STUDY OF PLANT TISSUES

Stems, Leaves, and Roots

+ Practical work in appendix

Introduction

This morpho-histological study focuses on the structural organization of higher plants (Angiosperms). It allows us to understand how tissues come together to carry out the functions of growth, conduction and photosynthesis. The study of botany should not be solely theoretical; It should serve as a basis for understanding plant toxicology (plants that are toxic to pasture) and animal nutrition (feed value of forages).

1. **Functions:** Support of photosynthetic organs and transport of fluids.

[Image of the primary structure of a broadleaf stem]

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3. Study of Leaves

The leaf is the organ specialized in the capture of light energy and gas exchange.

1. **Morphology:** It generally consists of a **blade** (flat part), a **petiole** (which connects it to the stem) and veins (conduction).
2. **Internal Structure:**
    1. **Epidermis:** Presence of **stomata** (pores allowing the exchange of CO2, O2 and water vapour).
    2. **Mesophyll:** Central tissue rich in chloroplasts. A distinction is often made between palisade parenchyma (under the upper epidermis, which is very active for photosynthesis) and lacunate parenchyma (which promotes the circulation of gases).
    3. **Veins:** Extension of the conductive tissues of the stem (xylem upwards, phloem downwards).

[Image de coupe transversale d'une feuille montrant le mésophylle et les stomates]

Note for the examination of samples: Observation under the microscope of thin sections stained (often with Mirande carmine-green) makes it possible to clearly distinguish the xylem (coloured green because it is lignified) and the phloem (coloured pink because it is cellulosic).

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I. Plant histology: the fundamental tissues

Histology is used to diagnose the presence of certain plants in rumen contents during an autopsy or forage analysis.

1. Support Fabrics (The Underwire)

1. **Collenchyma:** Living, flexible tissue, rich in cellulose. It is found in young growing organs (petioles, green stems).
2. **Sclerenchyma:** Tissue that is dead at maturity, impregnated with **lignin**. It is what gives hardness to mature stems.
   Veterinary note: Excess lignin decreases the digestibility of forage for ruminants.

2. Conductive tissues (the circulatory system)

1. **Xylem (Wood):** Made up of lignified vessels. It carries the raw sap. Its structure (striped, spiral or punctate vessels) is a microscopic identification criterion.
2. **Phloem (Liber):** Living cells (sieve tubes) carrying the elaborated sap.

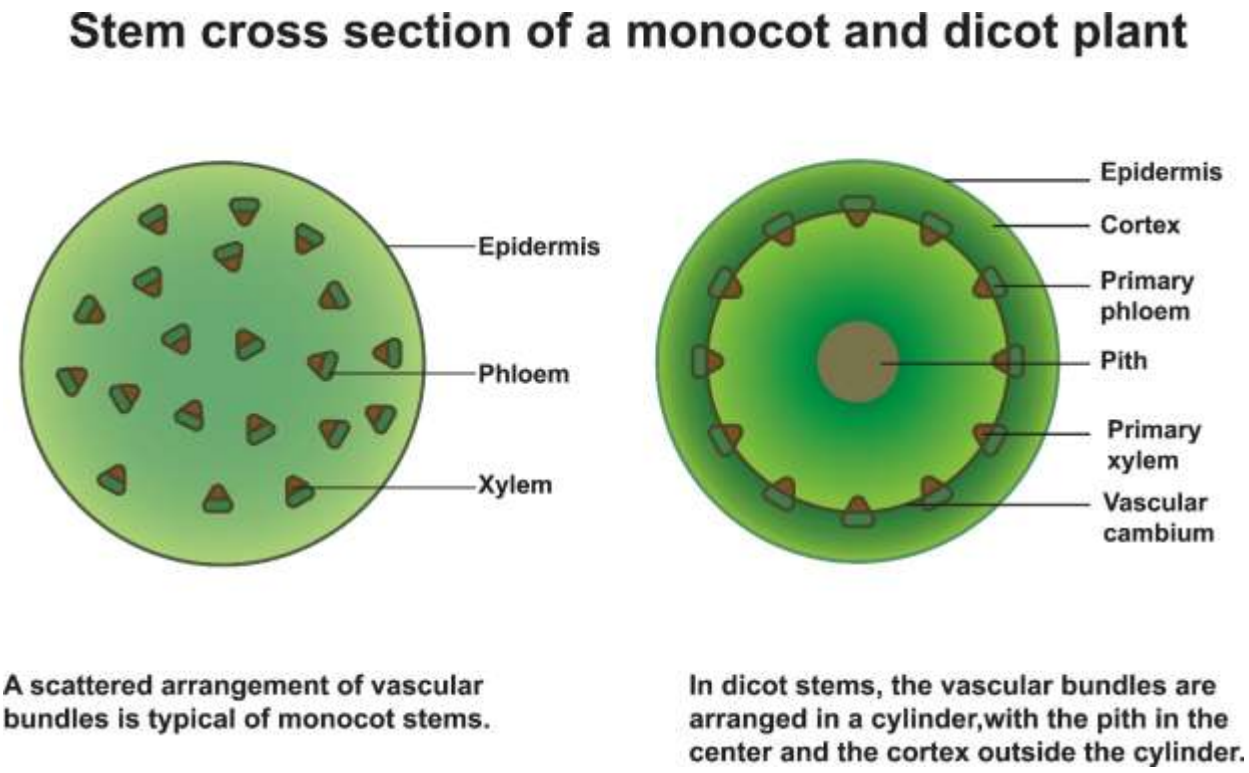
II. Study of the Stem (Cauline Apparatus)

The stem is the support of the carbohydrate reserves. In grasses (Poaceae), the structure is peculiar.

1. Comparative Anatomy (Cross-section)

1. **Dicotyledons:** The libero-ligneous bundles are arranged in a **single circle** (eustele). This often allows for growth in thickness (cambium).

2. **Monocots (e.g. Corn, Wheat):** The bundles are arranged in **several circles** or diffusely. There is usually no secondary growth (no real wood).



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2. Key points for the student:

1. **The Node and the Internode:** Areas of active growth.
2. **The Epidermis:** Often covered with a waxy cuticle that limits perspiration, but can also bear hairs (trichomes) that are sometimes irritating to the oral mucosa of animals.

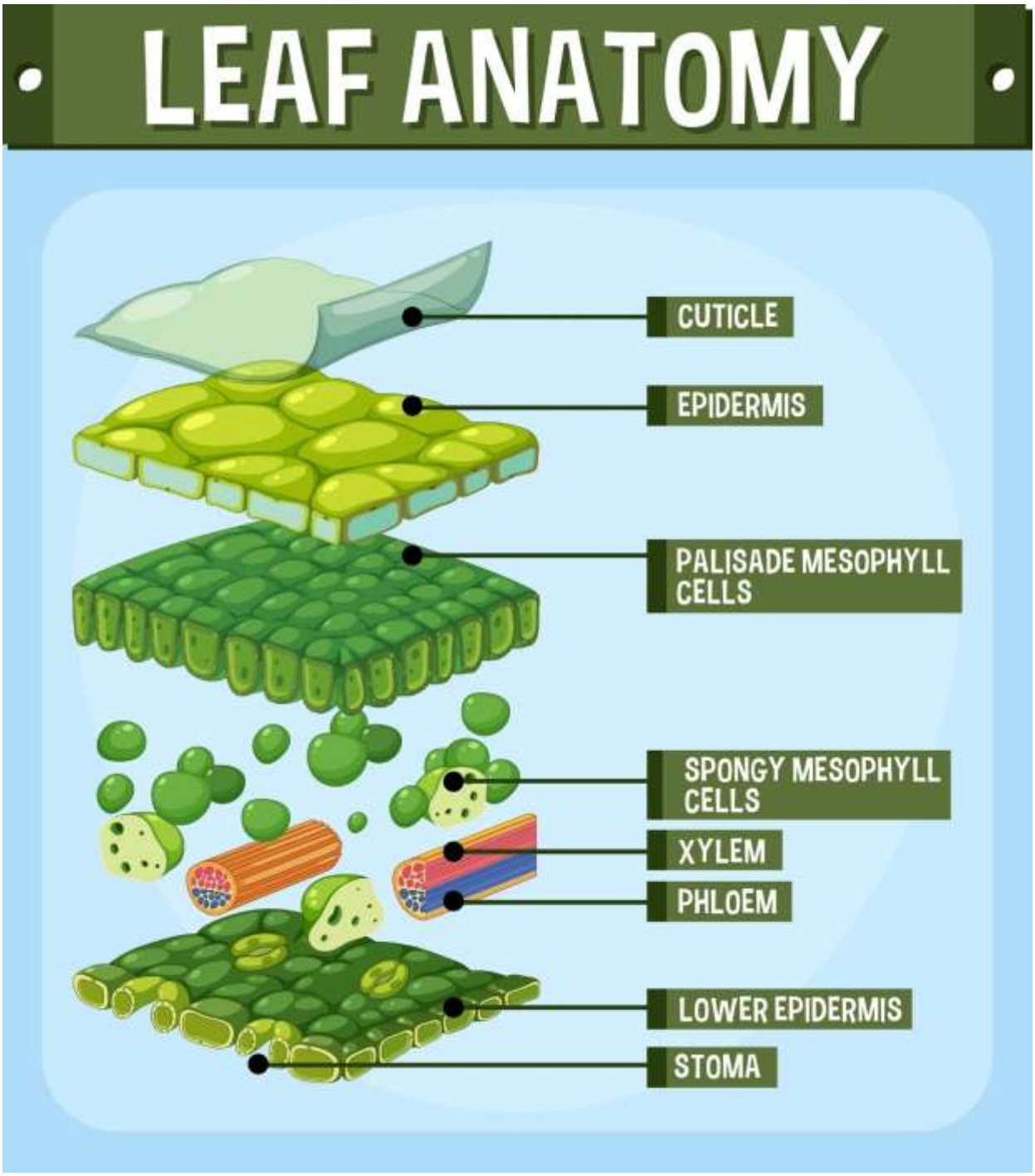
III. Study of the Leaf (Organ of Synthesis)

The leaf is the most nutritious part for livestock (proteins, vitamins).

1. Structure of the Mesophyll

1. **Palisade parenchyma:** Rich in chloroplasts, located under the upper surface. This is the photosynthetic factory.

1. **Lacunate parenchyma:** Located towards the underside, it provides empty spaces (meatus) for the circulation of gases (CO_2, O_2).



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2. The stomatal apparatus

The **stomata** regulate gas exchange and transpiration.

1. **Ecological significance:** The density of stomata varies according to the plant's adaptation to water stress (arid or semi-arid areas).

3. Types of ribs

1. **Pennae or webbed** in the Dicotyledons.
2. **Parallel** in Monocots (major characteristic of forage grasses).

IV. Practical Application: "The Veterinarian's Eye"

To make this course interactive, add a section on structure recognition:

1. **Microscopic identification:** Recognize a toxic broadleaf mixed with grass hay.
2. **Structure-Nutrition Relationship:** Explain why a "seeded" stem (highly lignified) is less palatable and less nutritious than a young leaf.

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Macroscopic morphology of plants.

Some essential criteria for the identification of species in the field, in particular to distinguish forage plants from toxic plants.

I. Morphology of the Twigs and Branching

The arrangement of the branches (twigs) determines the habit of the plant.

1. **Monopodial branching:** The terminal bud ensures the indefinite growth of the main axis (e.g. conifers).
2. **Sympodial branching:** The terminal bud aborts or turns into a flower, and growth is resumed by the lateral buds (e.g. the majority of deciduous trees).
    - **Types de bourgeons :** Terminaux (croissance en longueur) et axillaires (à l'aisselle des feuilles, donnant naissance aux branches ou aux fleurs).

II. The Sheet: Diversity and Disposition

The leaf is the most polymorphous organ. Its study is the basis of plant systematics.

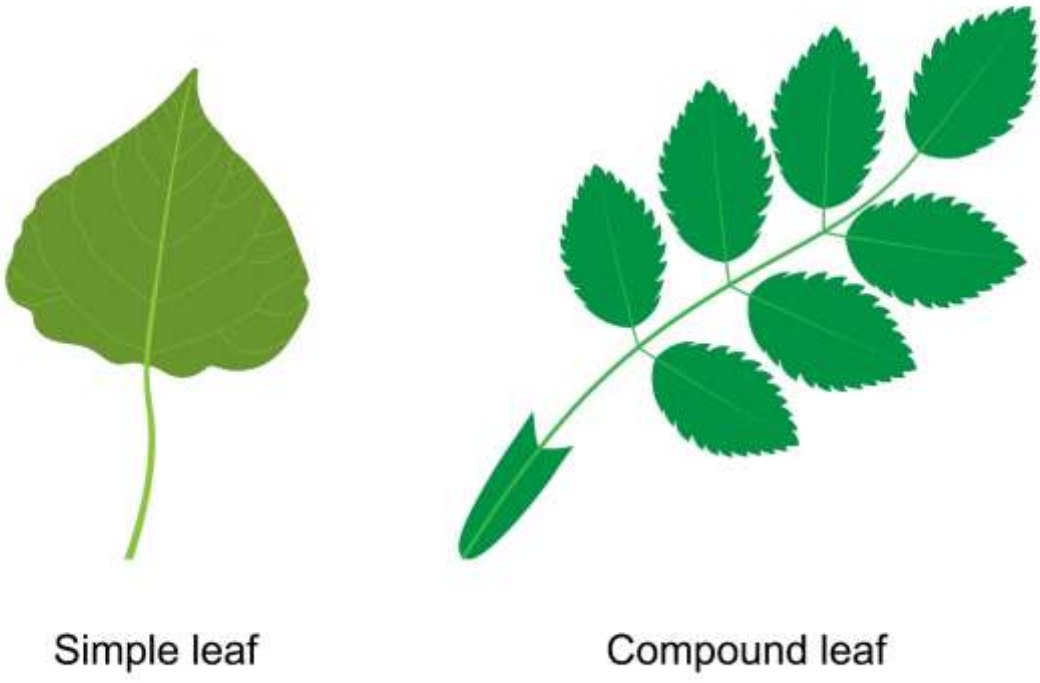
1. Phyllotaxy (Arrangement on the stem)

This is a quick identification criterion:

1. **Alternate:** Only one leaf per node (e.g. grasses).
2. **Opposite:** Two leaves facing each other at the same node (e.g. Mint).
3. **Whorl:** More than two leaves inserted in a circle at the same node (e.g., oleander — *very toxic*).

2. Shape of the Leaf Blade

1. **Single leaves:** The blade is in one piece (e.g. Oval, Lanceolate, heart-shaped).
2. **Compound leaves:** The blade is cut into several leaflets.
    1. *Pennea:* Leaflets arranged like the barbs of a feather (e.g. vetch).
    2. *Palmate:* Leaflets starting from the same point (e.g. Clover).



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3. The Margin (Edge of the Sheet)

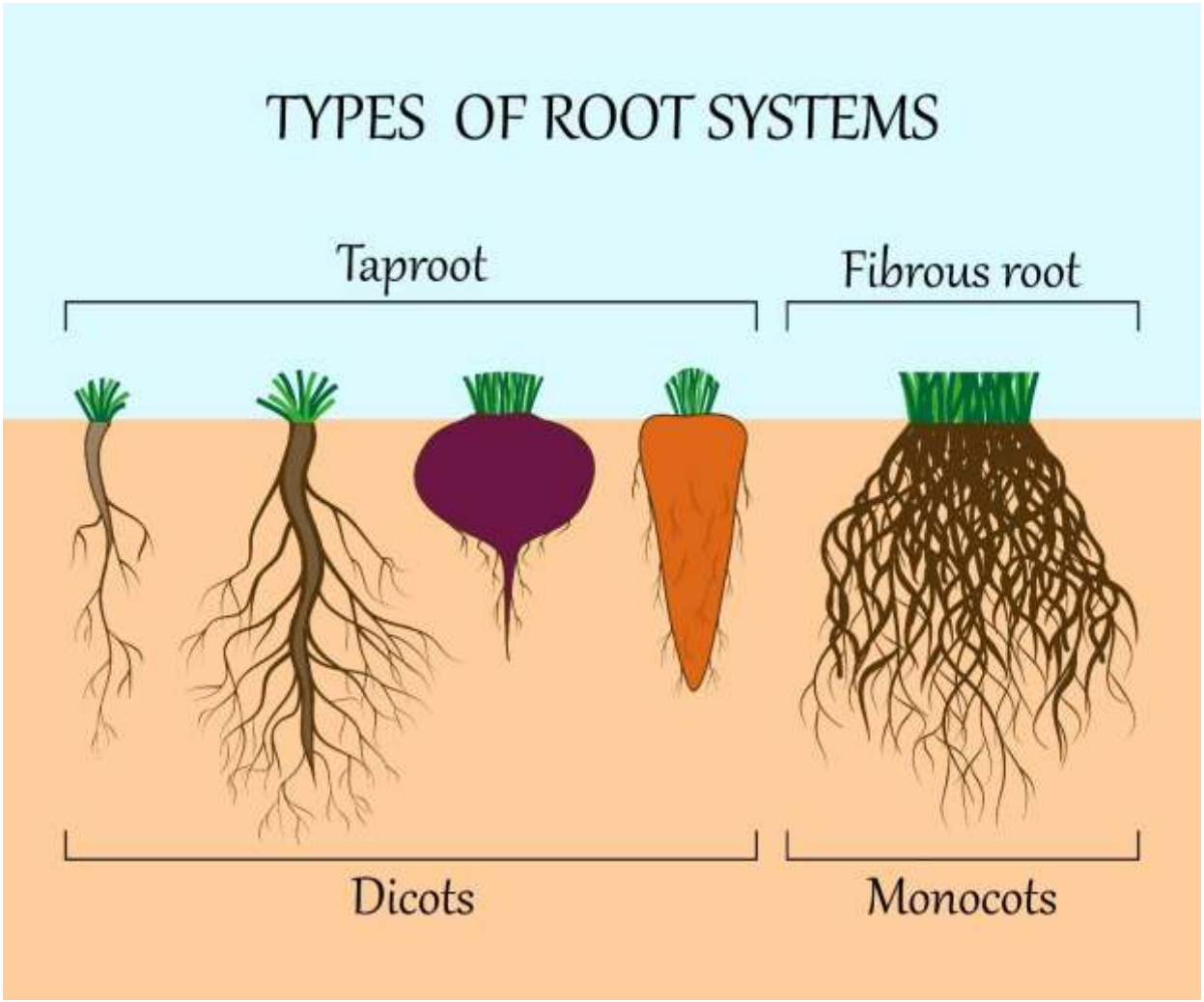
It can be **whole** (smooth), **serrated**, **lobed** (e.g. Oak) or **spiny** (e.g. Thistle).

III. Root Systems and Underground Organs

The root system is crucial to understanding the resistance of plants to grazing and drought.

1. The different types of roots

1. **Taproot (pivot):** A vertical main root that sinks deep with thinner secondary roots (e.g., alfalfa, carrot). Very resistant to drought.
2. **Fascicled Roots (clump):** A bundle of roots of equal importance starting from the base of the stem (e.g. wheat, barley, meadow grasses). They stabilize the soil well.
3. **Adventitious roots:** Roots that appear on organs other than the root (on stems or leaves).



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2. Subterranean Stems (Often Confused with Roots)

For the veterinarian, these organs are concentrated nutrient reserves:

1. **Rhizome:** Horizontal underground stem (e.g. couch grass). Allows for rapid vegetative propagation, making the plant difficult to eradicate.
2. **Tubercle:** Bulge of an underground stem loaded with reserves (e.g. potato).
3. **Bulb:** Underground bud surrounded by fleshy leaves (scales) storing reserves (e.g. onion, asphodel).

IV. Educational Interest for Veterinarians

It is relevant to insist on the following points :

1. **Palatability and Morphology:** Plants with thorny leaves or highly lignified stems are abandoned by livestock (refusal), which modifies the floristic composition of overgrazed rangelands.
2. **Toxicity:** Learning to recognize the whorled phyllotaxy of **the oleander** or the shape of the leaves of the **ferrule** can save a flock.
3. **Resistance to trampling:** Plants with fascicled roots (grasses) are more resistant to trampling by animals than plants with taproots, which are more fragile at the crown.

This table will allow students to quickly distinguish the two major classes of Angiosperms in the field or in the laboratory.

Comparison Table: Monocots vs Dicots **Character**	**Monocots (e.g. Poaceae/Grasses)**	**Dicotyledons (e.g. Fabaceae/Legumes)**
Seed	1 single cotyledon (reserve)	2 cotyledons
Roots	Fasciculates (tufted), homorhizae.	Pivoting (central pivot), allorhizia.
Stem (Anatomy)	Conductive bundles numerous and scattered over several circles.	Conductive bundles arranged in a single circle (eustele).
Leaves (Veins)	Parallellines (parallel ribs).	Pennate or webbed (reticulated veins).
Leaves (Attachment)	Often sessile with an embracing sheath.	Often with a petiole.
Flowers	Type 3 (3 sepals, 3 petals or multiples of 3).	Type 4 or 5 (multiples of 4 or 5).
Growth	Generally no secondary growth (no wood).	Secondary growth possible (wood and liber) via the cambium.

1. Interest in Animal Nutrition

1. **Monocots (Poaceae):** Constitute the energy base of grasslands (rye grass, corn, barley, oats). Their silica-rich structure and their bottom-up growth system (interlayer meristems) allow them to resist defoliation by herbivores.
2. **Dicotyledons (Fabaceae such as alfalfa, clover):** Are sought after for their richness in protein (nitrogen) and calcium. Their taproots allow them to draw water from deep down, remaining green when the grasses turn yellow in summer.

2. Interest in Pathology and Toxicology

1. **Rapid recognition:** In the case of suspected poisoning, the examination of plant remains in the rumen often makes it possible to decide. If the fragments have parallel veins, look for monocots (e.g. wild onion or certain toxic irises).
2. **Vegetative system:** Hollow stems (culms) are typical of grasses, while full, branched stems are more common in broadleaf grasses.

Suggestions for your Practical Work (TP)

To illustrate this table, you could organize a macroscopic recognition exercise:

1. **Material:** A wheat or corn plant (Monocot) and a bean or clover plant (Dicot).
2. **Instructions:** Diagram the root system and leaf insertion.
3. **Observation:** Make a cross-section of the stem and stain with carmine-green to visualize the arrangement of the bundles.

Practical Work Sheet : Histology and Plant Morphology

Audience: 2nd year Veterinarian
Objective: To master fine cutting and staining to identify support and conduction structures.

I. Materials and Samples

1. **Plants:** Stems and leaves of Poaceae (e.g., Wheat, Corn) and Fabaceae (e.g., alfalfa, broad beans).
2. **Cutting Equipment:** New razor blades (or manual microtome), elderberry pith (to hold the samples fine).
3. **Reagents (Mirande stain):**
    1. **Bleach** (Sodium Hypochlorite): to empty the cells of their contents.
    2. **Dilute acetic acid** (10%): to neutralize bleach.
    3. **Carmino-vert de Mirande** (mixture of carmine aluné and iodine green).

II. Colouring Protocol (Double Colouring)

The aim is to differentiate between cellulose (pink) walls and lignified (green) walls.

1. **Making the cuts:** Make the thinnest possible (almost transparent) cross-sections.
2. **Emptying (15-20 min):** Place the cups in bleach. They should become white/transparent.
3. **Rinse 1:** Pass the cups in distilled water.
4. **Neutralization (1 min):** Soak in acetic acid (CH_3COOH) to fix the dyes.
5. **Colouring (3-5 min):** Immerse the cuts in the Carmino-vert de Mirande.
6. **Final rinse:** Wash with distilled water until the water is no longer coloured.
7. **Mounting:** Place between the blade and the slat with a drop of water or glycerin.

III. Microcopic Observation and Interpretation

1. Colour code to remember

1. **Pink / Red:** Cellulosic wall cells (Parenchyma, Phloem, Collenchyma). Soft, digestible tissues.
2. **Green / Blue:** Cells with lignified walls (Xylem, Sclerenchyma). Hard tissues, poorly digestible, rich in crude fibre.

2. Diagnostic criteria (Monocots vs Dicots)

Have students draw a portion of the section and identify:

1. **The arrangement of the beams:** Are they on a single circle (Dicot) or scattered (Monocot)?
2. **The Xylem/Phloem Ratio:** The xylem is generally internal to the phloem.

IV. Veterinary application (the "why?")

1. **Rumen content analysis:** Explain to students that lignin (in green) is resistant to rapid digestion. Forage that is too "green" under the microscope (too lignified) means a decrease in the energy value for the animal.
2. **Identification of toxicants:** The presence of certain types of hairs (trichomes) or calcium oxalate crystals (visible in polarized light) can indicate a poisonous plant that has been ingested.

V. Student Exercise

Observed structure	Color after coloring	Main function
Epidermis	Light pink	Protection
Xylem	Intense green	Conduction sève brute
Sclenchyma	Green	Support (rigidity)
Parenchyma	Pink	Reserve / Photosynthesis

Instructions: "Observe the alfalfa stalk. Why is sclerenchyma more abundant at the base of the stem than at the top?"

Examination of stomata on the surface of leaves?

The study of **stomata** is a key step in understanding how plants breathe and regulate their water, especially in climatic contexts such as that of the wilaya of Medea, where water stress is an important factor for rangeland quality.

Here is a methodological and theoretical supplement to insert into your practical worksheet.

V. Study of the Stomatal Apparatus

Stomata are epidermal structures that regulate gas exchange (CO_2, O_2 and transpiration H_2O).

1. Structure of a Stomata

A stomata is composed of:

1. **Two guard cells (stomatal cells):** Kidney-shaped chlorophyll cells that delimit the opening.
2. **The ostiole:** A central pore whose opening varies according to the turgidity of the guard cells.
3. **Companion cells (or appendages):** Epidermal cells surrounding guard cells.
4. **Substomatal chamber:** Empty space located under the ostiole in the lacunate parenchyma.

2. Typology and Layout

1. **Hypostomatic Plants:** Stomata only on the underside (frequent case in trees and Dicotyledons). This limits the loss of water by direct evaporation in the sun.
2. **Amphistomatic Plants:** Stomata on both sides (frequent case in Poaceae/Grasses).
3. **Morphology:** * In **Dicotyledons**, the stomata are often scattered randomly.
    - In **Monocots** (grasses), they are usually aligned parallel to the veins.

VI. Practical Protocol: Observation of the Stomata

There are two simple methods for students:

Method A: Direct Peeling

1. Take a fresh leaf (example: Iris or Leek, easy to peel).
2. Break the sheet gently and pull with a thin pair of tweezers to loosen a thin film of transparent epidermis.
3. Rise in a drop of water between the blade and the coverslip.

Method B: The Varnish Imprint (Ideal for hard-to-peel sheets)

1. Apply a layer of **clear nail polish** to a small area on the underside of the sheet.
2. Let dry for 5 to 10 minutes.
3. Gently peel off the dry varnish film with transparent adhesive tape (Sctoch).
4. Glue the tape to a glass slide for observation. This "moulding" allows us to see the exact imprint of the ostioles and the guard cells.

VII. Veterinary and Ecological Interest

1. **Adaptation to the environment (Xerophytism):** In semi-arid areas, some plants (such as esparto grass or certain steppe grasses) hide their stomata at the bottom of crypts or leaf folds to reduce transpiration. Explain to students that these "hard" plants are often less palatable and richer in silica.
2. **Stomatal Index:** The number of stomata per mm2 is a stable genetic trait. In veterinary forensic expertise, this makes it possible to formally identify a crushed or dried toxic plant (hay) by simply observing a fragment of epidermis under a microscope.

Module Conclusion

At the end of this course/practical work, the student should be able to:

1. Distinguish between digestible tissue (parenchyma) and supporting tissue (sclerenchyma).
2. Identify a plant as Monocot or Dicot by its morpho-histological characteristics.
3. Explain the relationship between the structure of the plant and its nutritional value to the animal.

Evaluation grid (MCQ or reflection questions) to validate the students' achievements on this module

I offer you **visual identification questions** for the histological part and **reflection questions** for the link with nutrition. Here is a proposal for an **evaluation grid**

1. Parenchyma vs. Sclerenchyma Distinction (Observation)

1. **MCQ:** "Under the microscope, you observe a tissue with uniformly thickened and lignified walls, without meatus. Is it parenchyma or sclerenchyma?"
2. **Application:** Ask to diagram the difference in cell wall thickness between these two tissues.

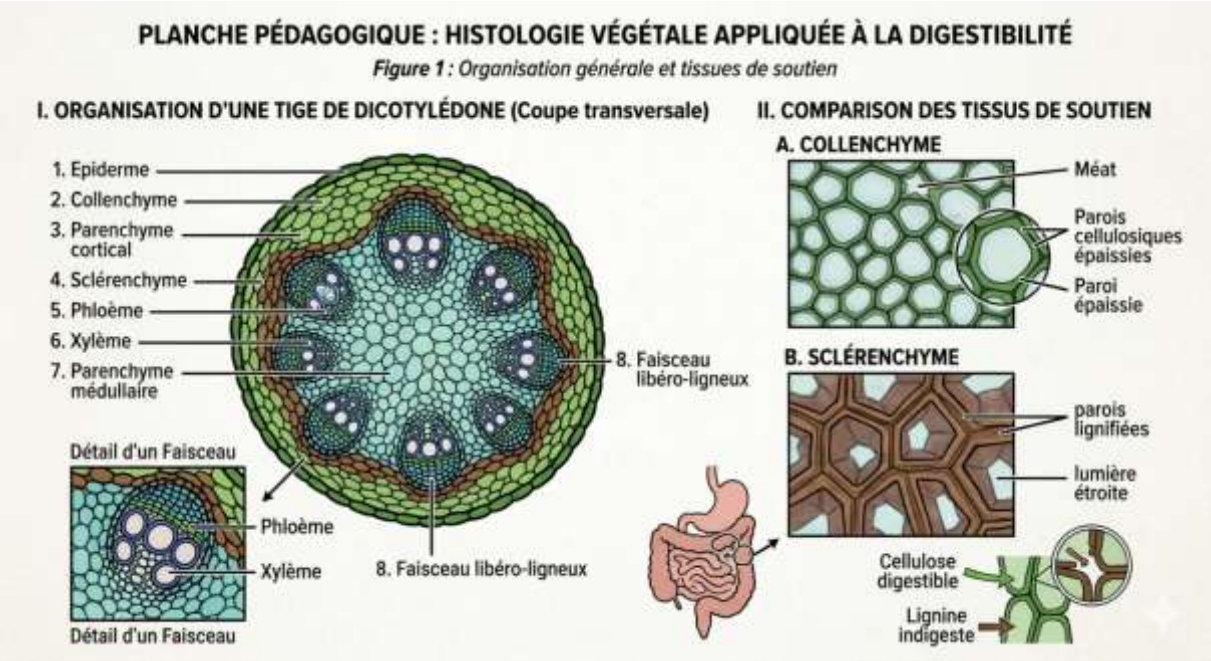
2. Mono vs. Dicot Identification (Morpho-Histology)

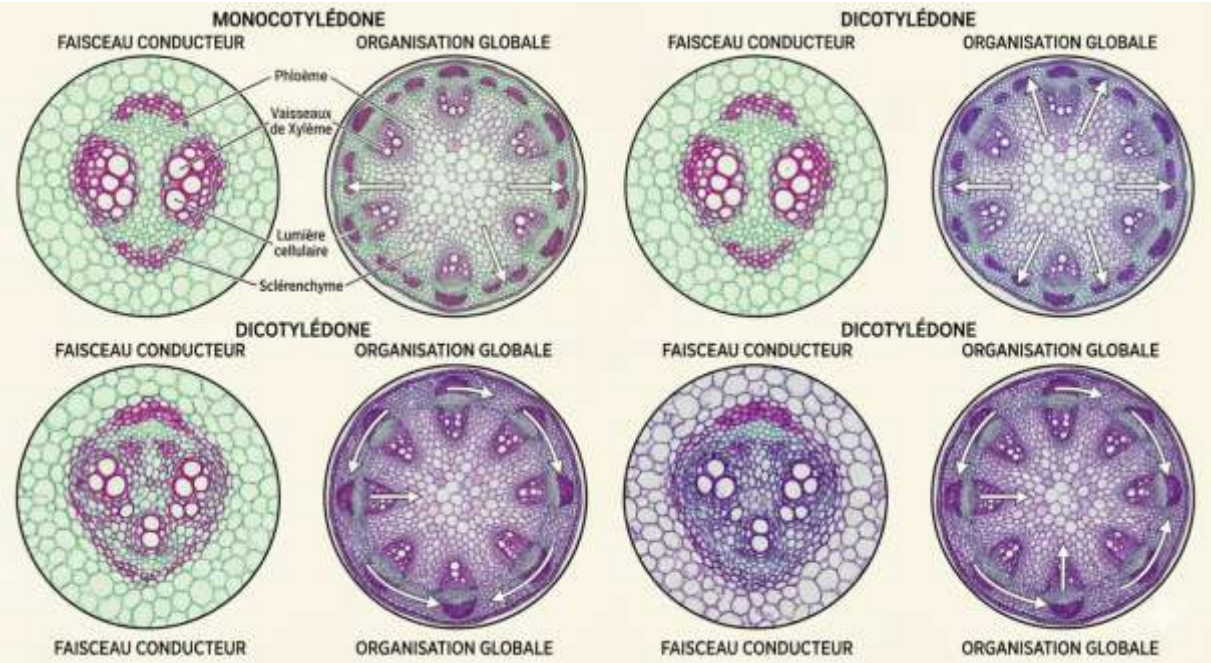
1. **MCQ:** "A stem section presents libero-ligneous bundles arranged on several concentric circles (atactostele). Which group does this plant belong to?"
2. **Observation:** Identify the type of venation (parallel or reticulated) on a given sample.

3. Structure and Nutritional Value (Reflection)

1. **Open-ended question:** "Why is a plant rich in sclerenchyma generally less digestible for a ruminant?" (Whereas: link between lignification and cellulose accessibility).
2. **Case analysis:** "Compare the nutritional value of a young grass (rich in parenchyma) versus a mature straw (rich in supporting tissue). Justify with histology."

Here is a **visual support structure** ready to be printed or projected. It is composed of three key figures corresponding to the pedagogical objectives.





📚 Revision Sheet: Histology & Digestibility

1. Support Fabrics: Support or Nourish?

There are two types of "skeletons" in the plant, with opposite impacts on digestion:

1. **Collenchyma (the "soft"):**
    1. **Appearance:** Living cells, walls thickened by **cellulose** only.
    2. **Nutritional value:** Fairly good. Cellulose is digestible by microorganisms in the rumen.
2. **Sclerenchyma (the "hard"):**
    1. **Appearance:** Dead cells at maturity, walls impregnated with **lignin** (red/brown colour). Very narrow cell light.
    2. **Nutritional value: Very low.** Lignin is an impenetrable barrier that traps nutrients.

2. The Parenchyma: The Energy Reservoir

This is the filling tissue (medulla, cortex).

1. **Structure:** Thin-walled cells with **meatus** (voids) for gas circulation.
2. **Role:** Storage of reserves (starch, sugars).
3. **Impact:** This is the most **digestible** and richest part of the plant.

1. Recognizing the Botanical Group (Stem Section)

Character	**Dicots**	**Monocots**
Beam arrangement	Arranged on **a single circle**	Scattered over **several circles**
Veining (leaf)	Reticulated (network)	Parallel
Cambium	Present (growth in width)	Absent

4. The link with Animal Nutrition

1. **Young Plant:** Rich in **parenchyma**, poor in sclerenchyma = High energy value.
2. **Aged Plant (Straw Stage):** Strong **lignification** (abundant sclerenchyma to carry the weight of the seeds) = useful for digestive congestion but low nutrient intake.
3. **Accessibility:** The more lignin there is, the less rumen bacteria can access cellulose and cell contents.

Tip for the examination: In a diagram, always look for the thickness and color of the wall to identify the fabric!

Technical glossary "toolbox" for the exam:

📘 **Glossary of Technical Terms**

1. **Phloem (Libber):** Tissue conducting the sap produced (sugars). On your schematics, it is located towards the **outside** of the beam. It is very digestible.
2. **Xylem (wood):** Tissue that conducts raw sap (water and mineral salts). On your diagrams, these are the big "ships" inwards. It is highly lignified (therefore not very digestible).
3. **Cambium:** Growth zone located between the phloem and the xylem in **Dicotyledons**. It allows growth in thickness.
4. **Atactostele:** A type of organization where the bundles are irregularly dispersed in the stem (characteristic of **Monocotyledons**).

    1. **Cell light:** The internal space of a cell. It is broad in the parenchyma and very reduced in the sclerenchyma because of the thickness of the walls.
