

CYTO-HISTOLOGICAL DATA CONCERNING THE INFLUENCE OF ATMOSPHERE POLLUTANTS ON FIVE SPECIES OF GYMNOSPERMS

LĂCRĂMIOARA IVĂNESCU, CONSTANTIN TOMA

In this paper we have studied five species of gymnosperms, spontaneous and cultivated ones – in areas adjacent to industrial platforms: **Borzești** (Bacău county), **Bicaz** and **Tașca** (Neamț county). Our investigations (have focused during the period 1996–2005) concerning the histo-anatomical changes induced by the (gaseous and solid) atmosphere pollutants on the lamina; the changes have been observed in protecting tissues, assimilatory tissues (the palisade layer and the lacunose tissue), vascular and mechanical tissues. Some leaves have presented disorders in all the component tissues, while in others, the structure changes have only appeared in part of the lamina.

Key words: gymnosperms, atmosphere pollutants, needles, cyto-histological changes, phenolic compounds.

INTRODUCTION

In agreement with the results of the experiments performed by various researchers, using various concentrations of several noxious substances on herbal and ligneous species, whatever the type of noxious substance may be, the cyto-histological changes in the leaf are almost the same, phenotypising as chlorotic and necrotic spots; in the latter case, the structure of all leaves is severely altered, which leads to the death and fall of the photo-assimilating organ and ultimately to the death of the entire plant (Ionescu, 1973; Barbu, 1991; Toma *et al.*, 1993, 1994, 1997; Toniuc *et al.*, 1994; Ivănescu, and Toma, 1997, 1999, 2003; Ivănescu; Yunus, Iqbal, 1996; Ivănescu, 1997).

Knowing the shape and structure of the gymnosperm leaves (Napp-Zinn, 1967; Toma *et al.*, 1979, 2003), their small dimensions and surface, we would expect less cyto-histological changes than in the case of the angiosperms influenced by various pollutants.

However, the results we have obtained determine us to say that the changes appeared in gymnosperms due to the action of pollutants are also extremely important and irreversible, although less phenotypised than those of deciduous plants. Also, the older leaves are more afflicted by pollutants than the young ones, due to the different number of stomata (which constitute one of the ways through which the noxious substances penetrate the plant), their size and, implicitly, their way of functioning.

That also depends on the concentration of noxious substances and on the stational conditions; this is how we can partly explain the early and massive fall of the leaves of various ligneous plants, even though they have shown phenomena of chlorosis or necrosis and irreversible morpho-pathogenic symptoms only partly or not at all.

MATERIAL AND METHODS

Leaves belonging to five species have represented the investigated material: *Pinus nigra* Arn., *Pinus sylvestris* L., *Abies alba* Mill., *Juniperus communis* L. and *Thuja occidentalis* L., found within the surrounding area of Borzești (Bacău county), Bicaz and Tașca (Neamț county); in the first case, the noxious substances are mainly gaseous (sulphur dioxide, chlorine, ammoniac), but also solid (carbon black, soot); in the other two cases, the noxious substances are mainly solid ones (lime or cement powders prone to sedimentation). The vegetal material has been collected throughout the period 1996–2005, each year from the month of May, until the end of September, making at the same time site observation, in order to monitor the vegetation state around the polluting sources.

The fixing, coloring and analyzing methods are customary ones made in the Phyto-anatomical Laboratory of the University of Iași.

The histo-anatomical analysis was made on permanent preparations which comprised cross-sections through the needles. The preparations were examined at Novex (Holland) microscope. Photos were taken with the Minolta photo camera.

RESULTS AND DISCUSSION

The use of photonic microscopy has allowed us to highlight some cyto-histological changes, the most important and most frequently observed ones being underlined as follows.

The **histo-anatomical changes** induced by the (gaseous and solid) atmosphere pollutants on the lamina have been observed in protecting tissues, assimilatory tissues (the palisade layer and the lacunose tissue), vascular and mechanical tissues. Some leaves have presented disorders in all the component tissues, while in others, the structure changes have only appeared in part of the lamina. In the analysed **gymnosperms**, the observed changes were the following (see Figs. 1–4):

- **in the epidermis:**

- ◆ thick, adherent local deposits of soot (in the material coming from Borzești) or lime and cement (in the material coming from Bicaz and Tașca); the closure of the supra-stomal chambers and sometimes of the sub-stomal ones, as well (*Pinus nigra*, *P. sylvestris* – Bicaz);
- ◆ deep tears on the abaxial face, sometimes reaching the endodermis;

- ◆ discontinuities on both epidermises; uneven surfaces (*Thuja occidentalis* – Bicaz, Taşca);

- ◆ structures appear here and there on the lower epidermis which resemble the lenticels formed on the petiole and young branches of the dicotyledons (*Abies alba* – Bicaz);

- ◆ epidermis cells with relatively wide lumina (*Pinus nigra* – Bicaz).

- **in the hypodermis:**

- ◆ few disconnected fibres with very thick walls (*Abies alba* – Bicaz);

- ◆ lumen filled with tannin (*Pinus nigra* – Bicaz);

- ◆ cells with moderately sclerified, slightly lignified walls (*Pinus nigra* – Bicaz).

- **in the assimilatory mesophyll:**

- ◆ the disappearance of the septa from the adaxial face (*Pinus sylvestris* – Borzeşti, *P. nigra* – Bicaz) or the presence of several septa in the cell walls (*P. sylvestris* – Bicaz);

- ◆ the hypertrophy and lignification of the cells in the mesophyll of the abaxial face; the cells of the internal layer are very long and curved, delimiting the torn portions (*Pinus sylvestris* – Borzeşti);

- ◆ the tanninisation and lignification of some cells (*Pinus nigra* – Borzeşti);

- ◆ the deformation of some palisade cells and the presence of tannin inside them (*Juniperus communis* – Bicaz);

- ◆ the change of the arrangement of the assimilatory cells under the hypodermis (*Thuja occidentalis* – Bicaz, Taşca); in this species, changes of the general structure sometimes occur: one side of the abaxial face presents typical two-layer palisade tissue, while the other side presents only an assimilatory parenchyma with isodiametric cells, which gives the leaf an asymmetric structure; sometimes, the cells of the central mesophyll constitute a real aquiferous parenchyma;

- ◆ entirely lacunose mesophyll, with deformed, flattened cells, with a tangentially lengthened shape, with a different orientation, the ones in the central area being extremely large; very large cavities are formed here and there; no trace of the palisade tissue is preserved and many of the cells of the hypodermic assimilatory parenchyma have a wall tissue undergoing a disorganisation – we can only distinguish parallel layers in the process of being torn (*Abies alba* – Bicaz).

- **in the secretory (resiniferous) ducts:**

- ◆ the deformation and narrowing of these ducts, the secreting cells being destroyed or having lignified walls (*Pinus sylvestris* – Borzeşti; *P. nigra* – Borzeşti; *Thuja occidentalis* – Bicaz);

- ◆ the mechanical pod has cells with very thin walls (*Pinus nigra* – Borzești, Bicaz);
- ◆ accumulation of tannin in the collecting channel (*Juniperus communis* – Bicaz);
- ◆ the flattening of the secreting channels, the deformation and flattening of all the mechanical cells (*Abies alba* – Bicaz);
- ◆ an asymmetry in the composition of the mechanical pod, made manifest by the asymmetry of the epithelial layer made – in some areas – of large normal cells and – in other areas – of very flattened and even disorganised cells (*Pinus sylvestris* – Bicaz).
- **in the primary (casparian) endodermis:**
 - ◆ the thickening of the cellular walls (*Pinus nigra* – Borzești);
 - ◆ the flattening of the cells, the Caspary thickenings in the radial walls being hard to observe (*Pinus nigra* – Borzești);
 - ◆ the tanninisation of the cells (*Pinus nigra* – Borzești);
 - ◆ heteromorphic cells (*Abies alba* – Bicaz);
 - ◆ sometimes, the endodermis is not at all visible (*Pinus nigra* – Bicaz).
- **in the tracheidal (transfusion) parenchyma:**
 - ◆ the lignification of the cellular walls (*Pinus sylvestris* – Borzești; *P. nigra* – Borzești, Bicaz).
- **in the vascular and mechanical tissues:**
 - ◆ the xylem of the two vascular bundles is very scarce compared to the phloem (*Pinus nigra* – Borzești) or the other way around (*P. nigra* – Borzești; *Pinus sylvestris* – Bicaz);
 - ◆ the individualisation of some wooden vessels in an inter-bundle position (*Pinus nigra* – Borzești);
 - ◆ on the internal face of the xylem, there are very large parenchymatous cells, separated into numerous compartments by walls with various orientations; in this position, some cells form “secreting channels” which are not surrounded by the typical sclerenchymatous pod (*Pinus nigra* – Bicaz);
 - ◆ the presence of a large number of crystals in the phloemic elements (*Pinus nigra* – Borzești) or the absence of these crystals (*P. nigra* – Bicaz);
 - ◆ many of the wooden vessels have very much thickened walls and tyloid structures are observed in some of them (*Abies alba* – Bicaz);
 - ◆ the elements of the phloem often have very much thickened walls, most of them being obliterated (*Abies alba* – Bicaz);

- ◆ the complete disorganisation of the vascular bundles by a shapeless mass of tanninised and lignified cells (*Pinus nigra* – Borzești) or the closer position of the vascular bundles to each other (*P. nigra* – Bicaz);
- ◆ the lignification of the mechanical elements in the peri-phloemic position (*Pinus sylvestris* – Borzești);
- ◆ the disappearance of the mechanical elements in the peri-phloemic and inter-bundle positions (*Pinus nigra* – Borzești, Bicaz);
- ◆ in the vicinity of xylem in the two vascular bundles, there are 1–2 fibres with very thick sclerenchyma walls (*Abies alba* – Bicaz).

A special situation occurs when individuals whose leaves were healthy – at least apparently – wither suddenly.

The cyto-histo-anatomical investigations performed on the leaves of *Pinus nigra* and *Pinus sylvestris* obtained from apparently healthy individuals which showed, however, signs of strong defoliation during our study, highlighted the presence (in the cells of the assimilatory parenchyma, the endodermis, the tracheidal parenchyma, in the cells of the phloemic parenchyma and, more seldom, in the mechanical cells of the pod of the resiniferous ducts and of the hypodermis) of defence compounds produced due to the aggression of atmosphere pollutants; these compounds are part of the category of phenols and are secondary products secreted only under stress, when the plants try to “adjust” to the changes of the external environment (see Figs. 5, 6).

From the category of phenols, the analysed species possess tannins which are usually present in the stems and branches, in the cells of which the mentioned compounds are compartmentalised in vacuoles, being separated from the hyaloplasm by the tonoplast, because they alter the structure of proteins and the activity of enzymes. As long as the tonoplast is intact, the phenolic compounds are not toxic for the cells which have produced them.

Even though the volume of noxious substances which may pass through the cuticular pores cannot be precisely evaluated, it remains incomparably smaller than the one which may penetrate the ostiola of the stomata, which are rightly considered to be the main passages for the atmosphere pollutants towards the inside of the leaves (the appearance of fissures at the level of the epidermis, produced by various traumatising factors, increases the number of passages for the pollutants inside the leaves).

The fact that the cells of the subjacent mesophyll change their shape, size and even their cellular content shows that they are the first to respond to the aggression of atmosphere pollutants, after which the reaction is centripetal.

Most cells in the assimilatory parenchyma contain grouped phenolic compounds (smaller or larger drops which may merge into islands) in their vacuoles. Most of the groups of phenolic compounds are placed next to the tonoplast, on whose integrity and functionality the life of the cell ultimately depends. The parietal hyaloplasm is often insignificant from the quantitative point

of view, the tonoplast being next to the plasmalemma and therefore next to the cellular wall.

The necroses inside the mesophyll, caused by the destruction of the cellular walls, provoke the release of these phenolic compounds into large spaces, where they tend to be concentrated due to the reaction of oxidation of the aromatic nuclei in their structure (the oxygen comes mainly from the air in the intercellular spaces), which later produces the brown or reddish-brown colour of the necrotic spots that are macroscopically visible.

Later, the necrotic areas may merge, occupying considerably large spaces in the dying mesophyll, even if the epidermis remains intact.

The resiniferous ducts generally maintain their structural integrity; the cells of the mechanical pod having a wide lumen, full of phenolic compounds or incompletely edified ducts or ducts opening directly on the exterior of the leaf are rarely observed.

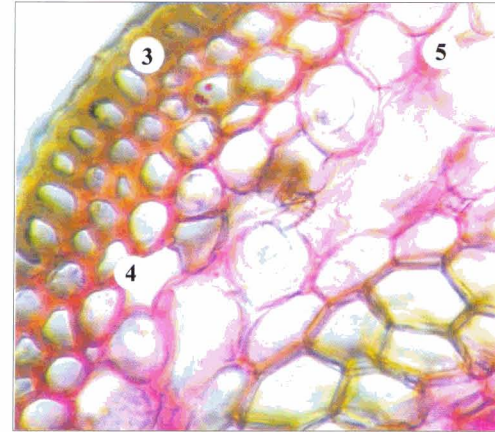
The endodermis constitutes a good temporary barrier, but once some of its cells have come into contact with the afflicted mesophyll, they begin to accumulate phenols, the same as the cells of the neighbouring tracheidal parenchyma. Sometimes, aeriferous spaces of various sizes, which are usually the result of the lysis of cells are found between the cells of the endodermis and those of the subjacent tracheidal parenchyma (these spaces probably constitute future accumulation places for the phenolic compounds after the latter have penetrated the tonoplast, precipitating in the hyaloplasma of the cell which has produced them provoking the death of the cell).

Zobel, 1996 (cf. Yunus and Iqbal, 1996) shows that the cells found next to the ones that die begin to produce phenols in less than a few minutes.

The vacuoles of various sizes containing phenolic compounds may be compared to "bombs" which may be detonated at any time by the alteration of the enzymatic mechanisms involved in the cellular metabolism; the gaseous or solid atmosphere pollutants are permanent stress factors that may determine the production of phenols in cells which do not naturally contain such compounds.

The excessive production of phenols by cells which are naturally specialised for performing other functions is a strong support for the suggestion according to which phenols serve as defence compounds and their very synthesis constitutes a bio-indicator of drastic environmental change.

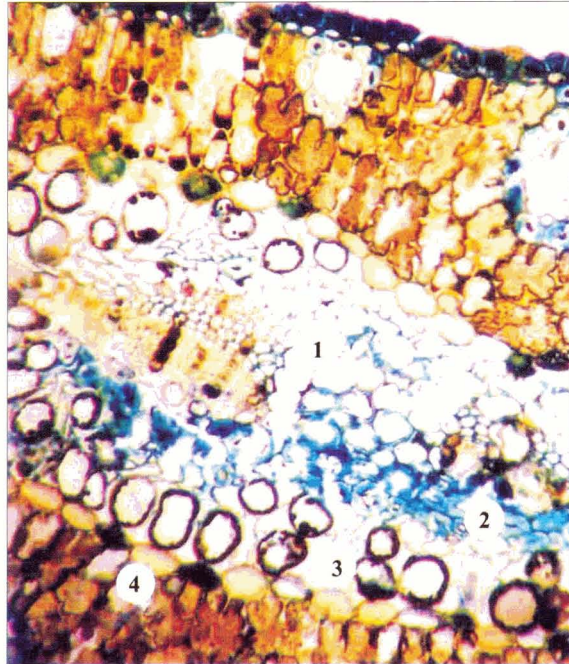
Unlike the gaseous pollutants, the cement dust does not have a rapid toxic action and does not produce an important damage to the vegetal tissue; the strong damage is produced by the lime dust, which is very fine and has a high basic reaction, thus having a more negative action than that of cement from this point of view. The basic reaction of lime ($\text{pH} = 8-12$) determines plasmolytic changes due to the tendency the vegetal cell has of balancing the concentration of the cellular liquid with the environment, as Smejkal has remarked in 1982.



Figs. 1, 2. Cross section of *Pinus nigra* needle, Bicaz ($\times 170$; $\times 300$); 1 – “resin duct”; 2 – phloemic parenchyma cell containing the crystals of calcium oxalate; 3 – epidermic cells with wide lumina; 4 – hypodermic cells with slightly lignified walls; 5 – the disappearance of the septa from the assimilatory mesophyll.



Figs. 3, 4. Cross section of *Pinus nigra* needle, Borzesti ($\times 300$): 1 – oxaliferous cells in the tracheidal parenchyma; 2 – mechanical cells with very thick walls; 3 – phloemic parenchyma cells containing the crystals of calcium oxalate; 4 – mechanical cell.



Figs. 5, 6. Semi thin cross section of *Pinus nigra*, Borzesti ($\times 170$; $\times 300$): 1 – spongy space resulted from disorganizing of the tracheidal parenchyma cells; 2 – atypical bundle; 3 – tracheidal parenchyma cells containing phenolic compounds; 4 – assimilatory parenchyma containing phenolic compounds; 5 – spongy space; 6 – tracheidal parenchyma containing phenolic compounds.

Certainly, when dealing with a chronic aggression due to noxious substances, whatever their nature is, the responses may be extremely various, but at the same time similar.

When dealing with the response reactions of plants to the action of noxious substances, we cannot consider any noticed change to be absolute; there is no uniformity among the individuals belonging to the same species, let alone among species belonging to different genera or families. The responses are individual ones; therefore, the studies concerning the impact of pollutants on vegetation should comprise a large variety of investigations for each individual in a polluted environment.

CONCLUSIONS

In the analysed gymnosperms, the histo-anatomical changes induced by the (gaseous and solid) atmosphere pollutants on the lamina have been observed in protecting tissues, assimilatory tissues (the palisade layer and the lacunose tissue), vascular and mechanical tissues. Some leaves have presented disorders in all the component tissues, while in others, the structure changes have only appeared in part of the lamina.

The cyto-histo-anatomical investigations performed on the leaves of *Pinus nigra* and *Pinus sylvestris* obtained from apparently healthy individuals which showed, however, signs of strong defoliation during our study, highlighted the presence (in the cells of the assimilatory parenchyma, the endodermis, the tracheidal parenchyma, in the cells of the phloemic parenchyma and, more seldom, in the mechanical cells of the pod of the resiniferous ducts and of the hypodermis) of defence compounds produced due to the aggression of atmosphere pollutants: these compounds are part of the category of phenols and are secondary products secreted only under stress, when the plants try to “adjust” to the changes of the external environment.

REFERENCES

1. Barbu I., 1991, *Moartea bradului – simptom al degradării mediului*. Ed. Ceres, București.
2. Ionescu Al., 1973 (edited by), *Efectele biologice ale poluării mediului*. Ed. Acad. R.S.R., București.
3. Ivănescu L., 1997, *Unele modificări morfo-anatomice induse de poluanții atmosferici asupra frunzei de la speciile Pinus sylvestris L. și Pinus nigra Arn. (Nota I)*, Bul. Grăd. Bot. Iași, t. 6, fasc. 1: 147–154.
4. Ivănescu L., Toma C., 1997, *Modifications morphologiques et structurelles induites par les polluants atmosphériques sur les aiguilles de Pinus nigra Arn. et Pinus sylvestris L.* An. șt. Univ. Iași, s. II a. (Biol. Veget.), **XLIII**: 31–38.
5. Ivănescu L., Toma C., 1999, *Responses of the plants to the action of atmosphere pollutants (I)*. An. șt. Univ. Iași, s. II a. (Biol. Veget.), **XLV**: 47–54.

6. Ivănescu L., C. Toma, 2003, *Influența poluării atmosferice asupra structurii plantelor*. Ed. Fundației Andrei Șaguna, Constanța.
7. Napp-Zinn K.L., 1967, *Anatomie des Blattes*. 1. *Gymnospermen*. In: *Handbuch der Pflanzenanatomie*, **8**, 1, Gebrüder Borntraeger, Berlin.
8. Toma C., Toma L., Diaconescu C., 1979, *Structura frunzei la unele gimnosperme din flora României*. Culegere de stud. și artic. Biol., Univ. "Al. I. Cuza" Iași (Grăd. Bot.), **1**: 315–331.
9. Toma C., Toma L., Ivănescu L., 2003, *Comparative anatomy of some Pinus L. species*, An. șt. Univ. "Al. I. Cuza" Iași, s. II a, Biol. veget., **XLIX**: 1–10.
10. Toniuc A., Toma C., Aiftimie A., Niță M., Ivănescu L., 1994, *Modifications induced by the atmosphere pollutants on the leaf structure on some wood plants*. In vol.: Conferința științifică a Botaniștilor, Chișinău: 7–8.
11. Yunus M., Iqbal M., 1996, *Plant response to air pollution*, John Wiley & Sons, Chichester–New York–Brisbane–Toronto–Singapore.

"Al. I. Cuza" University of Iași,
Faculty of Biology,
mail: ivanescu67@yahoo.com