Cito-histological changes due to the action of atmosphere pollutants on three species of gymnosperms

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

***Keywords*—** gymnosperms, atmosphere pollutants, needles, cyto-histological changes, phenolic compounds

I. Introduction

In agreement with the results of the experiences 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 later 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 [3]-[9]-[1]-[4]-[5]-[6]-[15]-[7]-[8].

Knowing the shape and structure of the gymnosperm leaves [11]-[13], 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 visible 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 way through which the noxious substances penetrate the plant), their size and, implicitly, their way of functioning.

That also depends on the concentration of the noxious substances and on the local 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..

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II. Material and Methods

Leaves belonging to three species have represented the investigated material: *Pinus nigra* Arn., *Pinus sylvestris* L.and *Abies alba* Mill., found within the surrouding area of Borzeşti (Bacău county), Bicaz and Taşca (Neamţ county), Romania; in the first case, the noxious substances are mainly gaseous (sulfur 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, up until the end of the month of September, making at the same time site observation, in order to monitor the vegetation state around the polluting sources. The vegetal material were fixed and conserved in ethanol 70%. The sections were made with free hand using a razor blade and colored with red-ruthenium and methyl-blue. The photos were made after the obtained permanent slides using an Olympus microscope with a C 330 photo camera.

III. 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, assimilator 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:

- in the epidermis:

• thick, adherent local deposits of soot (in the vegetal material coming from Borzeşti) or lime and cement (in the vegetal material coming from Bicaz and Taşca); 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;
• 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 assimilator 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, delimitating the torn portions (*Pinus sylvestris* – Borzeşti);
• the cell walls lignification and the presence of tannins of some cells (*Pinus nigra* – Borzeşti);
• 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 assimilator parenchyma have a wall tissue undergoing a disorganization – we can only distinguish parallel layers in the process of being torn (*Abies alba* – Bicaz).
- in the secreting (resin) ducts:
• the deformation and narrowing of these ducts, the secreting cells being destroyed or having lignified walls (*Pinus sylvestris* – Borzeşti; *P. nigra* – Borzeşti);
• the mechanical pod has cells with very thin walls (*Pinus nigra* – Borzeşti, 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* – Borzeşti);
• the flattening of the cells, the Caspary thickenings in the radiar walls being hard to observe (*Pinus nigra* – Borzeşti);
• the presence of tannin in some cells (*Pinus nigra* – Borzeşti);
• heteromorphic cells (*Abies alba* – Bicaz);
• some times, the endodermis is not at all visible (*Pinus nigra* – Bicaz).
- in the tracheidal (transfusion) parenchyma:
• the lignification of the cellular walls (*Pinus sylvestris* – Borzeşti; *P. nigra* – Borzeşti, Bicaz).
- in the vascular and mechanical tissues:
• the xylem of the two vascular bundles is very scarce compared to the phloem (*Pinus nigra* – Borzeşti) or the other way around (*P. nigra* – Borzeşti; *Pinus sylvestris* – Bicaz);
• the individualization of some wooden vessels in an inter-bundle position (*Pinus nigra* – Borzeşti);
• 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 sclerenchymatic pod (*Pinus nigra* – Bicaz);
• the presence of a large number of crystals in the phloemic elements (*Pinus nigra* – Borzeşti) or the absence of these crystals (*P. nigra* – Bicaz);
• many of the wooden vessels have very much thickened walls and tiloid 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 disorganization of the vascular bundles by a shapeless mass of cells with tannin and lignified walls (*Pinus nigra* – Borzeşti) or the closer position of the vascular bundles to each other (*P. nigra* – Bicaz);
• the lignifications of the mechanical elements in the peri-phloemic position (*Pinus sylvestris* – Borzeşti);
• the disappearance of the mechanical elements in the peri-phloemic and inter-bundle positions (*Pinus nigra* – Borzeşti, 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 – at least apparently – healthy 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 assimilator 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 defense 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.

From the category of phenols, the analyzed species possess tannins which are usually present in the stems and branches, in the cells of which the mentioned compounds are compartmentalized in vacuoles, being separated from the hyaloplasma by the tonoplast, because they alter the structure of proteins and the activity of the 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 stomatal opening, 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 traumatizing factors, increases the number of passages for the pollutants inside the leaves).

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

Most cells in the assimilator 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 hyaloplasma is often insignificant from the quantitative point of view, the tonoplast being next to the plasmalema 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 color 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 resin ducts generally maintain their structural integrity; the cells of the mechanical pod having a wide lumen, full of phenolic compounds.

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 neighboring 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 and provoking the death of the cell).

References [15]-[2], 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 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 specialized for performing other functions is a strong support for the suggestion according to which phenols serve as defense 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 (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 G. Smejkal has remarked (1982).

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.

If initially the changes in the physiological and bio-chemical activity are not deep and disappear with the elimination of their causes, later, because of the persistence of the noxious substances, they become irreversible, preceding the future histo-anatomical changes.

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.

IV. CONCLUSIONS

The histo-anatomical changes induced by the (gaseous and solid) atmosphere pollutants on the needles have been observed in protecting tissue (epidermis), assimilator tissues (the mesophyll), vascular and mechanical tissues. Some needles have presented disorders in all the component tissues (including the secretory ducts), while in others, the structure changes have only appeared in part of the needle (Photo 1 – 4).

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 assimilator 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 (Photo 5 and 6).The excessive production of phenols by cells which are naturally specialized for performing other functions is a strong support for the suggestion according to which phenols serve as defense compounds and their very synthesis constitutes a bio-indicator of drastic environmental change.

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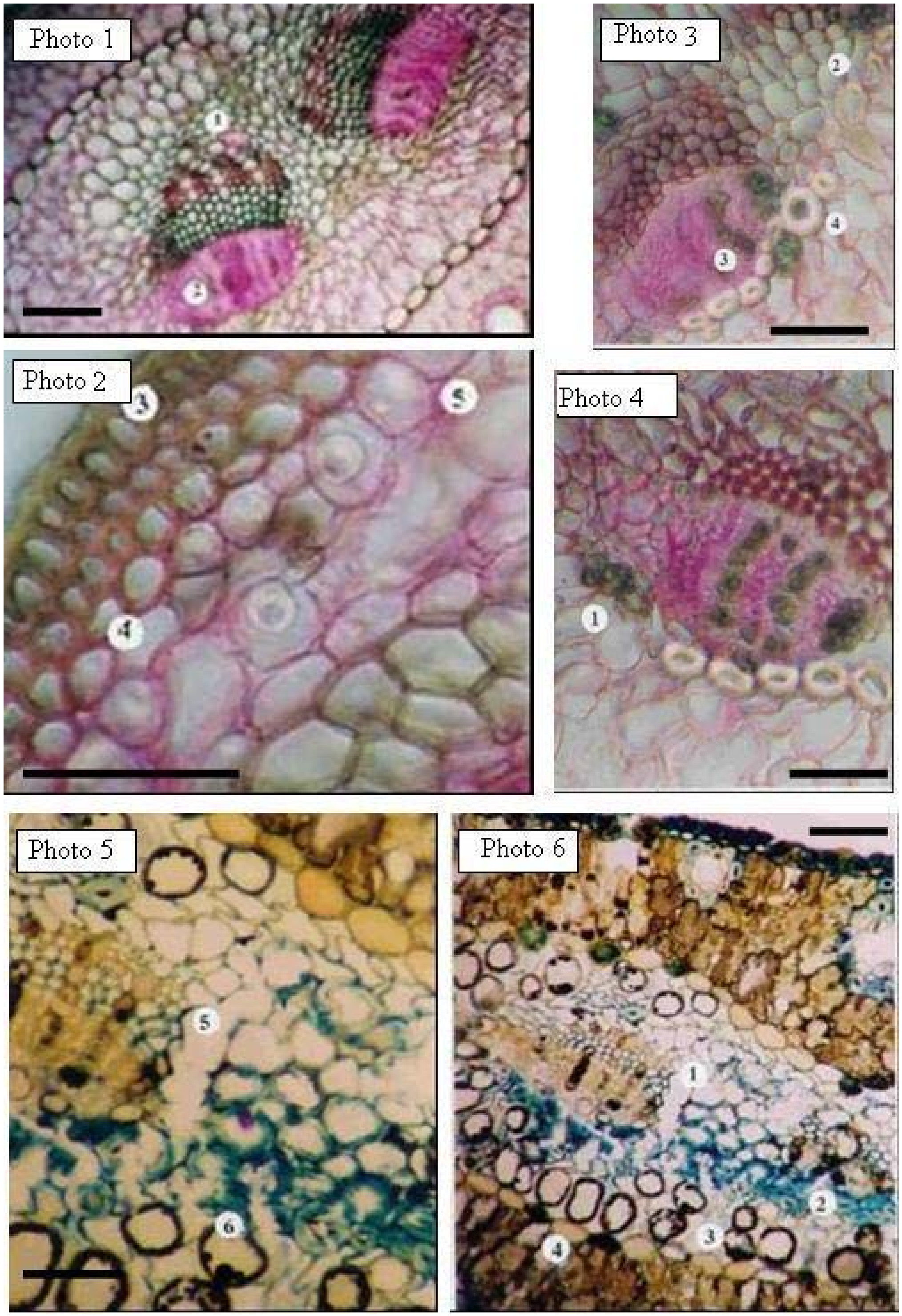
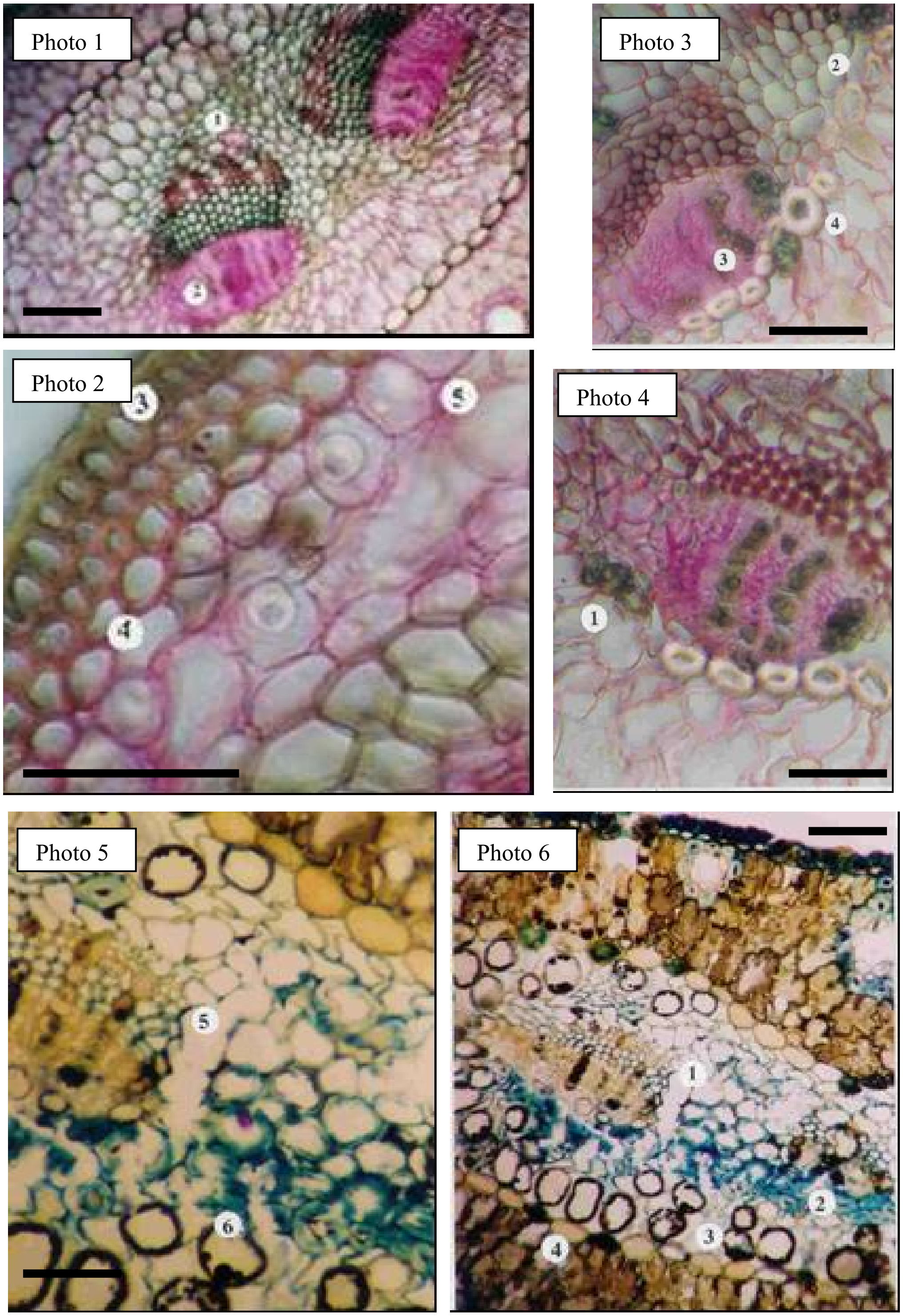


Photo 1, 2 – Cross section of *Pinus nigra* needle, Bicaz (x 170; x 300): 1 – “resin duct”; 2 – phloemic parenchyma cell containing the crystals of calcium oxalate; 3 – epidermic cells with wide lumen; 4 – hypodermic cells with slightly lignified walls; 5 – the disappearance of the sepa from the assimilator mesophyll; Photo 3, 4 – Cross section of *Pinus nigra* needle, Borzesti (x 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 ; Photo 5, 6 – Semi thin cross section of *Pinus nigra*, Borzesti (x 170; x 300): 1 – spongy space resulted from disorganizing of the tracheidal parenchyma cells; 2 – atypical bundle; 3 – tracheidal parenchyma cells containing phenolic compounds; 4 – assimilator parenchyma containing phenolic compounds; 5 – spongy space; 6 – tracheidal parenchyma containing phenolic compounds.



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