ULTRASTRUCTURAL FEATURES OF THE LEAVES IN SOME *SALIX* GENOTYPES, AND RESISTANCE TO ENVIRONMENT STRESS FACTORS

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INTRODUCTION

Genus *Salix* (willow) contain around 400 species of deciduous trees and shrubs, meet on moist soils in cold and temperate regions of the Northern Hemisphere. The willows present a large gamma of utilisations:

- **in agriculture - source of honey and food for animals;**
- **as medicinal plant (the bark contains salycin);**
- **manufacture of different objects of domestic utilisation;**
- **grown for biomass or fuel in energy forestry systems**
- **in environment for biofiltration, phytoremediation, soil erosion control, a/o.**

AIM

Analysis of leaf features in two willow species, with numerous utilization: phytoremediatory, energetic, medicine, decorative, manufacture of different domestic objects / sportive objects;

The interaction of polluted particles with cellular organelles;

The synthesis place of some bioactive substances;

Structural features implied in the resistance to environment.

BIOLOGICAL MATER

Four *Salix* populations belonging to two species:

Salix alba **L. , native from Europe, W & C Asia. Floresti population (Control, EM); cutting from Argineşti wood, cultivated on Rovinari waste dump. Tree up to 10-30 m tall, 1 m diameter, numerous hybrids and many cultivars selected for forestry and horticultural use.**

Salix viminalis **L., native from Europe and W-Asia. Shrub of 3-6 m. Argineşti wood, Rovinari dumps. Hyperaccumulator for Cd, Ch, Pb, Hg, Se, Ag, U, Zn, a/o; energy forestry; for sportive objects, a.o**

WORK METHODS

Heavy metal amount – spectrometric method

Radionuclide's activity – Duggan method

Ultrastructural investigation – classical method:

- **Prefixing in a 25% glutaraldehyde solution, 2 ½ h**
- **Fixing in a 1% Millonig solution 1 ½ h;**
- **Infiltration and embebbed in EPON 812;**
- **Ultrafine sections (90 nm thick) contrasted with uranyl acetate and lead citrate;**
- **Examination at a TEM of JEOL-JEM 1010 type (Centrum of Electron Microscopy, Cluj University).**

RADIONUCLIDE's ACTIVITY (Bq/kg soil)

Radionuclide	Arginesti wood	Rovinari waste dump	Rovinari, ash dump
U-238/Th-234	31.22 ± 3.48**	30.86 ± 3.53	97.70 ± 10.6
Ra-226	24.65 ± 1.10*	22.10 ± 1.31	99.20 ± 4.0
Pb-210	40.60 ± 2.88	36.60 ± 3.29	88.50 ± 6.0
Bi-214	22.40 ± 1.07	19.20 ± 1.42	66.20 ± 4.0
Pb-214	27.00 ± 1.08	25.10 ± 1.31	77.70 ± 3.75
U-235	3.54 ± 0.68	2.15 ± 0.47	5.78 ± 1.67
Ac-228 / Th-232	24.40 ± 1.87	26.90 ± 2.02	53.48 ± 3.82
Pb-212	40.10 ± 1.37	34.40 ± 1.26	81.67 ± 3.16
K-40	429.3 ± 21.9	401.5 ± 27.6	299.50 ± 3.59
Be-7	< 9.62	< 11.5	< 22.8
Cs-137	19.70 ± 0.93	13.60 ± 1.27	75.80 ± 3.44

HEAVY METALS AMOUNT (mg/kg soil)

Heavy metal	Agrinesti wood	Rovinari sterile dump	Rovinari, ash dump
Zn	205	58.8	66.6
Cu	35.2	19.0	52.8
Fe	326,108	22,995	26,705
Mn	578	422	215
Pb	36.9	15.6	38.3
Ni	85.8	29.6	42.6
Cr	46.0	26.1	39.4
Co	16.2	10.3	11.4
Cd	traces	traces	traces

EXOGENOUS PARTICLES vs. LEAF CELLS

The exogenous particles penetrate in cell leaf:

- **(a) at the epidermal cell level;**
- **(b) through the conducting vascular system.**

In leaf tissue the exogenous particles (granular or acicular shape) there are in all cells: on the cell wall, tonoplast, in the cytoplasm, as well as in some organelles as in nucleus, between chromatin fibbers.

In seedlings from Argineşti are present usually granular particles, while in seedlings from Rovinari are granular / acicular shape particles.

COMPOUNDS WITH STRESS RESISTANCE ROLE

FERRITIN - a metal-protein of 450 kDal, present in all organisms, rich in iron. The ferritin is associated with redox reactions, protecting the cell against the toxic effects of free iron, serving as a primary antioxidant. Is synthesized in chloroplast in structures of "crystal like bodies", as well as in mitochondria at the crysta level.

ANTHOCYANINS (flavonoids) - water-soluble pigments, present in all tissues of higher plants; protect the cells from the damage "high-light" by absorbing blue-green and ultraviolet light (high-light stress). Their synthesis takes place in chloroplast, the molecules being arranged on the thylakoid surface, later on being accumulated in cell vacuole together with other structures.

SALICYLLIC ACID, visible as amorphous blocks in leaf cytoplasm. Is mainly medicinal compound of *Salix* sp.

Other BAS: proteins, lipids, synthesized in cell organelles.

STRUCTURES vs. STRESS RESISTANCE

MULTIVESICULAR BODY's (*MVB's*) are endosomal organelles containing small vesicles (exosome), formed following the inward budding of the outer endosomal membrane, met in parenchyma cells or in the cells from the proximity of the conducting vessels. The plant cells can secrete endosomes derived from multivesicular bodies.

VACUOLE COMPARTMENTATION process, is met in plants and sessile organisms that synthesize secondary metabolites, which act as chemical defence and signal compounds (Wink, 1997).

DEPOSIT CELLS situated in lacuna parenchyma, near conducting system, single or together with

CONDUCTING VASCULAR SYSTEM are implied in removal of the cell rests, exogenous particles, a/o. If the amount of cell rests is better, these are accumulated in deposit cells, and then eliminate by conducting system.

CELLS AS A PROTEIN DEPOSIT, in palisade parenchyma tissue.

NUCLEOLAR ASSOCIATED BODY's (*NAB's*), is a metabolic nuclear structure, present in the cells with a bigger metabolic activity (normal and pathological).

SALIX ALBA - RESISTANCE FEATURES

Manifest a good adaptation to environment, having different mechanisms of resistance:

- **Polyploidy degree (4x);**
- **Small ICV value and DNA/chromosome (1.7 pg);**
- **Ferritin synthesis in mitochondria and in “crystal like bodies” in chloroplast**

SALIX ALBA, CONTROL

Palisade parenchyma present two regions:

- Near upper epidermis, 2-3 cells layer full with sallicyllic acid;

- 2-3 cell layers with many chloroplast parietal disposed, with starch grains.

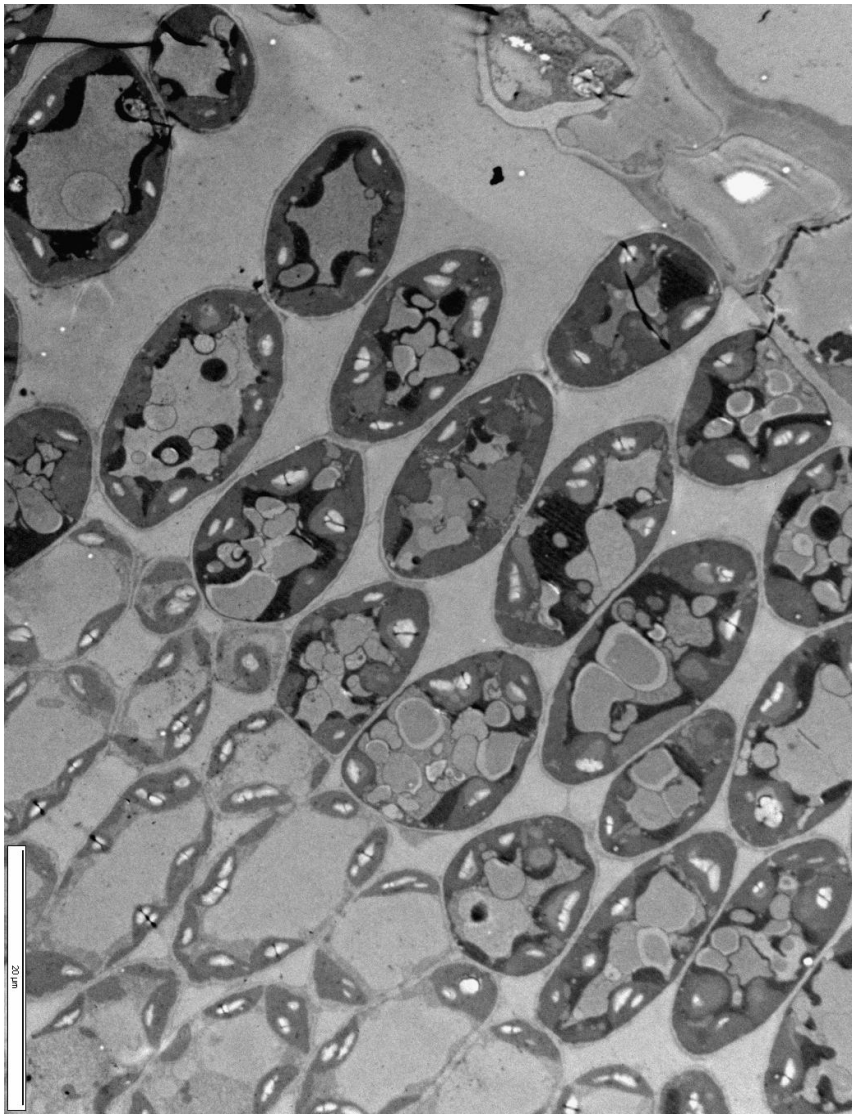
Cells are rich in cytoplasm and organelles: mitochondria, chloroplasts, dyctiosomes. a/o.

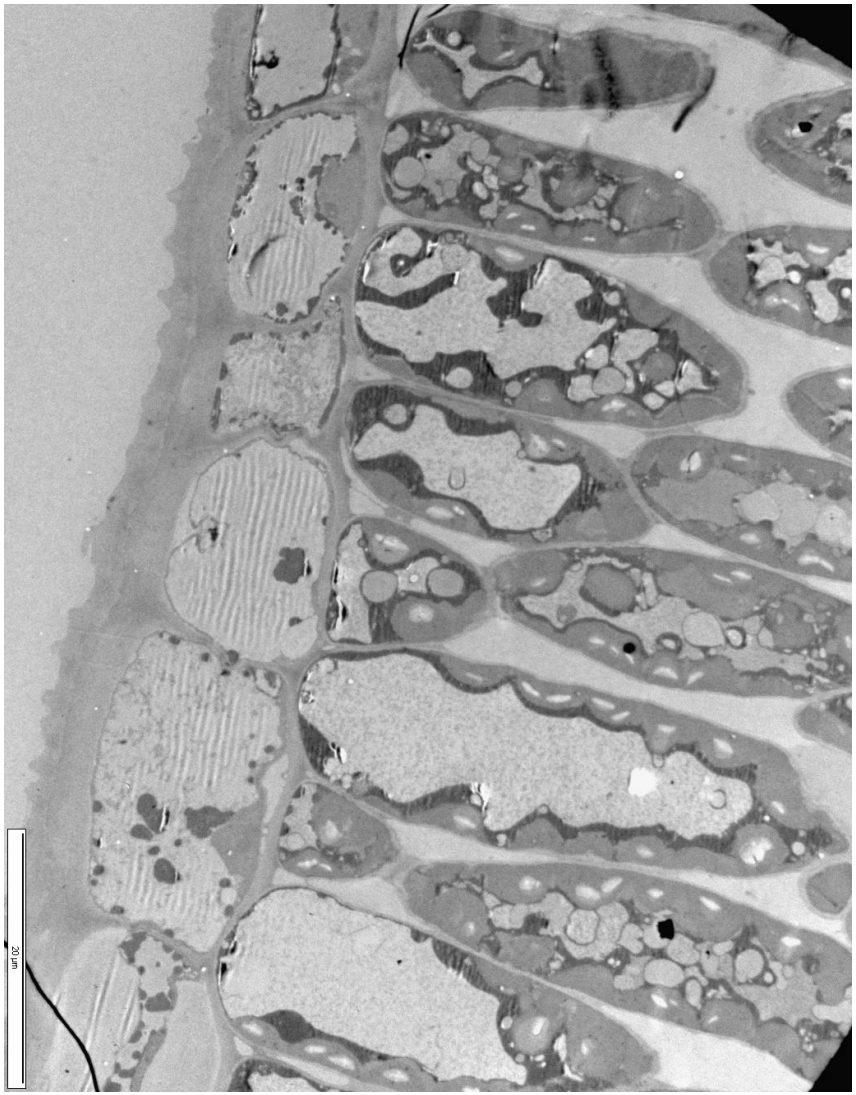
Euchromatic nucleus with electron-dense particles

Ferritin synthesis in mitochondria

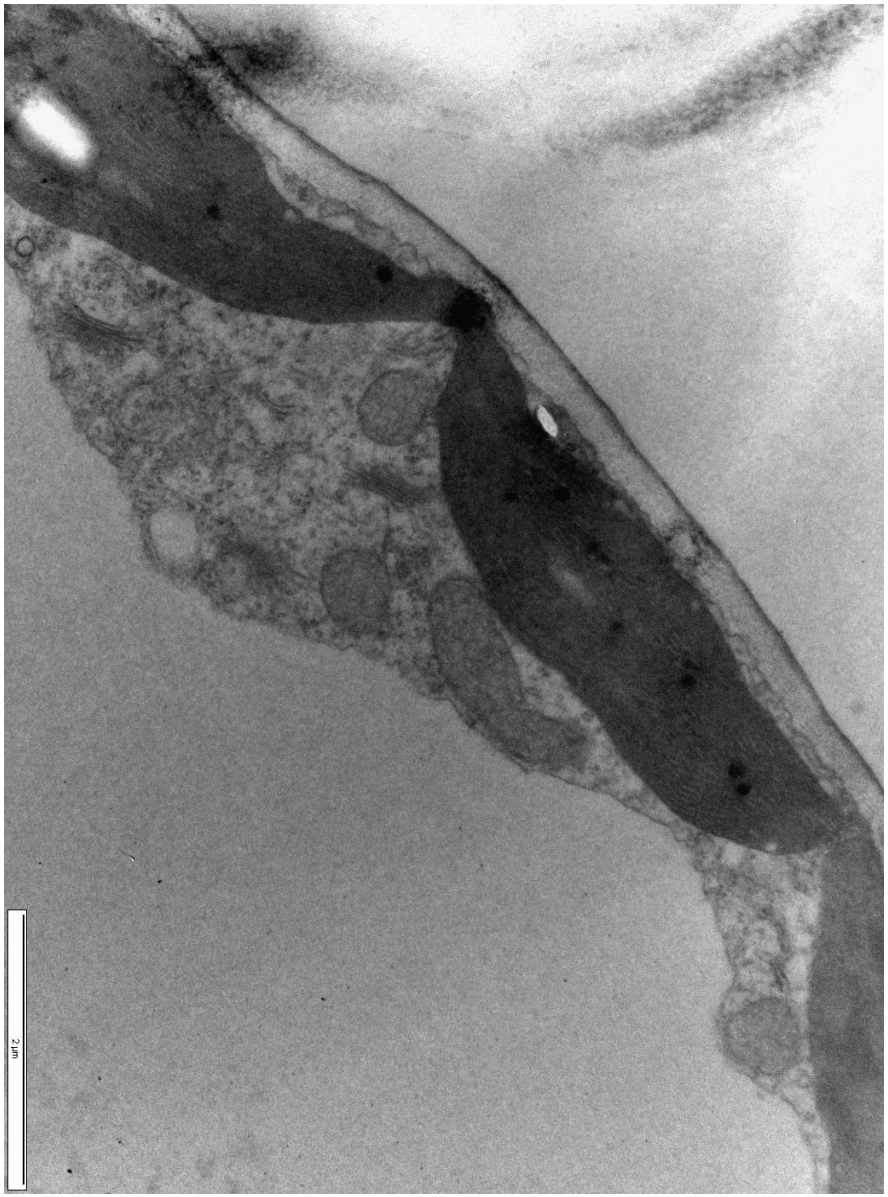
Lacuna parenchyma – 4-5 cell layers: chloroplasts with starch, SA, electron-dense particles, a/o

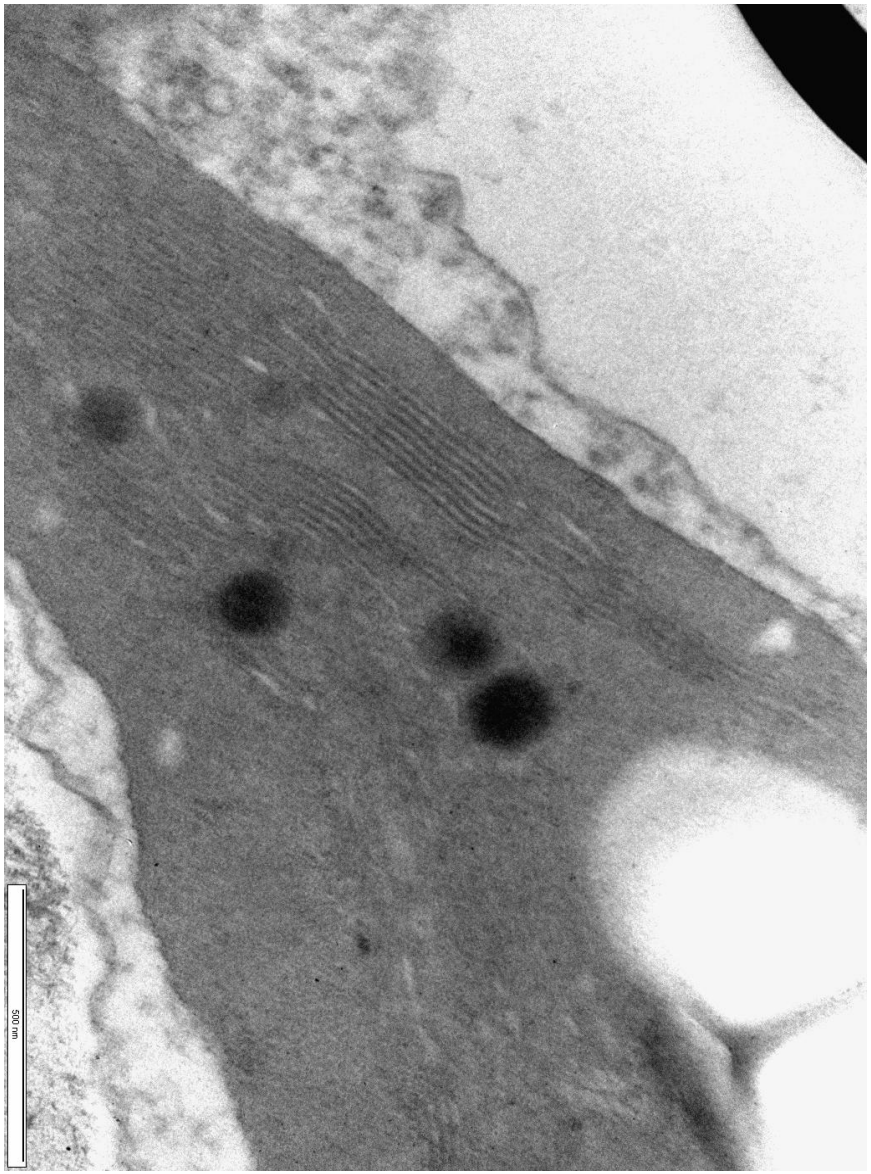
SALIX ALBA, Control





SALIX ALBA, Control



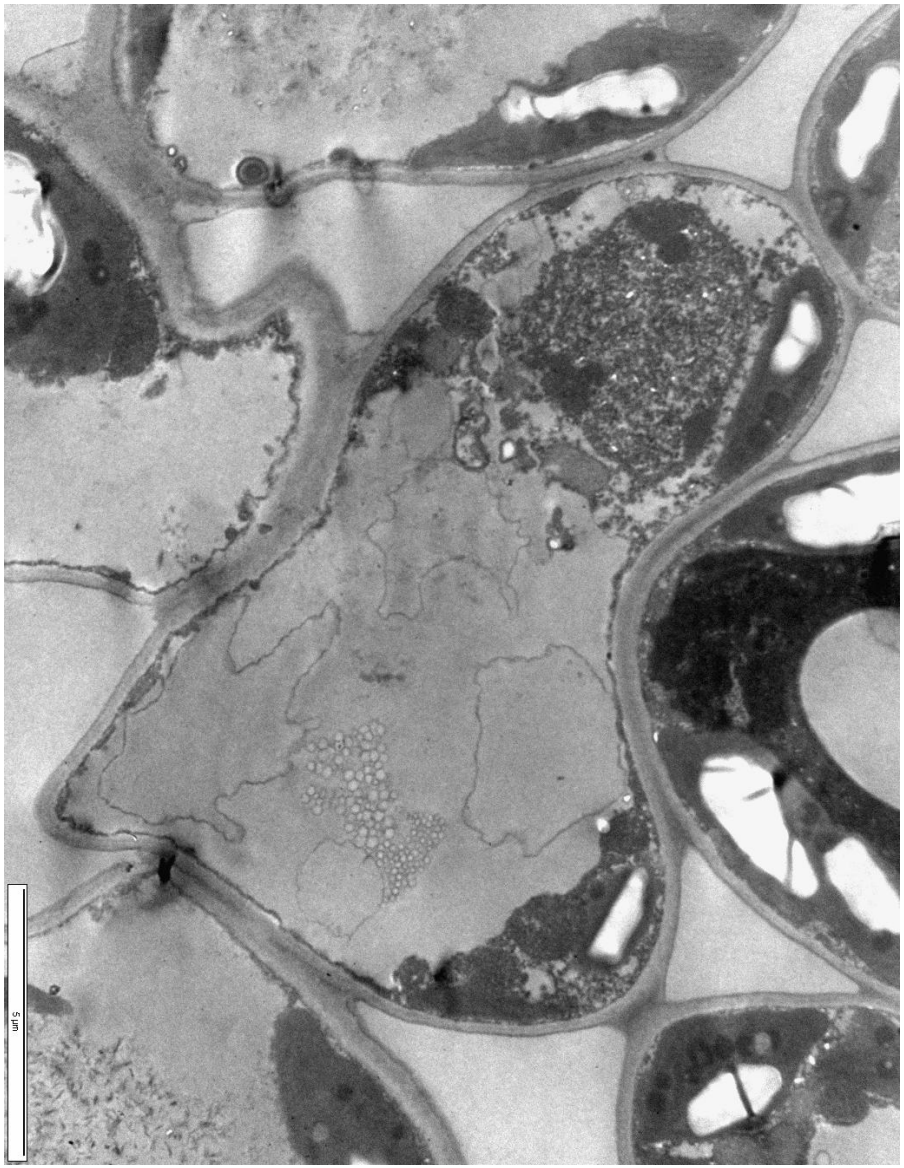


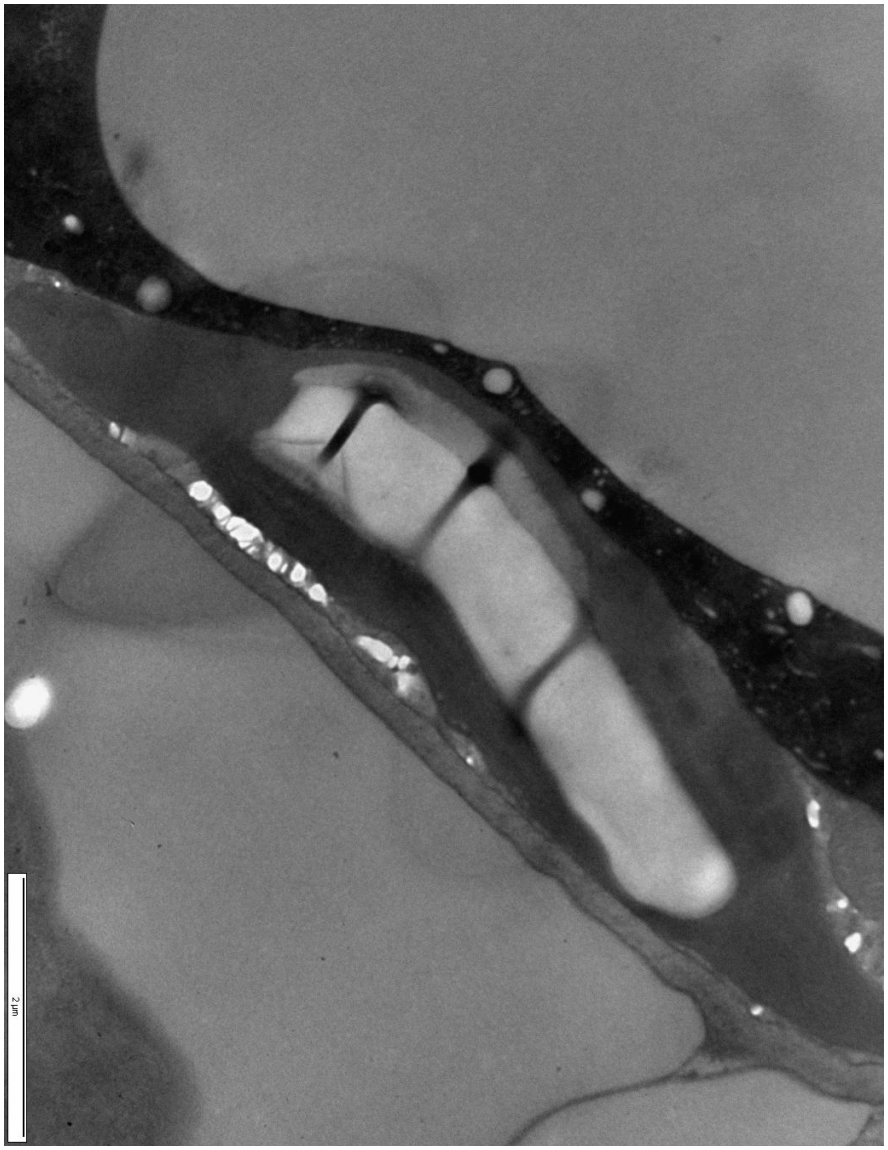
SALIX ALBA, fallow ash - Rovinari

Adaptation at stress factors (exogenous particles)

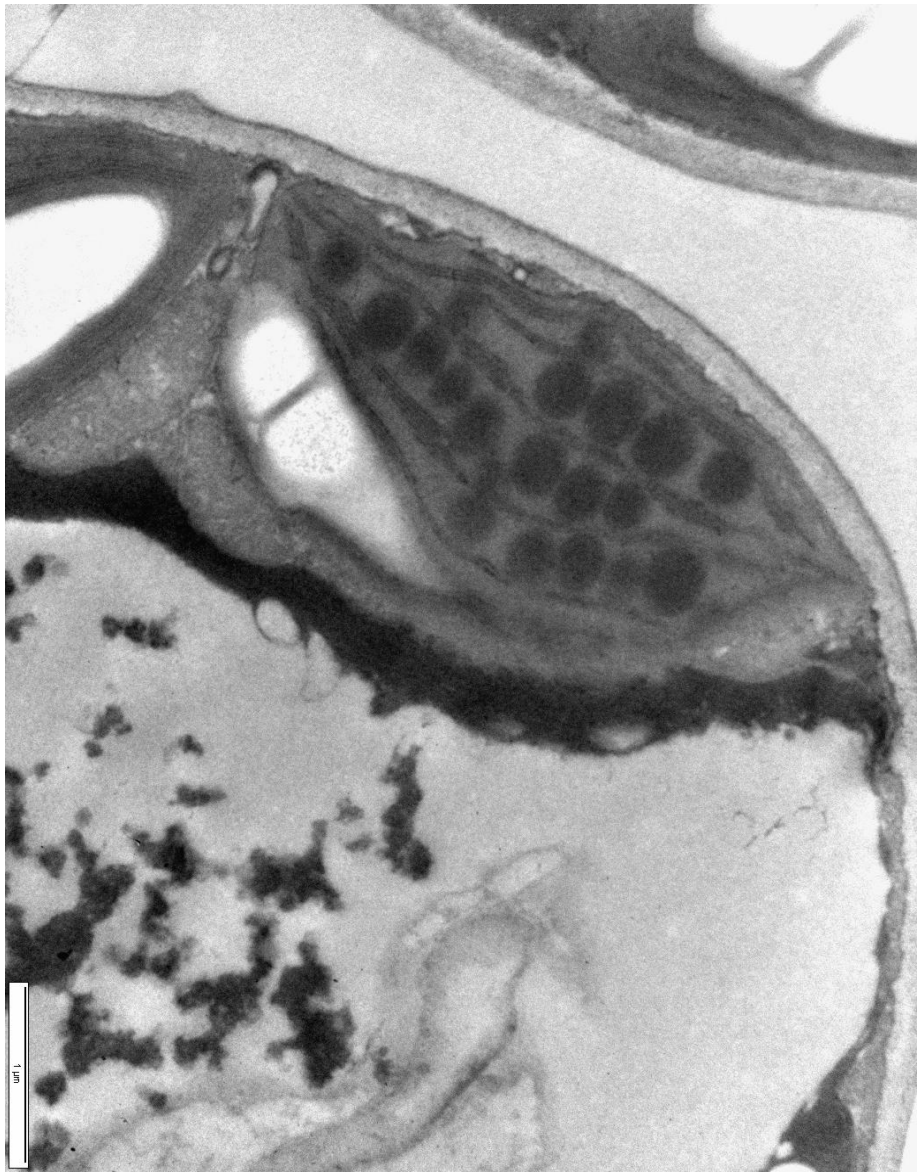
- **Compartmentation vacuole, and MVB's**
- **NAB's metabolic structures;**
- **Ferritin synthesis near crysta (Mt) and crystal like bodies (Chl);**
- **SA and other BAS synthesis in cytoplasm;**
- **Deposit cells for accumulation different matter**

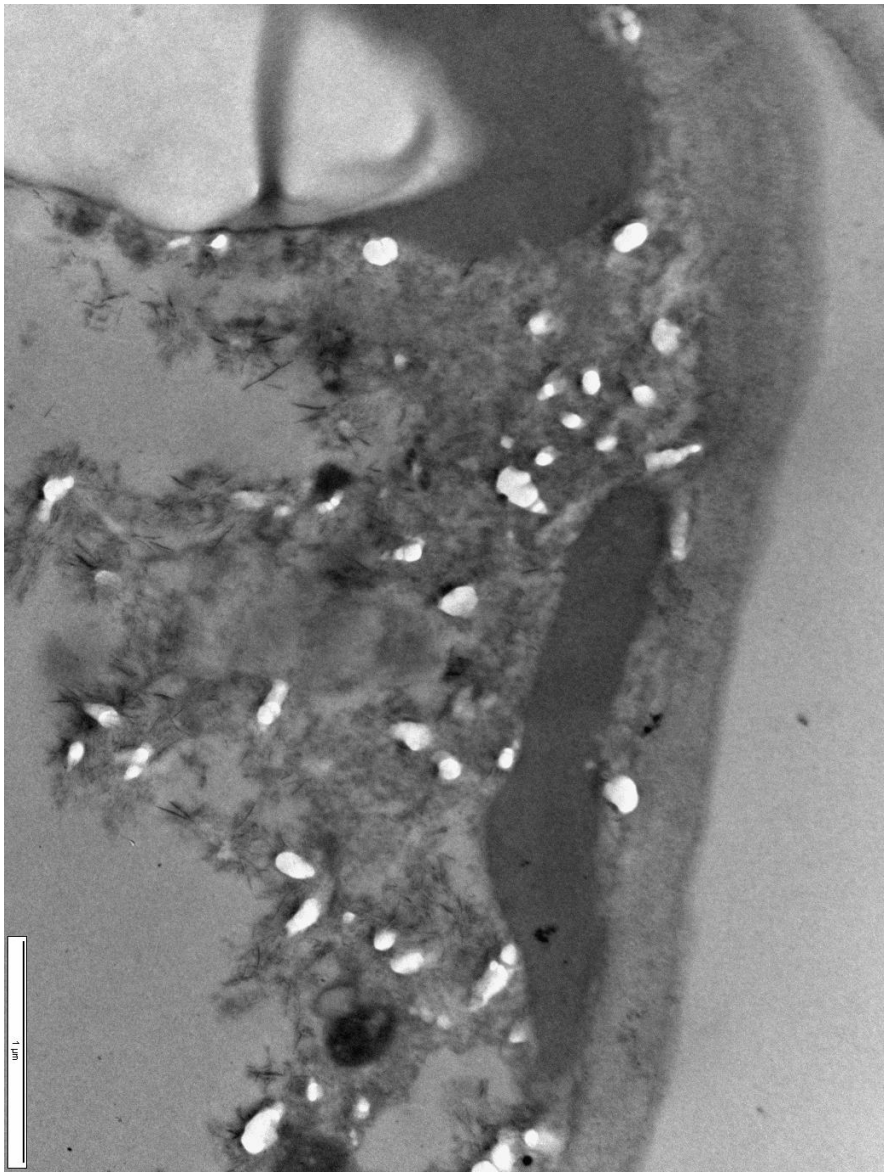
SALIX ALBA, fallow ash - Rovinari





SALIX ALBA, fallow ash - Rovinari





SALIX ALBA, waste dump - Rovinari

Palisade parenchyma - cells full with lipid drops.

Lacuna parenchyma – cells and cytoplasm parietal

Exogenous particles in the cell wall

Chloroplast in active synthesis: ferritin, anthocyan

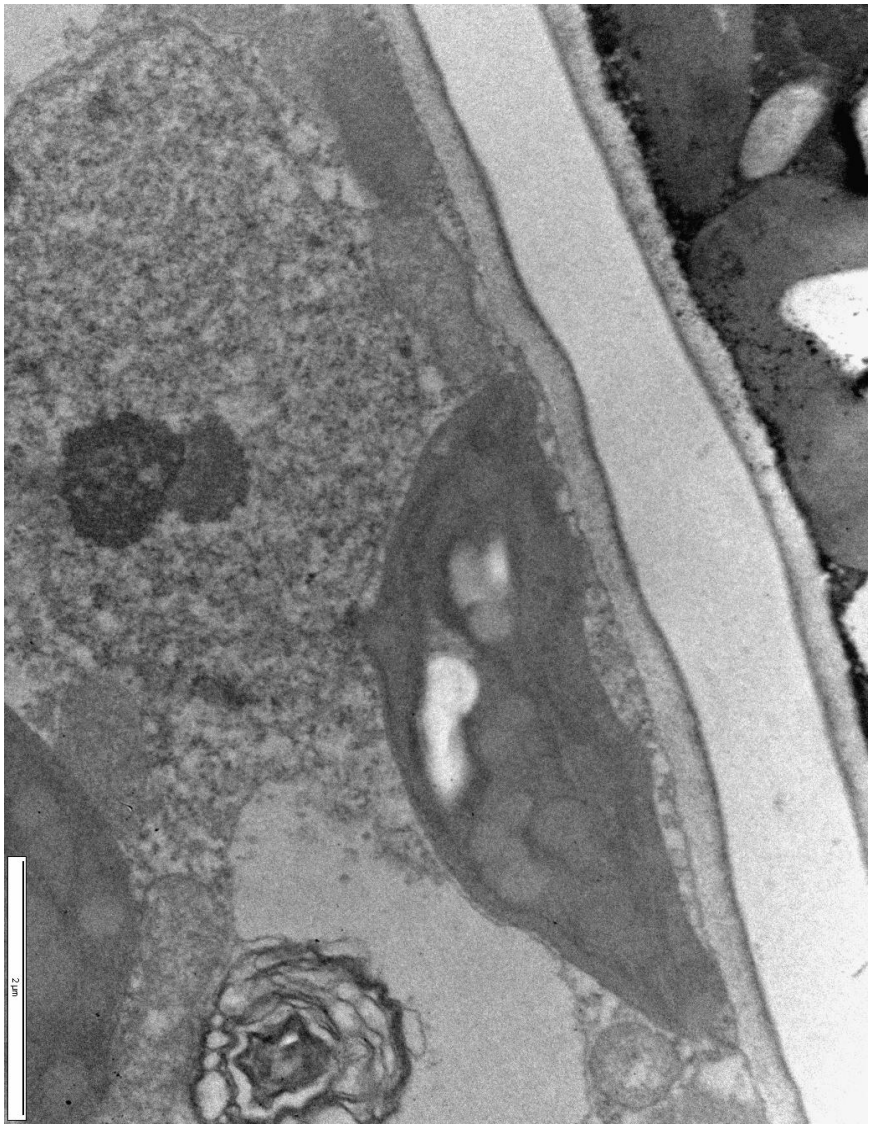
Mitochondria with ferritin synthesis

Nucleus with a NAB's structure.

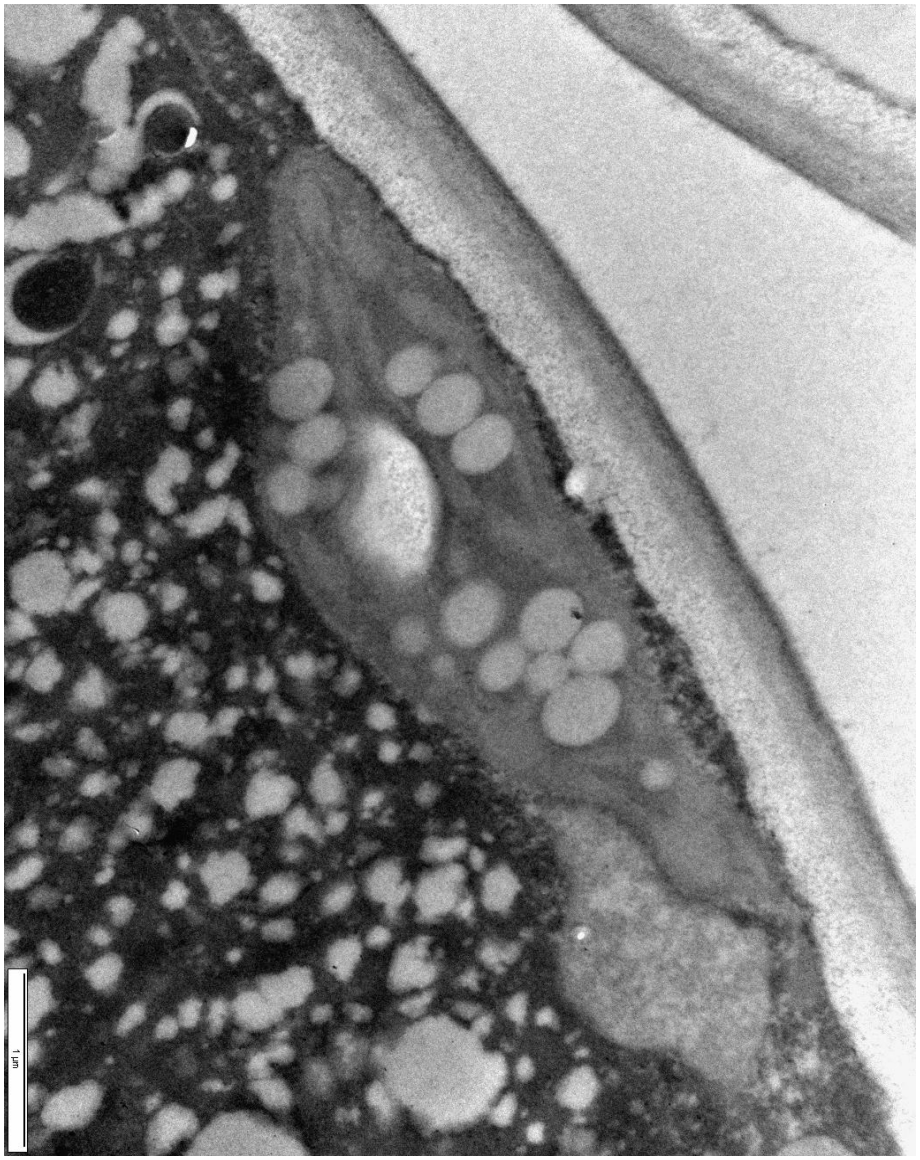
Mielinic structures in cytoplasm.

Central cylinder with vessels and fundamental parenchyma

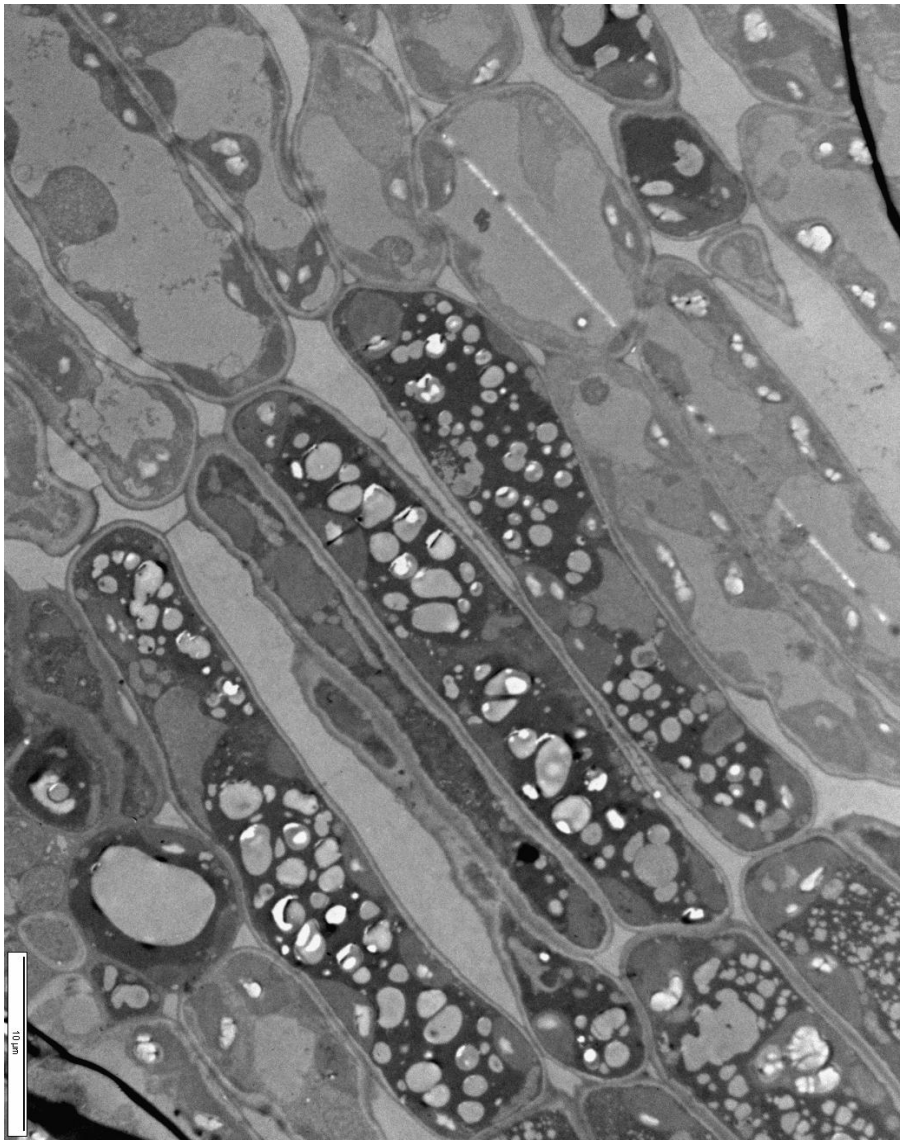
SALIX ALBA, waste dump - Rovinari



SALIX ALBA, waste dump - Rovinari



PARALELLISM – cell biology / painting



Life-net [Plasa vietii] –

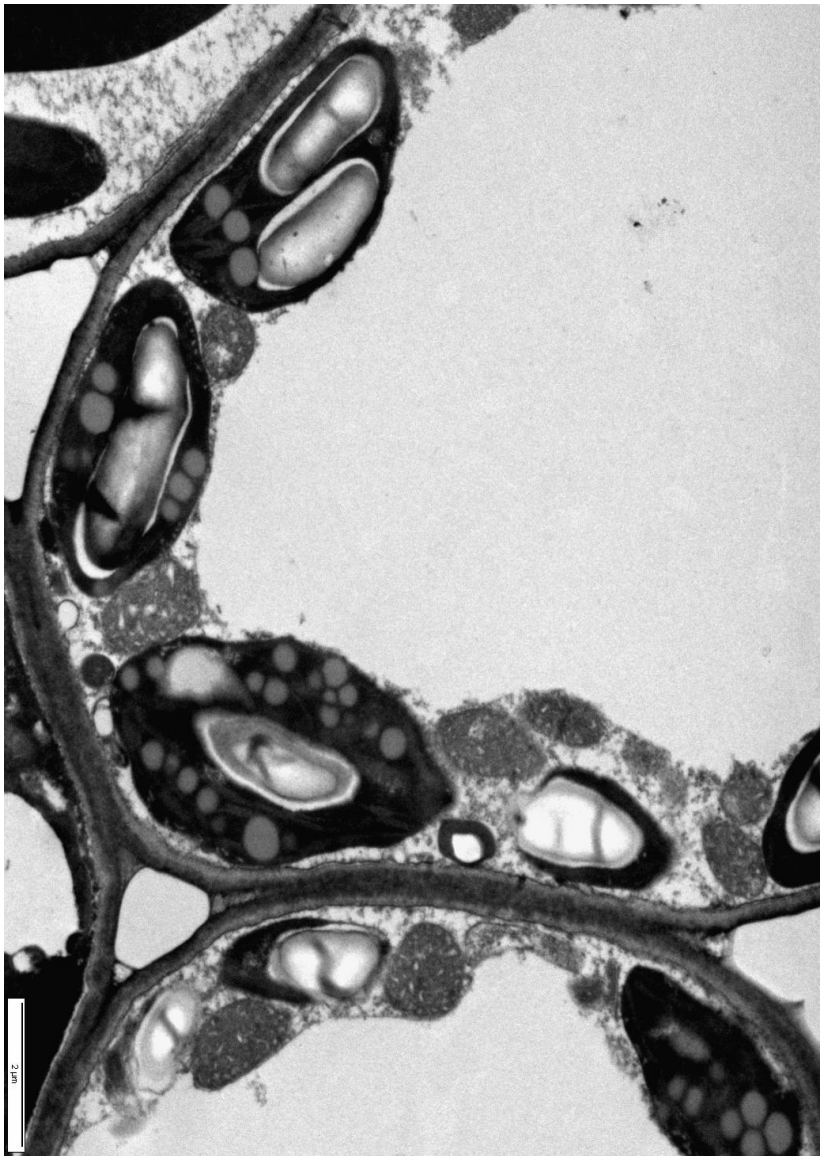
Emilia Burlan - Fira

SALIX VIMINALIS, Control

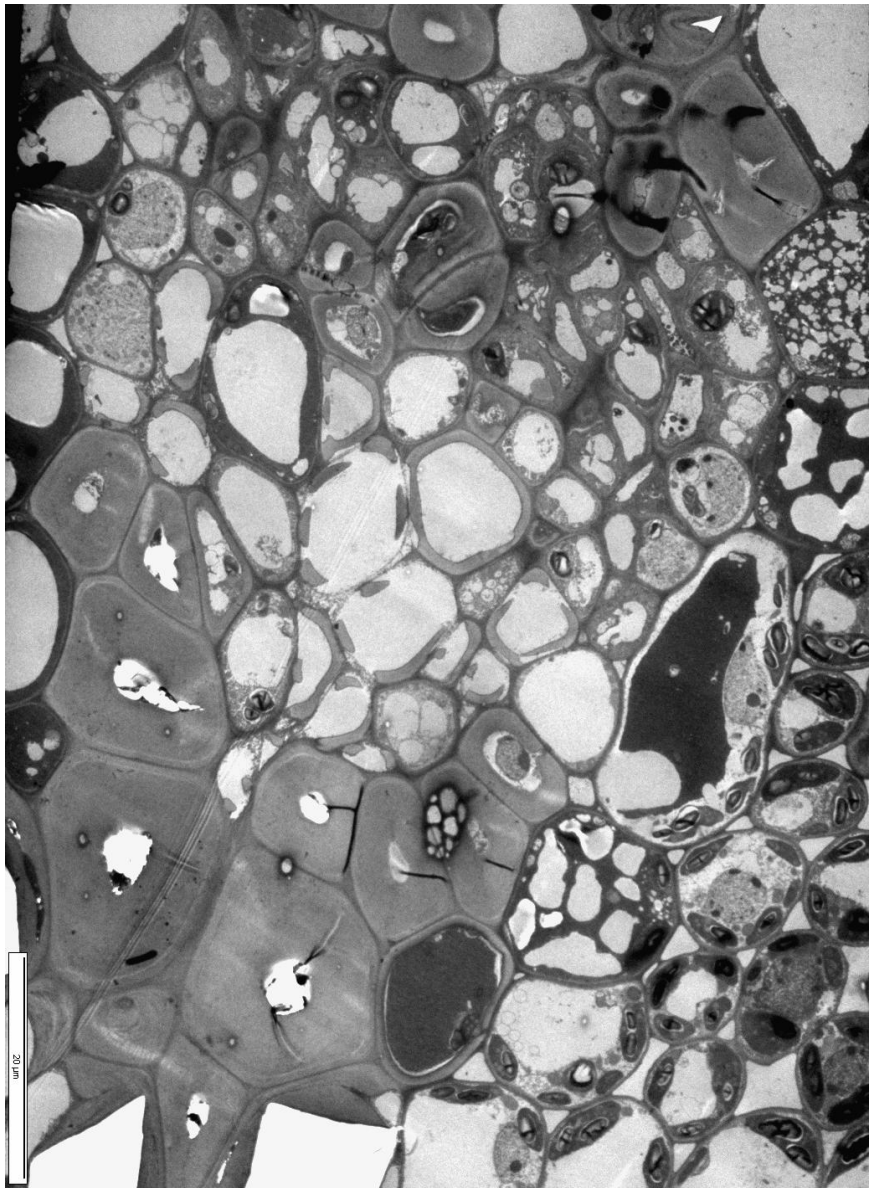
Normal structure for leaf

- **Palisade parenchyma: cells with ferritin in Mt, MVB's and mielin structures in vacuole;**
- **Different synthesis in chloroplast**
- **Nucleus in metabolic activity**

SALIX VIMINALIS, Control



SALIX VIMINALIS, Control



SALIX VIMINALIS, ash dump, Rovinari

Cells from central cylinder in mitotic activity:

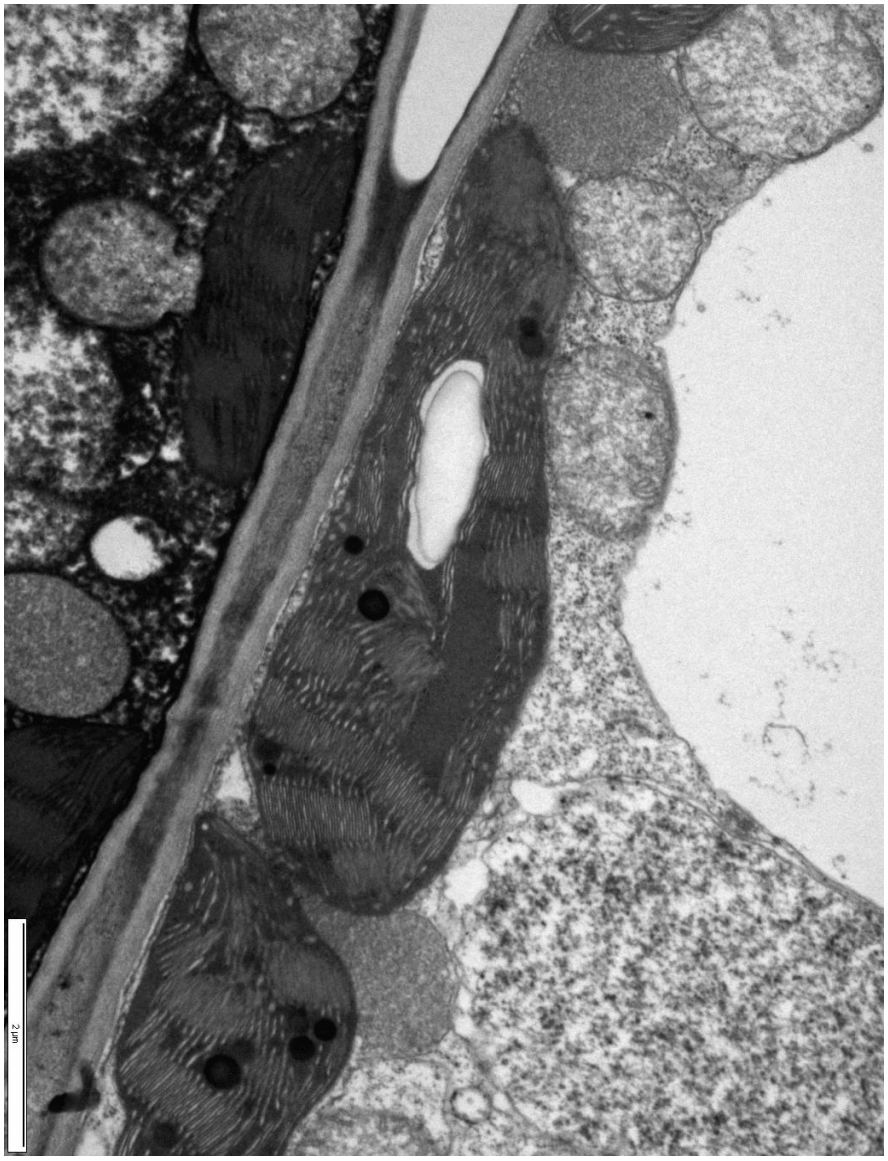
- **Mitotic division (prophase, prometaphase);**
- **Endomitosis in fundamental parenchyma**

Cell in active metabolic:

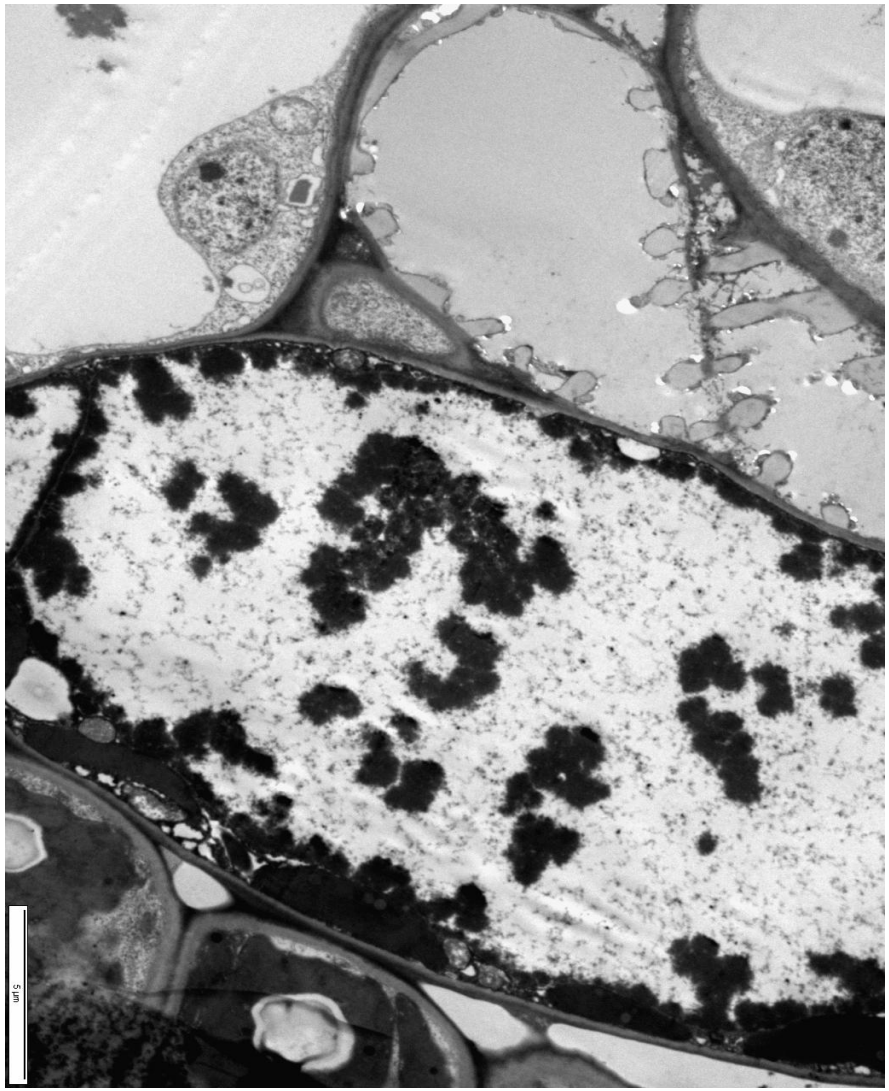
- **Synthesis in chloroplast; mitochondria**

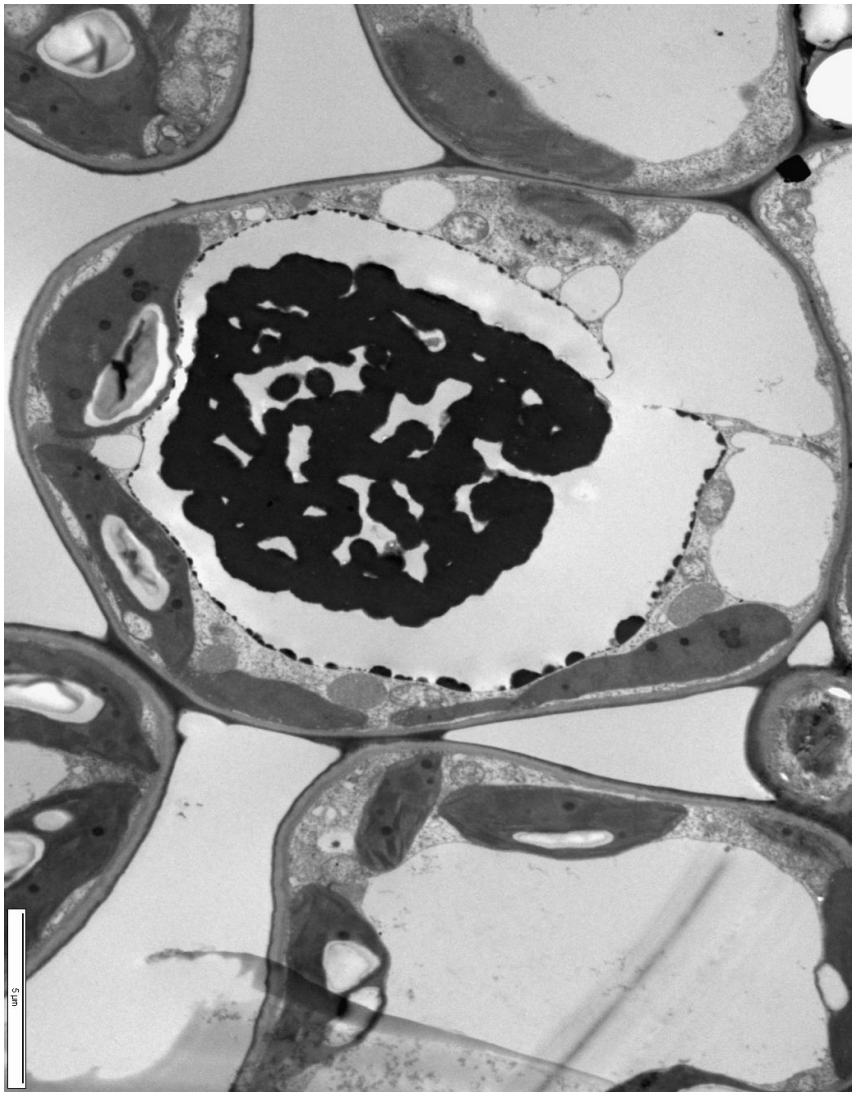
The cellular residues and exogenous matter, accumulate in cells near conducting vessels.

SALIX VIMINALIS, ash dump, Rovinari



SALIX VIMINALIS, ash dump, Rovinari





SALIX VIMINALIS – BETTER RESISTANCE

***S. viminalis*, manifest a better resistance:**

- **Diploid species, with small DNA amount (0.4 pg);**
- **Mitotic division in some parenchyma cells;**
- **Ferritin synthesis in mitochondria/chloroplast;**
- **Protein vegetative synthesis and accumulation in parenchyma cells**
- **Anthocyanin synthesis and spread in all cells;**
- **Salicyllic acid synthesis near chloroplast**
- **NAB's corpuscles near nucleus (a bigger amount of exogenous particles.**

CONCLUSIONS

The ultrastructural features of the mature leaves from *Salix alba* and *Salix viminalis*, underlined their resistance and adaptation at a great amount of heavy metals and radionuclide's activity.

In these species are synthesized substances which offer resistance to stress: ferritin (in chloroplast and mitochondria), anthocyanin, salicyllic acid, a/o.

In leaf cell there are structures which offer resistance: MVB in exocytose, deposit cells and conducting vascular system; like-crystalloid body in chloroplast

The cells are in high metabolic activity (in mitosis).

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