic structure of petioles of dicotyledonous plants

We start here with presenting the principal structure of the mid-section of a petiole from a tree species (Fig. 2). The epidermis forms a unicellular layer. The epidermis cells vary in form and size and are covered with a cuticle of variable thickness. The epidermis of some species features trichomes. The external cortex forms a large parenchymatic cellular part (Fig. 2). Cells are fairly uniform, thin-walled and unlignified. The external cells of the belt are often smaller than those towards the center and often collenchyma-like. Sclerenchymatic groups or belts, ducts and excretions often occur in the cortex (Fig. 3a). The parenchyma cells of the cortex are filled with chloroplasts and starch grains. Inside of the cortex is the xylem/phloem complex. This part often consists of single vascular bundles or crescent forms of xylem/phloem. The base of petioles is longer than its middle part. Characteristic is a

Figure 1a. Petiole of the dicotyledonous plant *Laurus nobilis*, with an open collateral vascular bundle containing vessels. Characteristic is the cambium between xylem and phloem (dashed line).

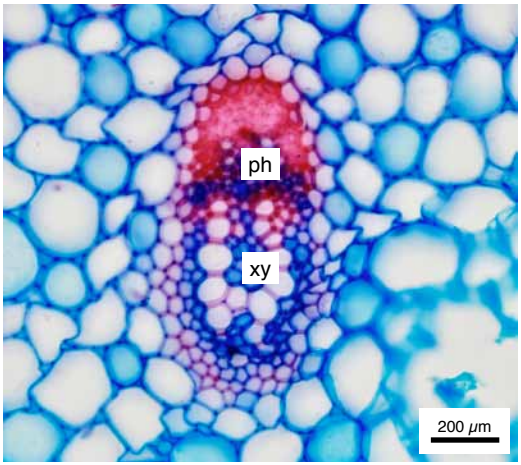


Figure 1b. Petiole of the monocotyledonous plant *Convallaria majalis*, with a closed vascular bundle containing vessels. The cambium between xylem (xy) and phloem (ph) is absent.

fb

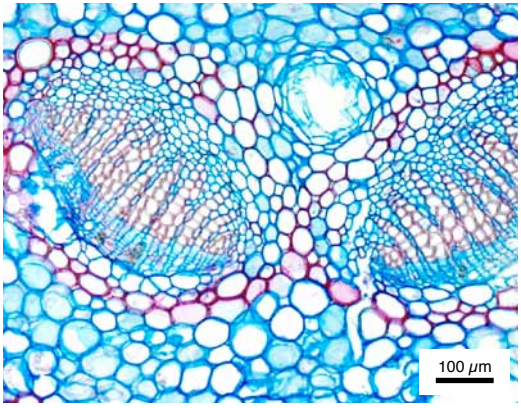


Figure 1c. Petiole of the Coniferopsida *Ginkgo biloba*, with an open collateral vascular bundle containing tracheids.

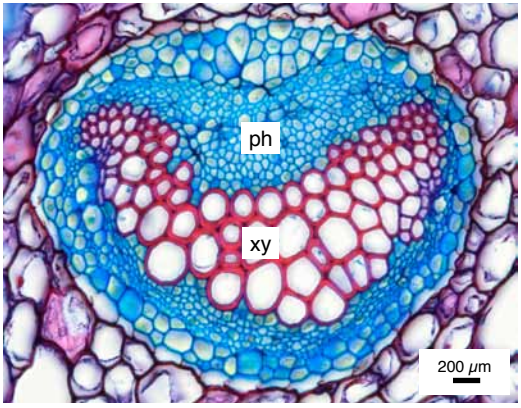


Figure 1d. Petiole of the fern *Asplenium adiantum-nigrum*, with a concentric vascular bundle containing tracheids. The cambium between xylem (xy) and phloem (ph) is absent.

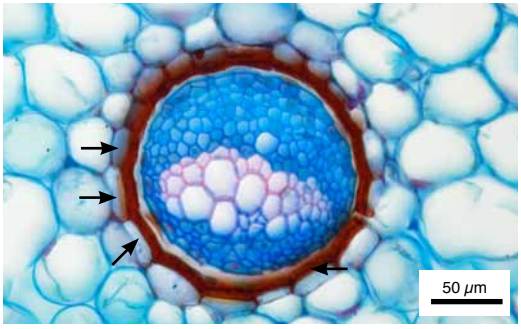


Figure 1e. Petiole of the fern *Cystopteris fragilis*, with a concentric vascular bundle containing tracheids. The vascular bundle is surrounded by an endodermis (arrows).

large parenchymatic zone and a reduced sclerenchymatic part (Fig. 3b).

Width and proportions between the parenchymatic belts vary between pinnate leaves and the main axis.

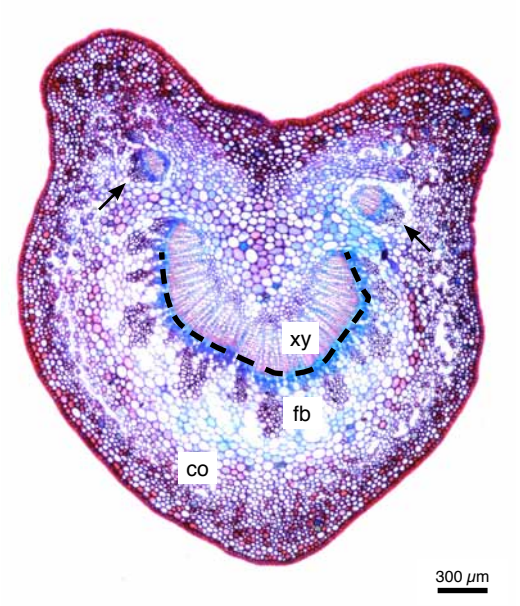


Figure 2. Principal structure of the mid-section of a petiole of *Prunus laurocerasus*. Characteristic are the epidermis, the uniform cortex (co), discontinuous fiber band (fb), the lignified xylem (xy), the small vascular bundles (arrows) and the presence of cambium (dashed line).

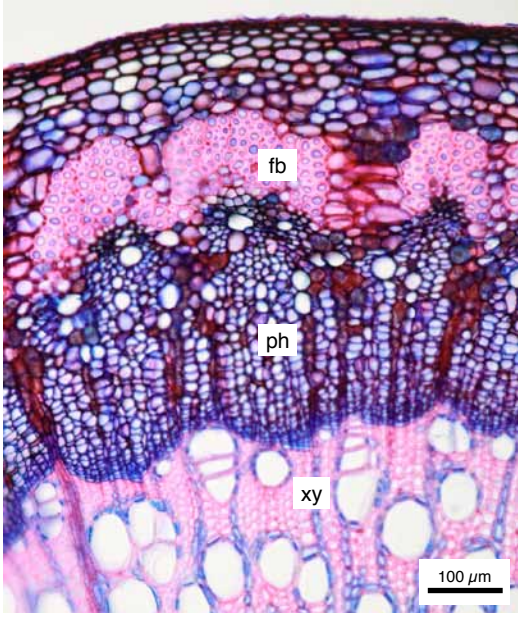


Figure 3a. Cross section in the middle of a petiole of *Juglans regia*. The principal structure is the same as in Fig. 2. In addition, a continuous fiber band (fb) occurs in the cortex.

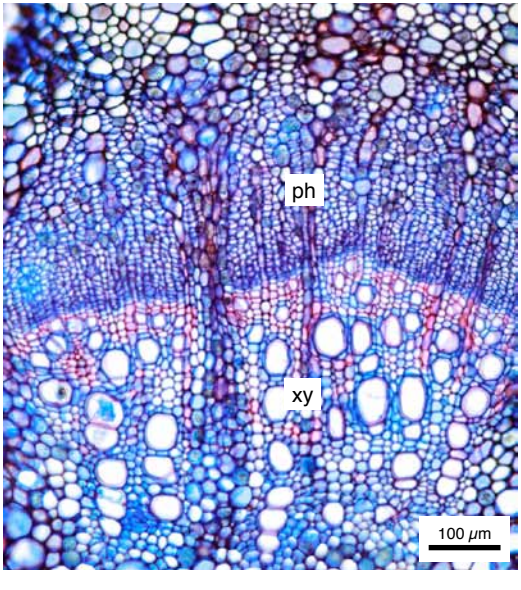


Figure 3b. Cross section at the base of a petiole of *Juglans regia*. The principal structure is the same as in Fig. 3a. Characteristic are the absence of the fiber band in the cortex and the smaller, hardly lignified xylem vessels (xy, diameter 40–50 µm).

5. 2. Structural variation of the xylem/phloem in the mid-section of petioles of pteridophytes, conifers, dicotyledonous and monocotyledonous plants seen in cross sections

The xylem/phloem system structure greatly varies within the taxonomic system and from species to species. In ferns, multiple central vascular bundles are dominant (Fig. 4a), which may be surrounded by a well differentiated endodermis (*Davallia canariensis*) or not (*Gymnocarpium dryopteris*). The presence of a single vascular bundle is the most frequent characteristic for Cycadaceae, Ginkgoidea (Spermatophytina) and conifers (Fig. 4b).

The structure and arrangement of vascular bundles in dicotyledonous woody plants varies. Major forms are shown in Fig. 4c. Dominant are crescent forms with or without external or internal vascular bundles. A special case is the double crescent form in *Quercus* and *Castanea* (Fig. 4c). Rather rare are types with circular xylem/phloem belts, e.g. *Carpinus* (Fig. 4c). Another special case is *Aesculus* with a circular belt and central concentric vascular bundles (Fig. 4c). Most dicotyledonous herbs have single vascular bundles, rarely circular arranged (*Aruncus dioicus*) or crescent forms (*Helianthemum nummularium*).

Single closed collateral vascular bundles with few sclerenchymatic parts are observed in herbaceous monocotyledonous plants (Fig. 4d). Their arrangement varies from crescent (*Allium ursinum*) to circular (*Convallaria majalis*) and irregular (Fig. 4d). Vascular bundles in petioles with a thick sclerenchymatic sheath are characteristic for Bambusaceae (bamboo, Fig. 4e) and Arecacea (palms) with closed vascular bundles (Fig. 4f).

The whole range of forms is shown in the monographic part (pag 81).

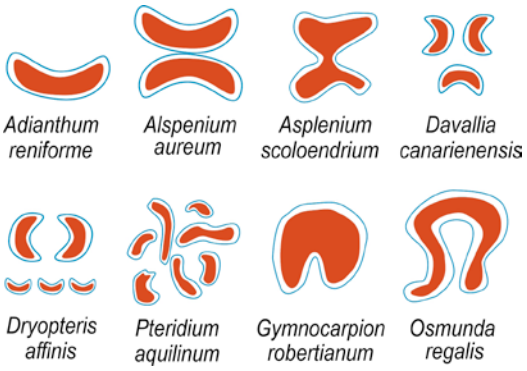


Figure 4a. Schematic representation of major forms and distribution of the vascular system in ferns. Red xylem, blue phloem, green cambium.

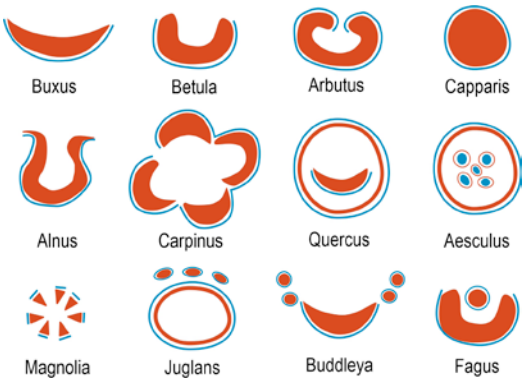


Figure 4c. Vascular bundle system of dicotyledonous trees and shrubs.

Figure 4b. Single and Multiple central vascular bundles of the needle base in conifers.

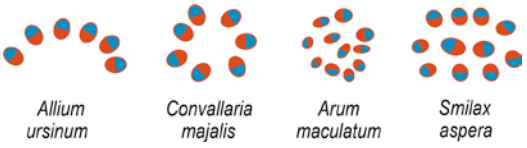


Figure 4d. Distribution of vascular bundles in monocotyledonous herbs.

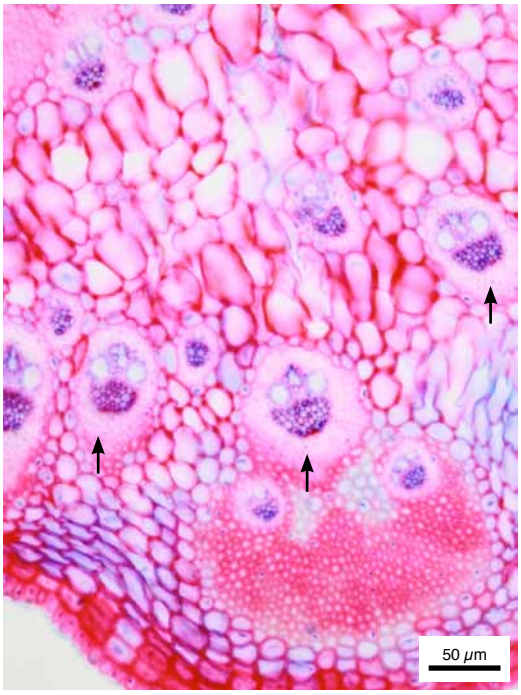


Figure 4e. Closed vascular bundles of petioles of *Phyllostachys* sp. (Bambusaceae) are surrounded by a thick fiber band (arrows) and arranged in a crescent shape.

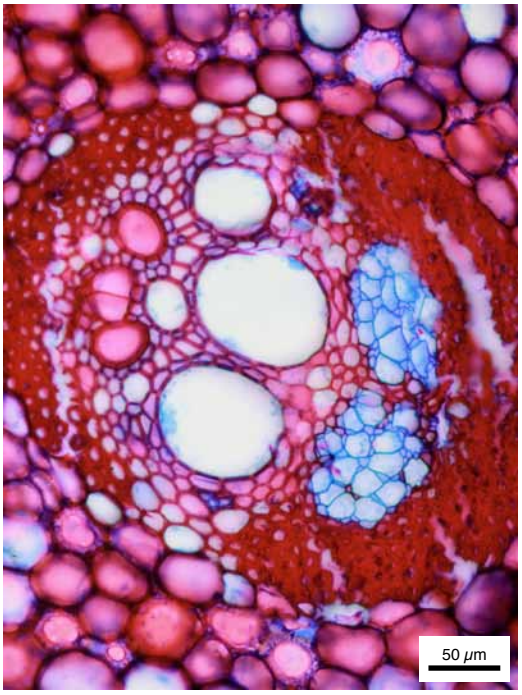


Figure 4f. Closed vascular bundles of petioles of the palm *Sabal palmetto* (Arecaceae) are distributed across the whole petiole.

5. 3. Longitudinal variations of the conductive elements from the leaf to the base of petioles and the abscission zone in dicotyledonous and monocotyledonous plants

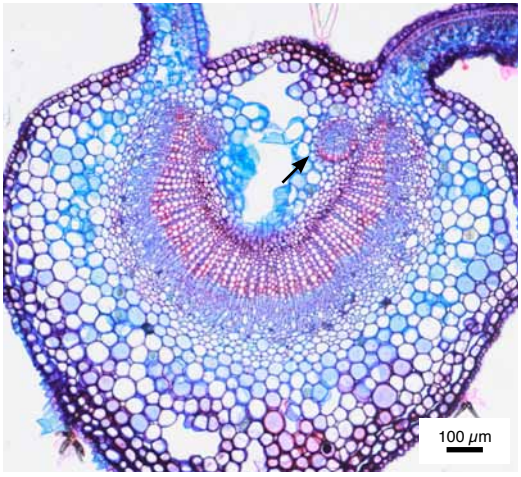
The structure of the vascular system changes from the midrib in the leaf towards the middle and base of the petiole in dicotyledonous plants. Here we show two types: a) when the petiole vascular system is structurally a continuation of the midrib (*Viburnum lantana,* Figs. 5a and b); and b) when the grouping of vascular bundles in the petiole is an aggregation of two or more ribs (*Populus* species, Figs. 5c and d).

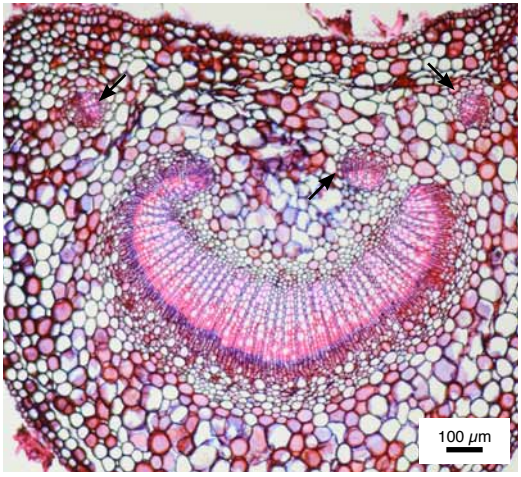
The final form of vascular systems at the abscission zone can be observed macroscopically at the bud scale scar, however, the superficial structures become indistinct within a short time after the leaves drop (Fig. 5e). Form and distribution of vascular bundles in abscission zone are normally different. Circular forms (Fig. 5f) dissolve into isolated vascular bundles (Fig. 5g) and sclerenchymatic parts in the cortex are absent at the base of petioles (Fig. 5g).

The frequency and size of vascular bundles varies in monocots. Isolated vascular bundles are arranged in horizontal lines in leaves and arranged in an ellipse around a few central bundles (*Ruscus aculeatus*, Figs. 5h and i), in a circle (*Convallaria majalis*), v-shaped lines (*Polygonatum multiflorum*) or are irregularly distributed (*Arum maculatum*). See monographic part.

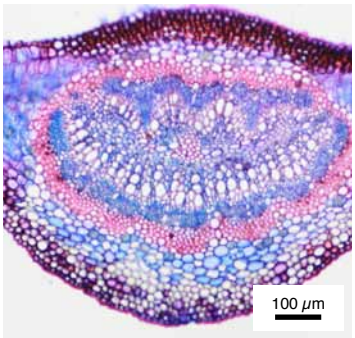
The vascular system is different in the parenchymatic zones from those in the pith and cortex. The abscission zone of a petiole is microscopically characterized by an abrupt interruption of the vascular system by a periderm with a large phellem (Figs. 6a and b). The phellem seals the parenchymatic and the conductive tissue from external influences, especially from destruents. The sealing process seems to progress very fast which is indicated by the absence of chemical compartmentalization effects (Fig. 6).

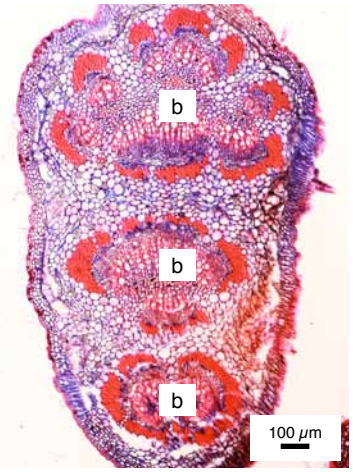
Figures 5a-i. Structural variation between the midribs, the middle and base of petioles.





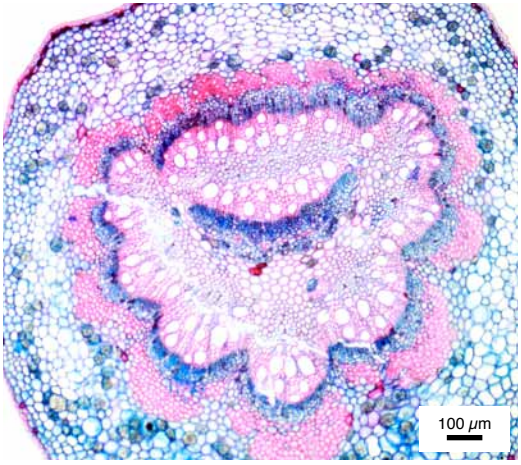
Figures 5a (left), b (right). Little variation between the midrib (a) and the middle of the petiole (b) in *Viburnum lantana*. The crescent form of the main xylem/phloem complex is accompanied by traces of small veins in leaves (arrows).





Figures 5c (left), d (right). Major structural changes between the midrib and the petiole in *Populus nigra*. Three veins of the leaves aggregate to a vertical line of vascular bundles (b) in the petiole.

Figure 5e. Bud scale scar (arrow) with a distinct crescent xylem in *Rhamnus alpina*. The base mainly reflects the distribution and form of vascular bundles in the abscission zone.



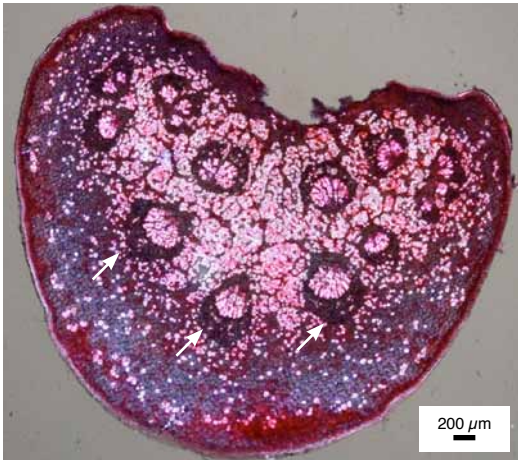


Figure 5f, g. Major structural changes between middle and base of the petiole of Quercus rubra. Vascular bundles are encircling in a continuous belt in the middle of the petiole, while in the base vascular bundles are isolated (arrows), but maintaining the encircling organization. Schlerenchyma bands next to the phloem are absent at the base of the petiole.

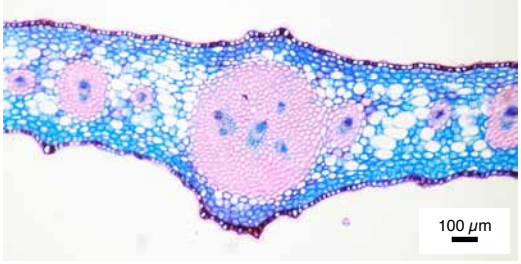


Figure 5h. Vascular bundles in the middle of the petiole in *Ruscus aculeatus*. A circle of laterally connected collateral open vascular bundles and a centric bundle are separated.

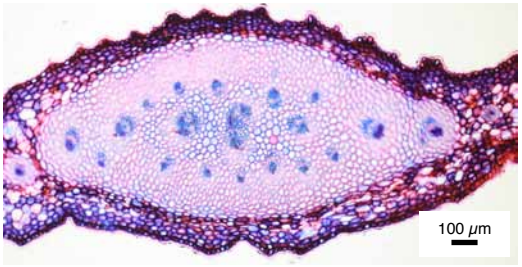


Figure 5i. Vascular bundles at the base of the petiole in *Ruscus aculeatus*. Single vascular bundles are arranged in a compressed circle. Sclerenchymatic parts are absent.

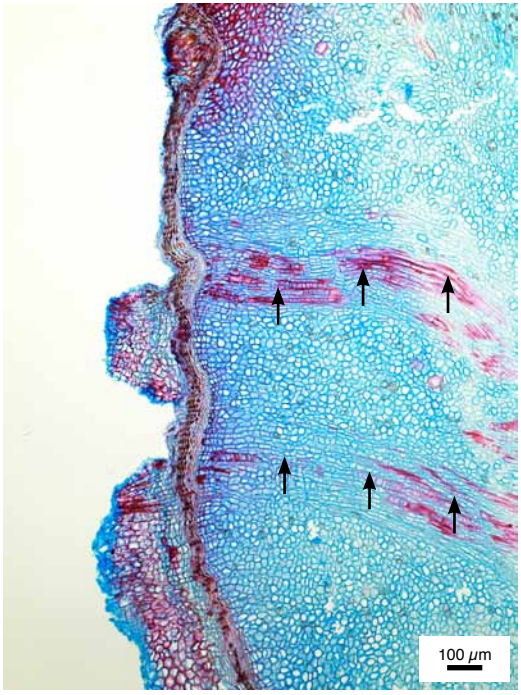


Figure 6a. Abscission zone of a leaf of *Castanea sativa*. Two vascular bundles (arrows) within a parenchymatic tissue are sealed by products of a phellogen (cork).

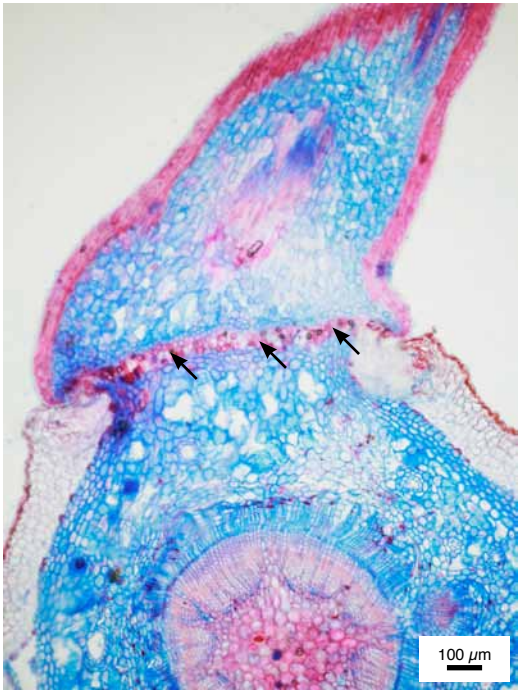


Figure 6b. Figure 6b. Abscission zone of a needle of *Abies nordmanniana*. The abscission zone (arrows) separates the phellem and the needle from the cortex of the shoot.

5. 4. Proportions between conductive, storage and stabilization elements

This section discusses the proportions between the maximum vessel diameter, in the middle and the base of a few petioles, the phloem and the parenchyma of one leaf and the petiole (Tab. 2). Despite the low number of analyzed materials some trends become obvious. The parenchymatic area is the dominant part in petioles and annual shoots. It occurs in petioles primarily in the cortex (Fig. 7) and in shoots in the cortex and the pith. The vessel diameter in the middle of the petiole varies approximately from 15–60 μm; vessel diameter in the base varies in the same range but is generally reduced by maximum of 60% (Tab. 2), except for *Parthenocissus tricuspidata*, where vessel diameters on the base of petiole was 33% wider compared to the middle region (Tab. 2).

Vessel diameters of petioles, annual shoots and stems are compared in Table 3. Selected are a few species with large, medium and small leave sizes (Tab. 4). Vessels are small in petioles, varying between 10–30 μm, but they are larger in annual shoots of the same specimens, varying between 20–40 μm (Tab. 3). The average vessel diameters in stems of different species are much larger, varying between 50 to more than 100 μm. There is a clear trend: Vessel diameters in stems are large, vessels are approximately 4 times bigger than those in annual shoots and 5–6 times bigger than those in petioles. The proportion of conductive xylem varies 5–15% in petioles and 20–30% in annual shoots.

The conductive portion of the phloem is indeterminable because phloem cells cannot be normally differentiated from parenchyma cells and rays, and it is unknown how long the sieve tubes are active.

Stabilization elements have not been studied in detail. Fibers, never sclereids, occur in the cortex and in the xylem to various amounts. In the cortex of *Populus* fibers amount to approximately 15% but they can also be absent, e.g. in *Prunus laurocerasus*. Similar variations occur in the xylem. The amount of fibers is very high in the xylem of *Buxus sempervirens* (Fig. 7), while it is low in *Alnus cordata*.

Crystals, mostly calcium oxalate druses, can occur in all the elements in large amounts but their frequency varies quite unpredictably. Crystals are constantly larger in the cortex than in the phloem.

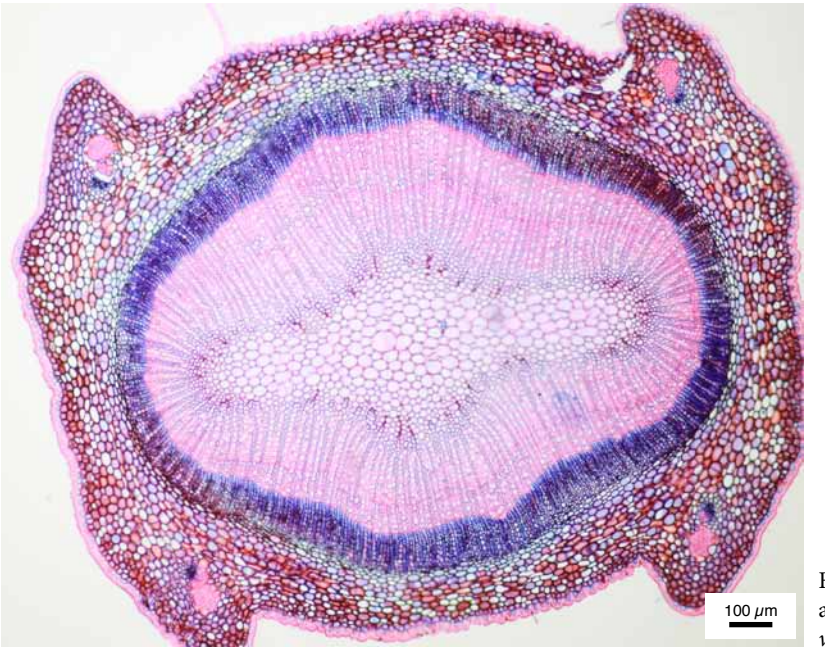


Figure 7. Cross section of a shoot of *Buxus sempervirens*.

Table 2. Change in maximum vessel diameters from the middle of the petiole to its basal zone. The measured values at the base can vary depending on the position of the section in relation to the abscission zone.

Species	Family	Maximum vessel diameter (μm)		
		Middle of petiole	Base of petiole	Change (%)
Acer pseudoplatanus	Sapindaceae	20	20	0
Aesculus hippocastanum	Hippocastaneaceae	50	20	60
Alnus cordata	Betulaceae	25	12	52
Amorpha fruticosa	Fabaceae	60	60	0
Cotinus coggygria	Anacardiaceae	15	15	0
Cydonia maliformis	Rosaceae	25	15	40
Fraxinus excelsior	Oleaceae	40	20	50
Gleditsia triacanthos	Fabaceae	30	20	33
Juglans regia	Juglandaceae	60	50	17
Olea europaea	Oleaceae	25	20	20
Ostrya carpinifolia	Betulaceae	15	10	33
Parthenocissus tricuspidata	Vitaceaae	30	40	-33
Populus nigra	Salicaceae	25	17	32
Ribes rubrum	Grossulariaceae	22	15	32

Table 3. Increase in mean vessel diameters from the middle of the petiole to the annual shoot close to the breaking zone and the stem at breast height. Petioles and annual shoots were sampled from the same individual. Earlywood vessel diameters of stems were measured at cross sections of different individuals.

Species	Family	Mean vessel diameter (μm)		Increase (%)	Vessel diameter (μm)	Increase from petiole (%)	Increase from annual shoot (%)
		middle of petiole	annual shoot		stem at breast height		
Arbutus unedo	Ericaceae	16	25	56	50–80	213–400	100–220
Arbutus andrachne	Ericaceae	12	20	67	40–60	233–400	100–200
Quercus coccifera	Fagaceae	12	20	67	60–90	400–650	200–350
Ceratonia siliqua	Fabaceae	25	30	20	90–120	260–380	200–300
Cercis siliquastrum	Fabaceae	25	32	28	80–100	220–300	150–213
Myrtus communis	Myrtaceae	10	25	150	50–70	400–600	100–180
Pistacia lentiscus	Anacardiaceae	20	30	50	100–130	400–550	233–333
Pistacia terebinthus	Anacardiaceae	30	30	0	90–110	200–267	200–267
Nerium oleander	Apocyanaceae	18	30	67	70–90	289–400	133–200
Olea europaea	Oleaceae	10	20	100	50–60	400–500	150–200
Tilia cordata	Tiliaceae	12	29	142	50–60	317–400	72–107
Vitex agnu-castus	Lamiaceae	25	35	40	150–180	500–620	329–414
Smilax aspera	Smilacaceae	8	40	400	90–100	1025–1150	125–150

6. Ecophysiological interpretation of the petiole function

Leaf petioles connect the plant's photosynthetic machinery to the supporting shoot (Figs. 4 and 5). They play a mechanical role, they permit positioning of foliage towards the sun, they transport water, nutrients and biochemical signals from the shoot to the leaf, and photosynthates and other products from the leaf to the shoot. Commonly green, and thus also photosynthetically active, they may be stiff or flexible, long or short, and have to channel and connect both phloem and xylem conduits into the stem's conduit system. At their base, petioles are widened, and in woody taxa, they can activate an abscission meristem that ensures proper leaf shedding at senescence. Although part of the leaf, functionally, the petiole can be considered a stem, possessing all essential tissue elements such as pith, conduits (in various arrangements), sclerenchymatic tissue (fibers), cortical and bundle-sheet parenchyma and epidermis. Because leaf area specific metabolic activity, leaf toughness and duration and associated rates of vapor loss vary widely among taxa and within taxa with exposure to the sun (e.g. Poorter & Rozendaal 2008) the water conduit capacity of the petiole relative to the blade area supplied varies as well. Hence any assessment of petiole support capacity versus stem capacity must be standardized by leaf blade demand, or has to be conducted within a given shoot, where those variables can be assumed to equilibrate.

Hydrological functions are expressed by the dimension (Tab. 2) and number of vessels. The transition from the petiole to the base and the breaking zone is important in relation to the hydrological architecture. Since the same amount of water has to flow through the middle of the petiole as through its base proportions between vessel diameter and vessel number change. Vessels are smaller in the base than in the middle of the petiole (Tab. 2). The reduction of vessel diameters in the breaking zone has probably also a function in relation to cavitation. If a hydrological imbalance occurs in the leaf or the shoot the abscission zone and the petiole prevent the leaf or shoot from desiccation.

Three functions are on the foreground: 1) the leaf is the major photosynthetically active part; 2) the petiole is the crucial organ which connects the leaf to all other parts of the plant; and 3) the surface of single petioles in relation to leaf surfaces varies between 0.15–3%, e.g. in *Laurus nobilis*, cross section 0.14% versus 100% leaf surfaces (Tab. 4). In the same range are results between twigs with many leaves, e.g in *Laurus nobilis*, cross section 1.46% versus 100% leaf surfaces of all leaves (Tab. 5).

Petioles must conduct water from the xylem of the shoot to the assimilating part of the leaf and all parts with chloroplasts to all parenchymatic cells in the whole plant. The surface of the total xylem in relation to the total petiole surface varies between 15–30% (Tab.4). Within the xylem only 6–30% are conductive elements (Tab. 5). Due to measurement problems of the surface of conductive vessels within the xylem it is not possible to precisely relate the conductive area to the leaf surface. We estimate that only 0.01% of the petiole surface is responsible for the regulation of hydrological processes in the leaf area.

The numbers given in Tabs. 2–5 are good reasons to focus on that 'missing' part when it comes to understand plant water relations under water shortage. A leaf can be viewed as an analogy to a human household, with the stem representing the mains network of electricity: the priority for an intact mains system makes it imperative that over-consumption at the household level does not impact the network. In electric networks, that problem is solved by fuses that prevent 'peripheral misbehavior' to affect the mains system, including power supply to neighbor households. This 'fuse' idea was first coined in a study by Zimmermann & Sperry (1983) in palm leaves, where 'mains' supply of water is at particular risk, because there is no secondary growth and fatally embolized conduits cannot be replaced. An enhanced hydraulic transfer resistance between the shoot stem and the attached leaf could also represent an early warning system, enforcing stomatal controls of flows before the stem's conduit system is at risk to break (cavitate). Finally, a narrowing of the conduits at one point could even operate similar to an orifice in flow mechanics, setting a maximum flow rate.

While the mechanical component (xylem and sclerenchyma) keeps the leaf in the optimal position towards the light, the parenchymatic component at the leaf base guarantees mobility. This is indicated by the different amounts of parenchyma

at the base of the principal petiole and its secondary orders. The parenchymatic part of the very mobile partial leaves of the secondary order of pinnate leaves is much higher than that of the basic part of the petiole (Figs. 9a and b).

The architecture of the soft unlignified parenchymatic tissues of the cortex and the hard-lignified xylem guarantees stiffness and mobility. Leaves must be mobile (shaking) due to mechanical effects (wind, water, snow) and reduced turgor after water shortage (drought, frost). The fiber belts in petioles and shoots keep leaves in mechanically imbalanced positions (gravitation effect). Various turgor pressures in peripheral parenchymatic tissues of the cortex allow short time positional changes of the leaf surface.

Petioles also function as a defense barrier against pathogens, which is expressed by decaying leaves with pathogenic features. Leaves decay but the petiole remains intact until time-relevant abscission occurs. Therefore, the shoot does not get effected by the pathogens, primarily fungi.

The function of crystals is not clear. Druses in adult petioles might prevent petioles from mechanical destructions by insects. However, the constant occurrence of crystals in buds and juvenile tissues make physiological functions probable.

Table 4. Relations between single leaf and petioles areas, and total area of xylem, phloem and parenchyma tissues.

Species	Single leaf area (mm^2)	Petiole cross-section area (mm^2)	Total area of xylem (%)	Total area of phloem (%)	Total area of parenchyma (%)
		Large leaves			
Eucalyptus sp.	3432	1.97	8	7	85
Ceratonia siliqua	2903	2.15	10.7	4.2	85.9
Viburnum rhthidiophyllum	4123	4.59	13.9	8.8	68
		Middle size leaves			
Arbutus andrachne	1397	2.3	15.3	9.3	75.4
Laurus nobilis	1513	1.78	8.9	7.8	83.3
Laurus nobilis	1232	0.87	10	5.6	-
Laurus nobilis	848	1.42	70	5.6	-
Ilex aquifolium	1388	2.11	4.2	1.14	95
Ilex aquifolium	915	0.55	14.5	5.54	-
		Small leaves			
Buxus sempervirens	135	0.39	16.4	10.2	74.4
Olea europaea	149	0.42	6.9	5.4	88.7
Olea europaea	180	0.51	5.8	6.8	87.4

Table 5. Relations between surface areas of all leaves of an annual shoot and total area of xylem, phloem and parenchyma tissues of the corresponding shoot.

Species	Total leaf area (mm^2)	Shoot cross-section area (mm^2)	Total xylem area (%)	Total phloem area (%)	Total parenchyma area (%)
Large size leaves			**Annual shoot**		
Eucalyptus sp.	9022	14.19	31.9	10.7	57.4
Ceratonia siliqua	5790	11.01	19.2	13.8	67
Viburnum rhthidiophyllum	15623	14.15	30.1	8.3	61.6
Middle size leaves			**Annual shoot**		
Arbutus andrachne	8627	9.1	23	12.9	64
Laurus nobilis	1179	4.23	17.7	12.8	70
Laurus nobilis	5826	3.1	18	9.3	3.5
Laurus nobilis	4581	2.4	19.6	11.25	11.3
Ilex aquifolium	5667	2.9	22	11.3	66.7
Ilex aquifolium	4964	2.9	23.1	6.5	10.3
Nerium oleander	5084	7.01	33.3	34	32.7
Small size leaves			**Annual shoot**		
Buxus sempervirens	1416	2.34	25.6	6.1	62.7
Olea europaea	1347	12.01	19.3	24.6	56.1
Olea europaea	1470	2.11	17	13.3	69.1

7. Ontogeny of petioles

Troll (1939) described the ontogeny of petioles on the basis of a few herbs. Here we concentrate on petioles of trees, shrubs and herbs from the temperate region. Longitudinal growth is not covered here because it is technically difficult to recognize the different developmental stages (Troll 1939).

Petioles develop in or outside buds in late summer and fall before bud breaking in the next spring (Figs. 8a and b). Bud formation is combined with the formation of a shoot with secondary growth (Fig. 8a). Radial growth in petioles occurs in most species unifacially, meaning radial growth is suppressed on the lower side. Therefore, the xylem/phloem zone appears as a crescent (Fig. 1).

7. 1. Timing of longevity of cambia

Initial shoots develop into petioles or flower stalks or twigs. This occurs at different times in different species, e.g. in late fall in *Viburnum lantana* without bud scale scars (Fig. 8a), or in the autumn of the previous year (Fig. 8b) in species with bud scales, e.g. in *Juglans regia*.

Petioles are principally composed of all major anatomical elements such as cortex, cambium, phloem and xylem containing vessels with secondary cell walls (Figs. 8c and d). Petioles reach their final form after cellular elongation during the foliation in spring. The final short differentiation process takes place during bud breaking und full leaf unfolding time. It is characterized by the enlargement of cells in the cortex and radial growth and lignification of the xylem (Figs. 8e and f). The cambium of the petiole is active before bud break until leaves are fully developed.

Anatomical differentiations also exist within pinnate leaves. Relations between the leaf base of different leaf orders are especially obvious (Figs. 9a and b). The final mobilization and stabilization seem to determine the amount of parenchyma, fibers and vessels.

The phloem zone develops very early, just before bud break, and remains in its initial size until leaf fall (Figs. 8e and f).

Figure 8a. Initial terminal shoot with an initial annual scale-less shoot and leaves with petioles in *Viburnum lantana*.

Figure 8b. Cross section of a bud with bud scale scars in *Juglans regia*. In the center of the bud is an initial polygonal shoot and outside of it are several initial petioles.

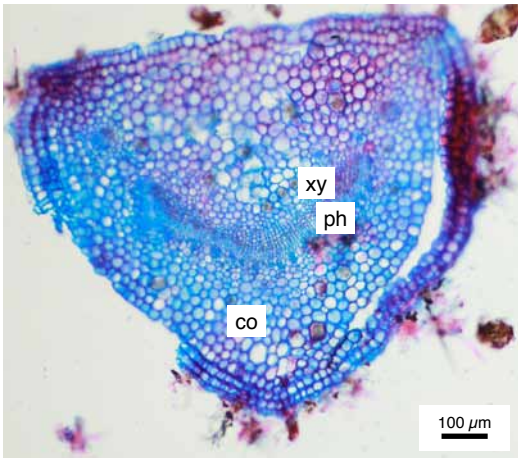


Figure 8c. Initial petiole at the base of the undeveloped leaves in *Viburnum lantana* in the middle of winter. All parts such as cortex (co), phloem (ph) and xylem (xy) are tructurally developed.

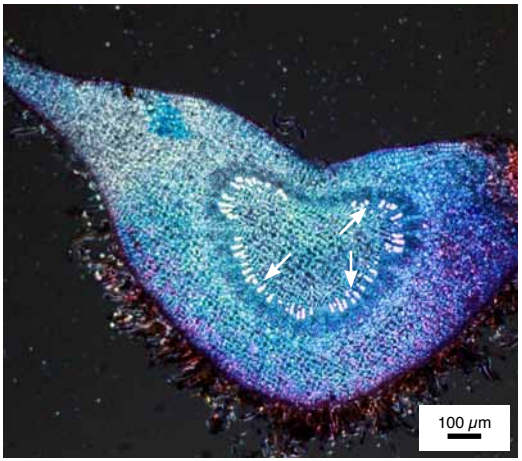


Figure 8d. Initial petiole in a bud with bud scars in *Fraxinus excelsior* in the middle of winter. Secondary walls of the xylem (arrows) are fully developed.

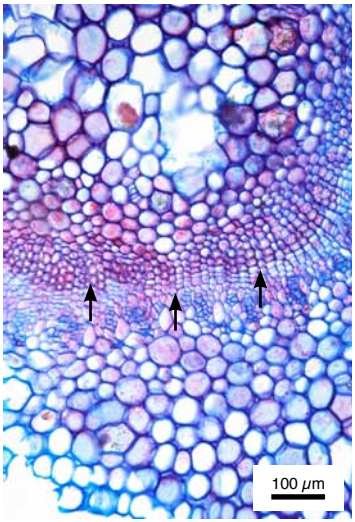


Figure 8e. undeveloped petiole in *Viburnum lantana* in the dormant season. The cambium (arrows) already formed a hardly lignified, vessel-less earlywood zone.

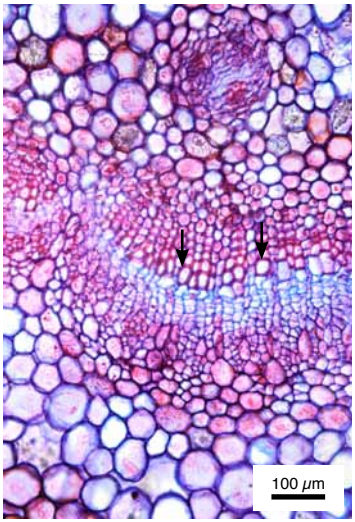


Figure 8f. Petiole in *Viburnum lantana* with 20% leaf development in early spring. The cambium already formed a first row of lignified vessels (arrows).

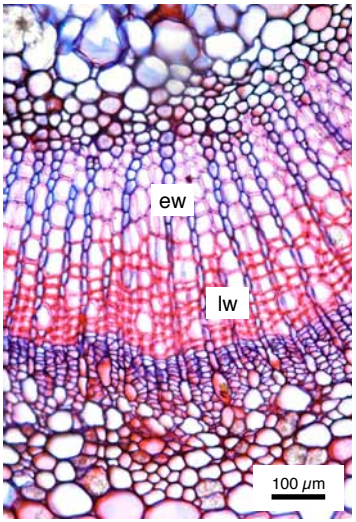


Figure 8g. Fully developed petiole in *Viburnum lantana* with 100% leaf development in late autumn shortly before leaf drop. The xylem now consists of a distinct earlywood (ew) and latewood (lw).

Figure 9a. Base of the first order of a pinnate leaf in *Fraxinus*. The relatively small conductive part (xylem and phloem, dashed line) is surrounded by a large portion of parenchyma.

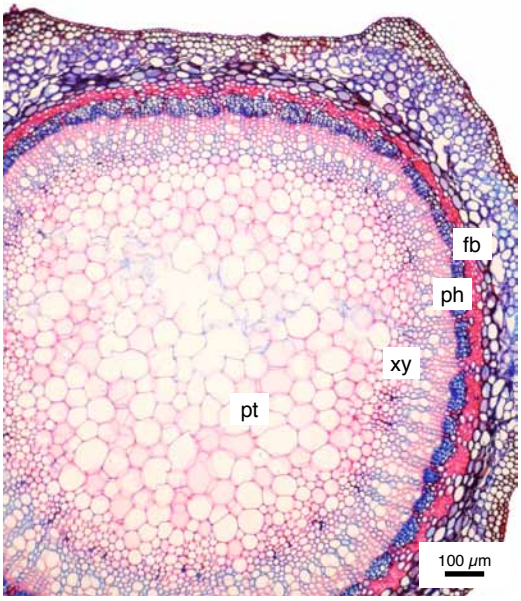


Figure 9b. Middle part of the petiole of the first order of a pinnate leaf in *Fraxinus*. The parenchymatic cortex is relatively small. A circle of thickwalled fibers (fb), followed by phloem (ph) and xylem (xy) surround a very large pith (pt).

7. 2. Relation between leaf phenology, radial growth of petioles, annual and biennial shoots

The formation of the xylem in the petiole is related to the annual and biennial shoot and to the phenology of leaves. This is demonstrated here on young twigs in the trees *Juglans regia* and *Tilia cordata* and in the shrub *Rubus idaeus*. All three species develop earlywood vessels in petioles, annual shoots and biennial shoots (near the annual shoot) at the beginning of leaf development.

There is no difference between proximal, middle and distal parts of the twig during the growing season. Two to four radial rows of earlywood vessels are already formed at the beginning of leaf development in *Juglans regia* and *Tilia cordata*. At the same date in spring leaves of *Rubus* are fully developed and the annual ring already shows latewood cells; radial growth is almost completed. The development of conductive elements continues during leaf maturation.

The differences shown in Figs. 9 and 11 indicate that the timing of radial growth in petioles varies between species and phenological stages and corresponds with secondary growth in twigs (Schweingruber & Poschlod 2005).

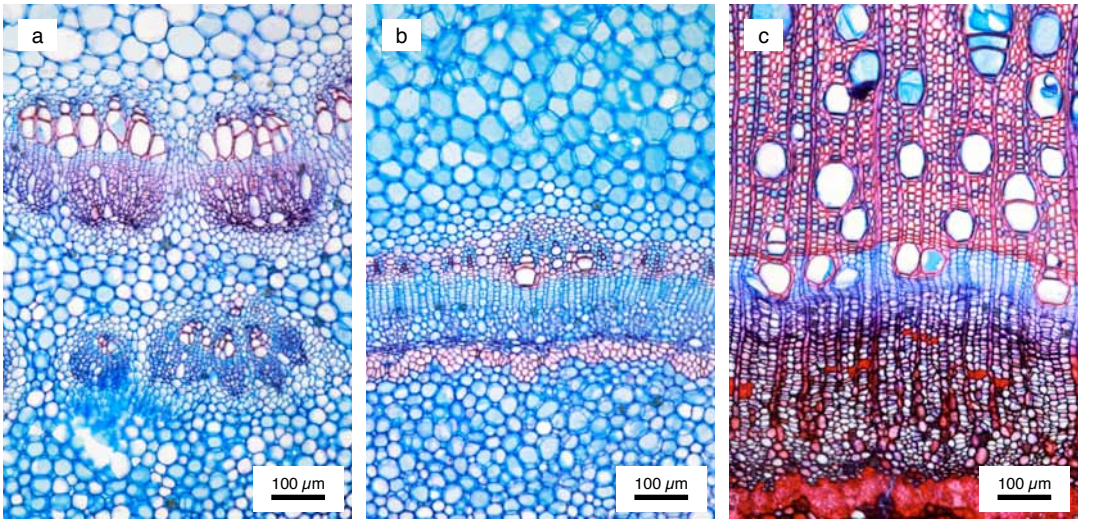


Figure 10. Xylem and phloem development in petioles, annual and biennial shoots of *Juglans regia* at the same date in spring. Plants have phenological stage 2, after bud breaking.

Figure 10a. Petiole: The xylem of the petiole shows a few lignified earlywood vessels and a fully developed phloem. Fibers are still absent. Vascular bundles are laterally separated by parenchyma cells.

Figure 10b. Annual shoot: The xylem shows a fully developed metaxylem, very few lignified earlywood vessels and a fully developed phloem, some sieve tubes have already collapsed. Fibers are still absent. The xylem is laterally almost continuous.

Figure 10c. Biennial shoot: A few unlignified and lignified vessels and parenchyma cells are formed. They are in contact with the latewood cells of the previous year. The phloem seems to be fully developed. Fibers are still absent. The xylem ring is incomplete.

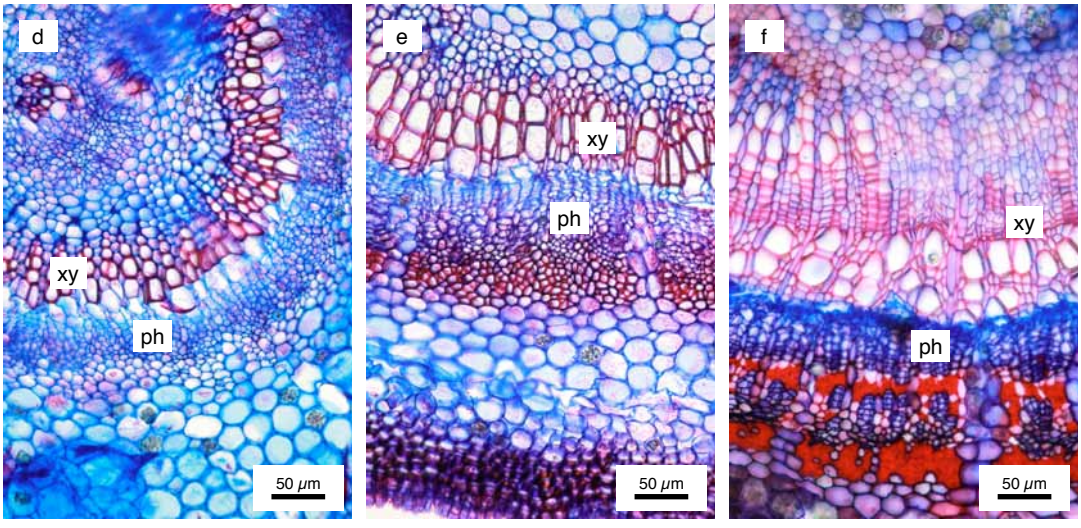


Figure 10 d-f. *Tilia cordata*: Phenological stage 2, after bud breaking.
Figure 10d. The xylem (xy) of the petiole shows a few rows of unlignified and lignified earlywood vessels and a fully developed phloem (ph).
Figure 10e. The xylem (xy) of the annual shoot shows a few rows of unlignified and lignified earlywood vessels and a fully developed phloem (ph). Outside of the phloem the first zone of lignified fibers is formed. The xylem ring is complete.
Figure 10f. The xylem (xy) of the biennial shoot shows a few rows of unlignified and lignified earlywood vessels and fibers and a fully developed phloem (ph). The xylem ring is complete.

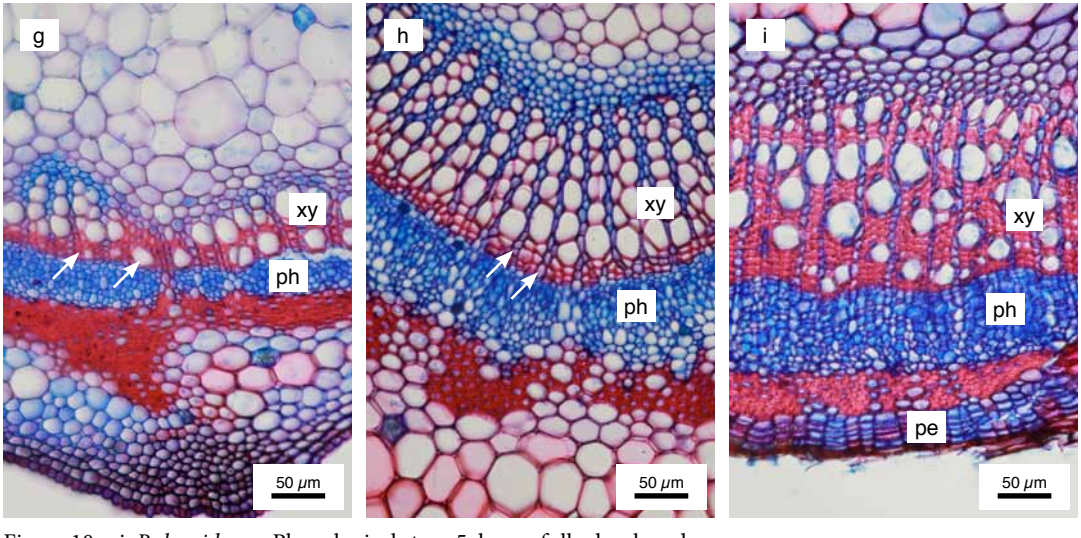


Figure 10 g-i. *Rubus idaeus*: Phenological stage 5, leaves fully developed.
Figure 10g. The xylem (xy) and phloem (ph) of the petiole is almost complete. First latewood cells are already formed (arrows).
Figure 10h. The xylem and phloem of the annual shoot are complete. A few rows of latewood cells are already formed (arrows). Fibers are still absent.
Figure 10i. The xylem and phloem of the biennial shoot is complete. The xylem of the second ring is in an adult stage. Fibers occur in the earlywood and latewood as well as in the first ring. The periderm (pe) is active.

8. The principal structure of the flowering system

8. 1. Overview

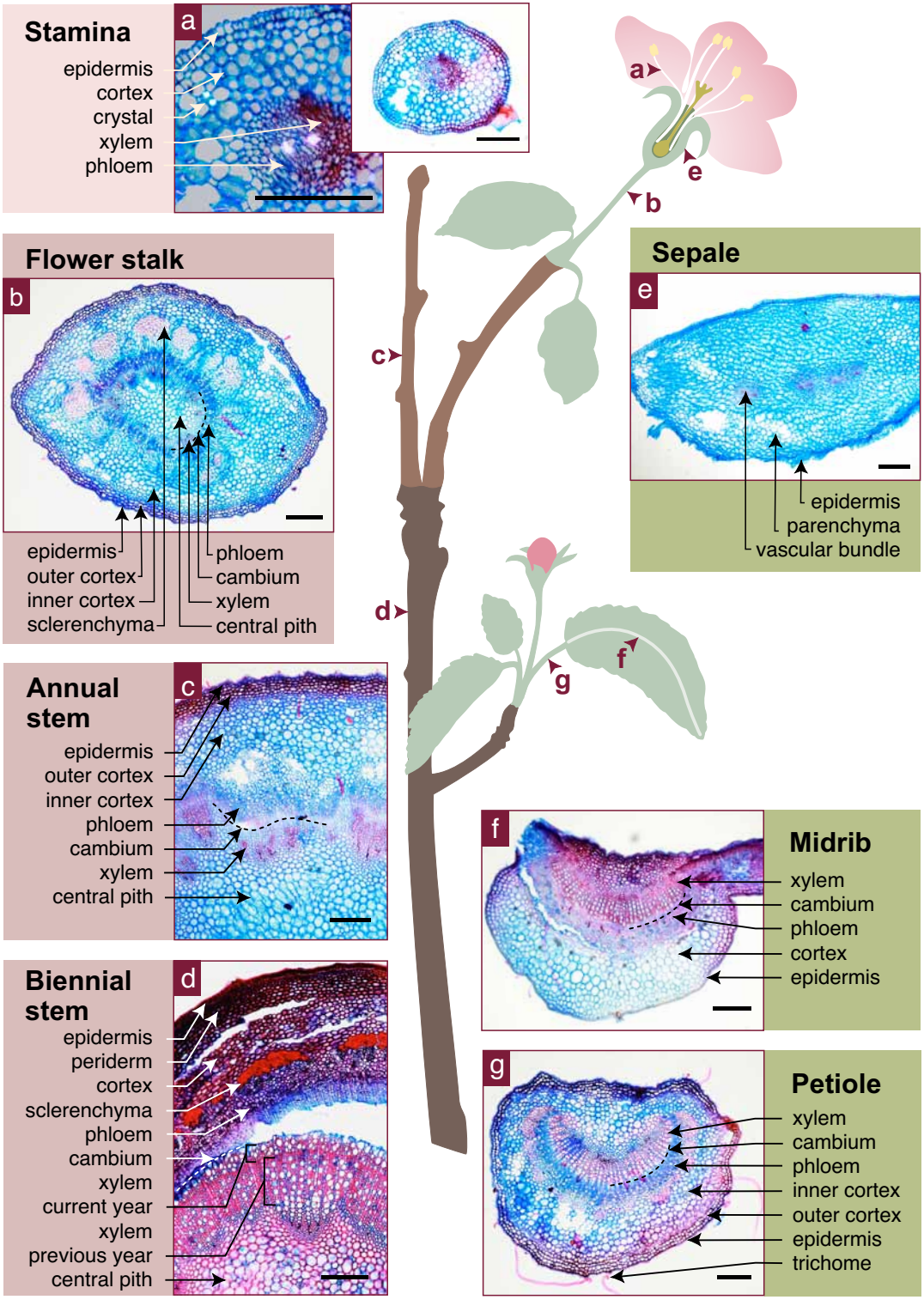
Here, we first show the anatomy of major anatomical parts of the flowering system of *Malus sylvestris* and then relate the structure of petioles to other stem-like plant parts. The flowering system is connected to an annual shoot, a biennial to perennial shoot, the petioles and the leaf.

The flower stalk and the annual and biennial shoot have a similar structure (Figs. 11 b-d). Open collateral vascular bundles are arranged centric around a pith and surrounded by a cortex. The flower stalks and the juvenile annual shoots are very similar (Fig. 11c). The sclerenchymatic parts in the flower stalk are a special feature (Fig. 11b).

The xylem of 2-year-old shoot already has a closed xylem/phloem ring and sclerenchymatic groups of cells in the cortex (Fig. 11d). The sepal base is flattened and consists mainly of cortex-like unlignified parenchyma cells and a few collateral vascular bundles containing lignified vessels and sieve tubes (Fig. 11e). The cambium in the shoot is active over several years, whereas cambium activity is limited to a few months in the flower stalk and a few days or weeks in sepals.

Petioles and midribs have a similar structure as older annual stems, however, the xylem/phloem zone remains in a crescent shape (Figs. 11f and g). The xylem/phloem part is laterally compact. The petiole is a continuation of the midrib of the leaves. The only difference is the presence of lateral leaf structures, e.g. palisade cells in the midrib (Fig. 11g). The cambium might be active for a few months.

The structure of stamens is different and, with the absence of a pith, reminds of roots. A central cylinder, consisting of vessel walls and sieve tubes is surrounded by a large unlignified parenchyma zone (Fig. 11a). A special feature is the presence of vessels with secondary walls.


Stamina
a
epidermis
cortex
crystal
xylem
phloem
Flower stalk
b
epidermis
outer cortex
inner cortex
sclerenchyma
phloem
cambium
xylem
central pith
Annual stem
c
epidermis
outer cortex
inner cortex
phloem
cambium
xylem
central pith
Biennial stem
d
epidermis
periderm
cortex
sclerenchyma
phloem
cambium
xylem
current year
xylem
previous year
central pith
a
b
c
d
e
f
g
Sepale
e
epidermis
parenchyma
vascular bundle
f
Midrib
xylem
cambium
phloem
cortex
epidermis
g
Petiole
xylem
cambium
phloem
inner cortex
outer cortex
epidermis
trichome

8. 2. The structure of stalks of male flowers

A few monoecious species have male inflorescences. Shown here are the stalks of the drooping male inflorescences of *Fagus sylvatica* and *Juglans regia* with pollinating stamens. In both cases the xylem/phloem part of the stalk forms a circle outside of the pith and inside of the cortex. Vessels are lignified (Figs. 12). Cambium cells in *Juglans* are still present (Fig. 12b). The formation of the stalk occurs within a week and its live span is 2–3 weeks.

8. 3. The structure of stalks of female and androgynous flowers

Many monoecious species have female inflorescences in horizontal or vertical positions, e.g. *Fagus sylvatica* (Fig. 13a) and *Alnus viridis* (Fig. 13b), while androgynous species have their flowers in a range of positions, e.g. *Prunus avium* (Fig. 13c), *Prunus padus* (Fig. 13d) and *Viburnum lantana* (Fig. 13e). Characteristic for all of flower stalks is their circular shape in cross section view. However, the form of the conductive tissue is different, separated vascular bundles occur in *Fagus sylvatica* (Fig. 13a), *Prunus avium* (Fig. 13c) and *Prunus padus* (Fig. 13d), while *Viburnum latana* (Fig. 13e) and *Alnus viridis* (Fig. 13b) form a closed circular xylem-phloem belt.

Corresponding with the changing weight of flowers and fruits over time, the stalks of androgynous species also change their structure. The juvenile flower stalk has single vascular bundles and a small lignified xylem/phloem belt. After pollination of the flower, the cambium remains active and forms a large continuous lignified belt of fibers, a few vessels and parenchyma cells in the cortex form large groups of sclereids. The process is the same in *Prunus domestica*, but due to this species' fruit being heavier than the fruit of *Prunus avium*, the xylem belt here consists of a dense fiber tissue with a few vessels. Sclereids are also present in the cortex.

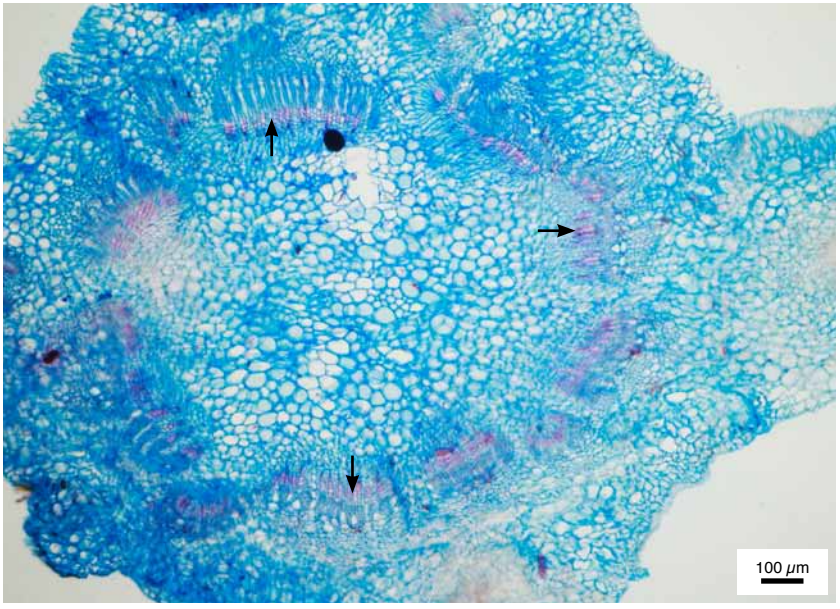


Figure 12. *Juglans regia*. 2-mm-long inflorescence in vertical position. Vessels are lignified (arrows).

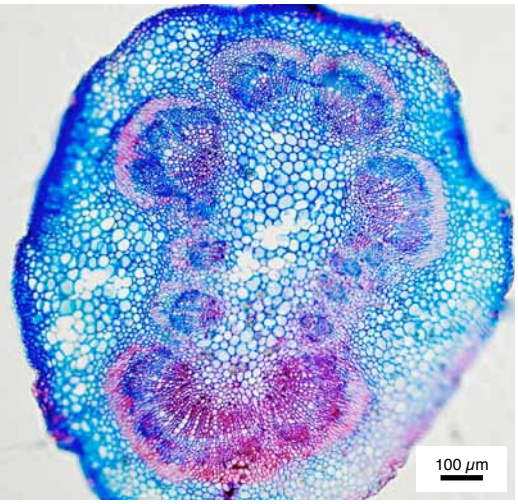


Figure 13a. *Fagus sylvatica*. Irregularly arranged large and small vascular bundles in a horizontally positioned stalk.

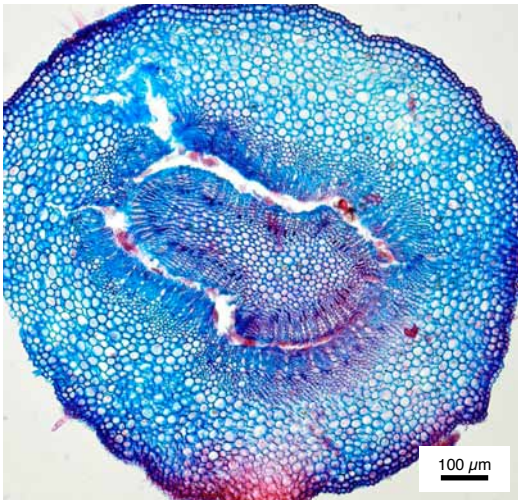


Figure 13b. *Alnus viridis*. Continuous band of xylem and phloem around the pith in an upright flower stalk.

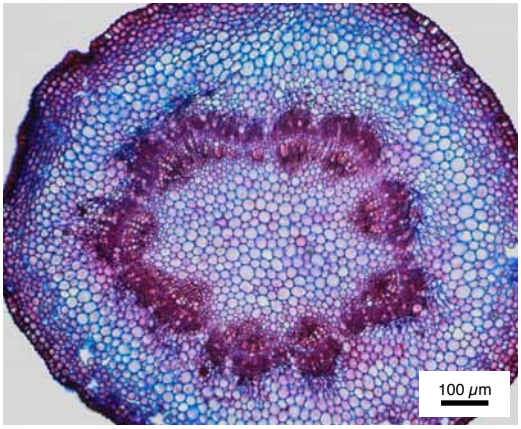


Figure 13c. *Prunus avium*. Centric arranged vascular bundles in a horizontally positioned stalk.

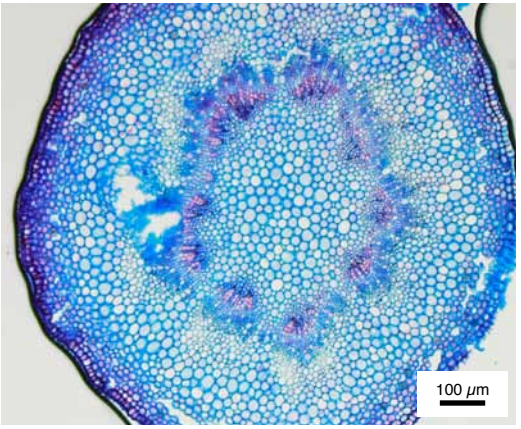


Figure 13d. *Prunus padus*. Centric arranged vascular bundles in an upright flower stalk.

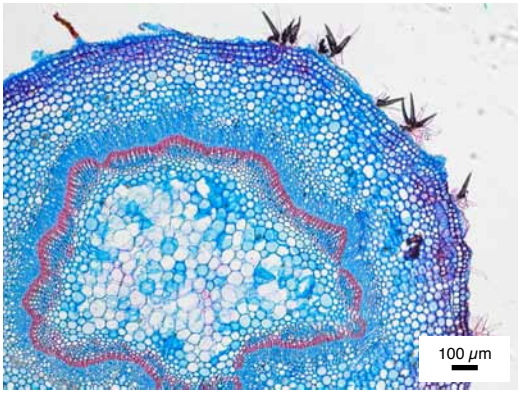
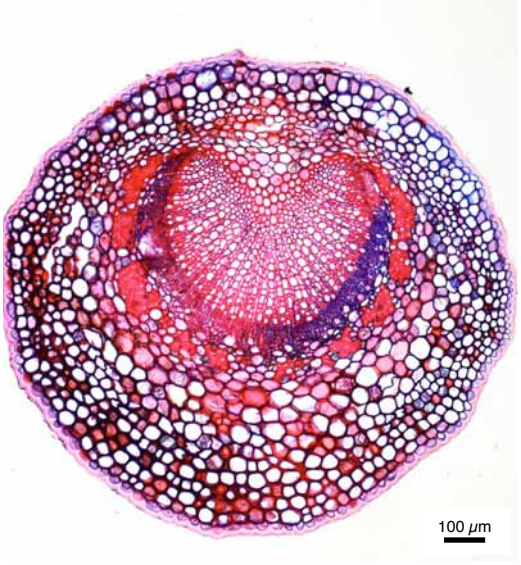


Figure 13e. *Viburnum lantana*. Continuous band of xylem and phloem in an oblique positioned stalk.

9. Definition of petiole features

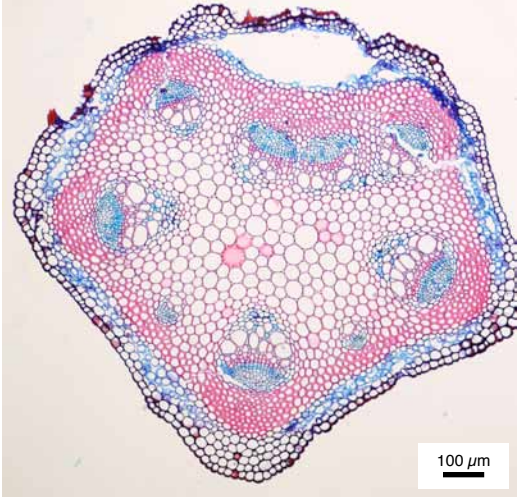
9. 1. Dicotyledons

Outline of petioles

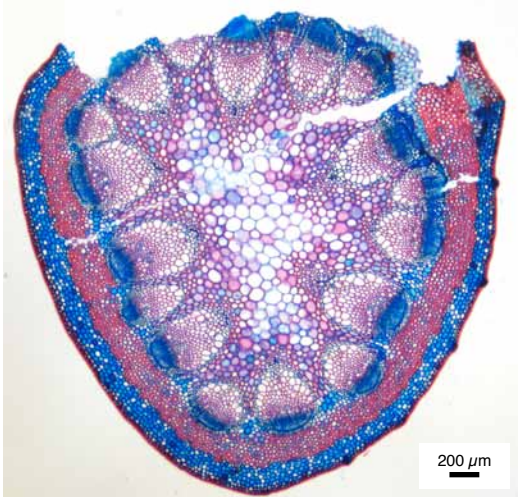


DP 1. Circular (*Amelanchier canadensis*)

DP 2. Almost circular, with flat part or indentation (*Cornus sanguinea*)



DP 3. Polygonal (*Clematis vitalba*)



DP 4. Triangular (*Mahonia aquifolium*)