



ACCUMULATION OF PHENOLIC COMPOUNDS IN THE NEEDLES OF *PICEA ABIES* KARST. AND *PINUS SYLVESTRIS* L.: BIOLOGICAL MARKERS OF AIR POLLUTION DAMAGE

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SYNOPSIS

In this paper we have studied two species of gymnosperms (*Picea abies* Karst. and *Pinus sylvestris* L.) – in areas adjacent to industrial platforms: Borzești (Bacău county), Bicaz and Tașca (Neamț county), Romania. Our investigations (have focused during the period 2000 – 2007) 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.

The cyto-histo-anatomical investigations performed on the leaves of *Picea abies* and *P. sylvestris* obtained from apparently healthy individuals who showed, however, signs of strong defoliation during our study, highlighted the presence of defense compounds produced due to the aggression of atmosphere pollutants. 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 changes.

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, phenotyping 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 (Ionescu Al., 1973; Barbu I., 1991; Yunus M., Iqbal M., 1996; Ivănescu L., C. Toma, 1997, 1999, 2003; Soukupova J., Cvikrova M., Albrechtova J., Rock B.N., Eder J., 2000; Ivănescu L., M.M. Zamfirache, 2005, 2006; I. Gostin, 2007).

Knowing the shape and structure of the gymnosperm leaves (Napp-Zinn Kl., 1967; Toma C. et al., 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 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 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 the two species have represented the investigated material: *Picea abies* Karst. and *Pinus sylvestris* L., found within the surrounding area of Borzești (Bacău county), Bicaz and Tașca (Neamț county), Romania; 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 2000 – 2007, 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 fixing, coloring and analyzing methods are customary ones made in the Phyto-anatomical Laboratory of the University of Iași by using the method described by Wulff A., Ahonen J. and Kärenlampi L., 1996.

The photos were made after the obtained permanent slides using a Novex (Holland) microscope and a Canon digital photo camera.

RESULTS AND DISCUSSIONS

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 cyto-histo-anatomical investigations performed on the leaves of *Picea abies* 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.

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 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 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 assimilator parenchyma contain grouped phenolic compounds (smaller or larger drops which may merge into islands) in their vacuoles (Photo 1). 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 plasmaleme 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.

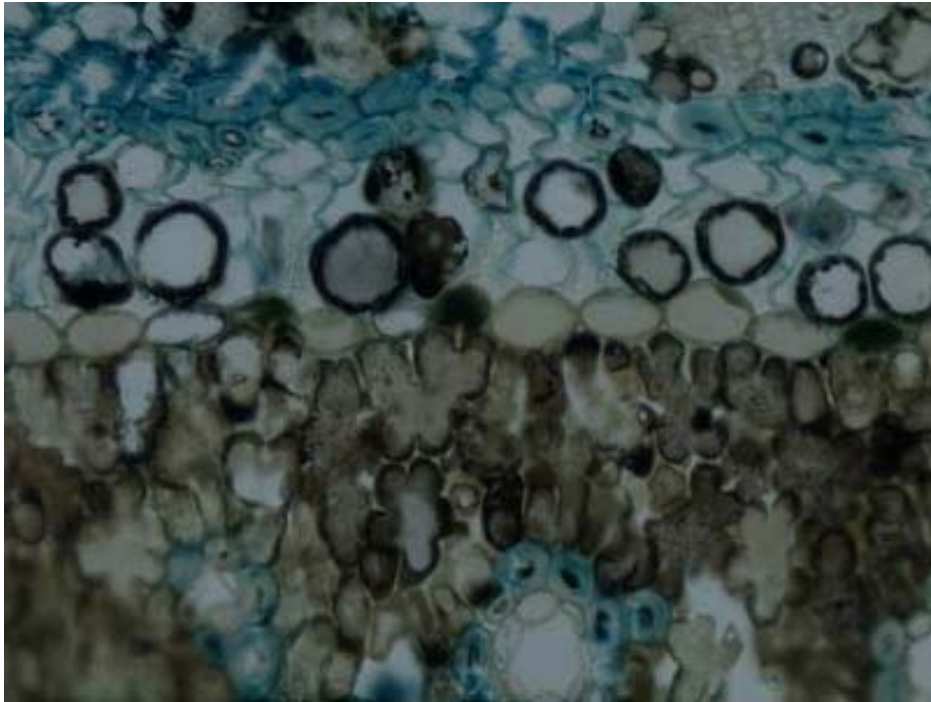


Photo 1. Semi thin cross section of *Pinus sylvestris* needle, Borzesti; we can see the assimilator parenchyma cells containing phenolic compounds and tracheidal parenchyma cells with droplets of phenolic compounds

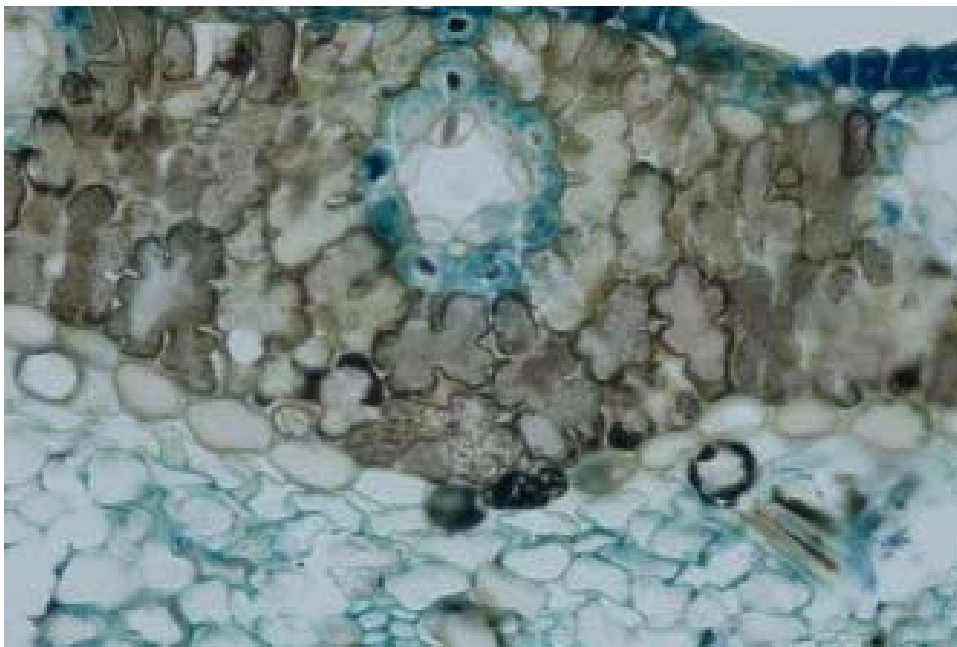


Photo 2. Semi thin cross section of *Pinus sylvestris* needle, Borzesti; we can see the presence of phenolic compounds in endodermis and little droplets in some tracheidal parenchyma cells.

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 (Photo 2). 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).

A.M. Zobel, 1996 (cf. M. Yunus, M. 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 (Photos 3-6).

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 (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.

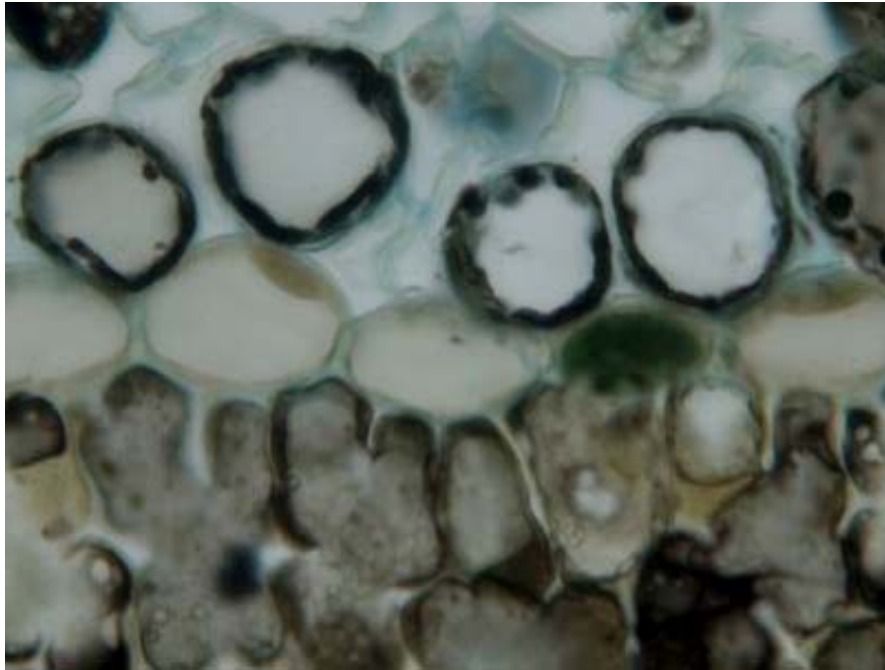


Photo 3. Semi thin cross section of *Pinus sylvestris* needle, Borzesti; we can see presence of phenolic compounds in the assimilator parenchyma cells and small droplets of these compounds in tracheidal parenchyma cells.

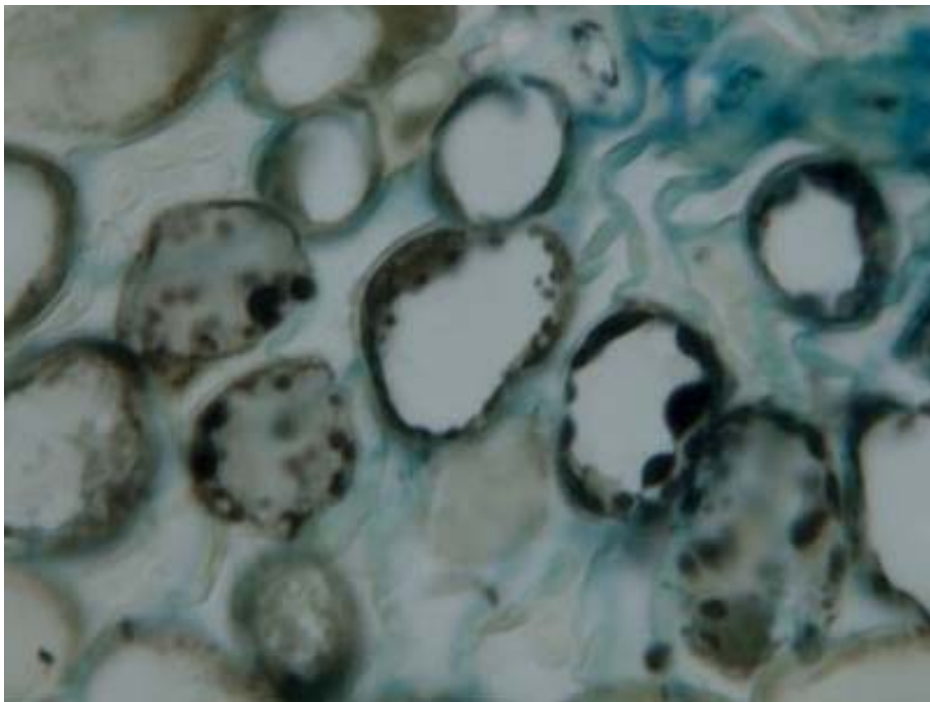


Photo 4. Semi thin cross section of *Pinus sylvestris* needle, Borzesti; we can see the droplets of phenolic compounds in in tracheidal parenchyma cells.

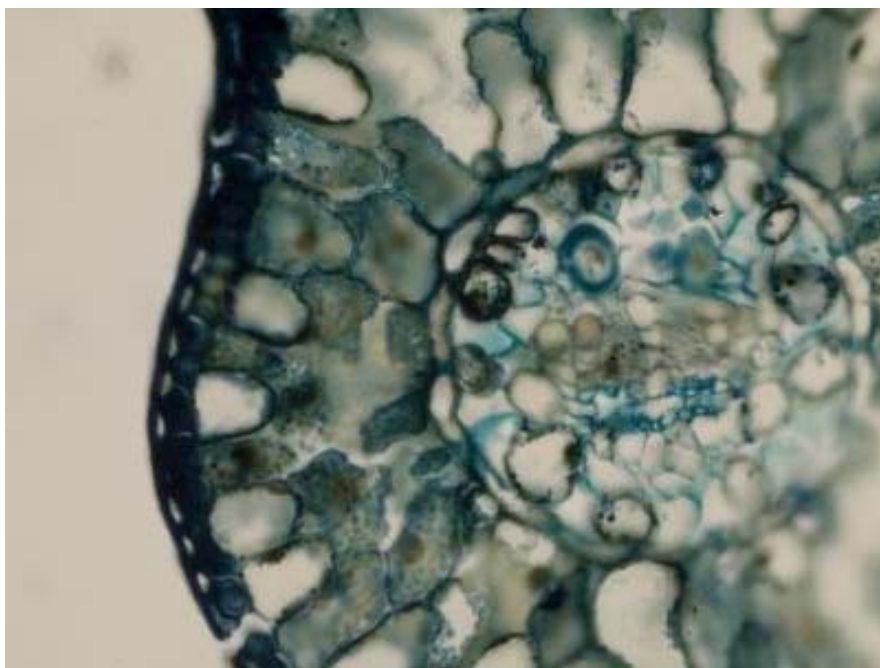


Photo 5. Semi thin cross section of *Picea abies* needle, Bicaz; we can see that only the central cylinder contain in some tracheidal parenchyma cells droplets of phenolic compounds.

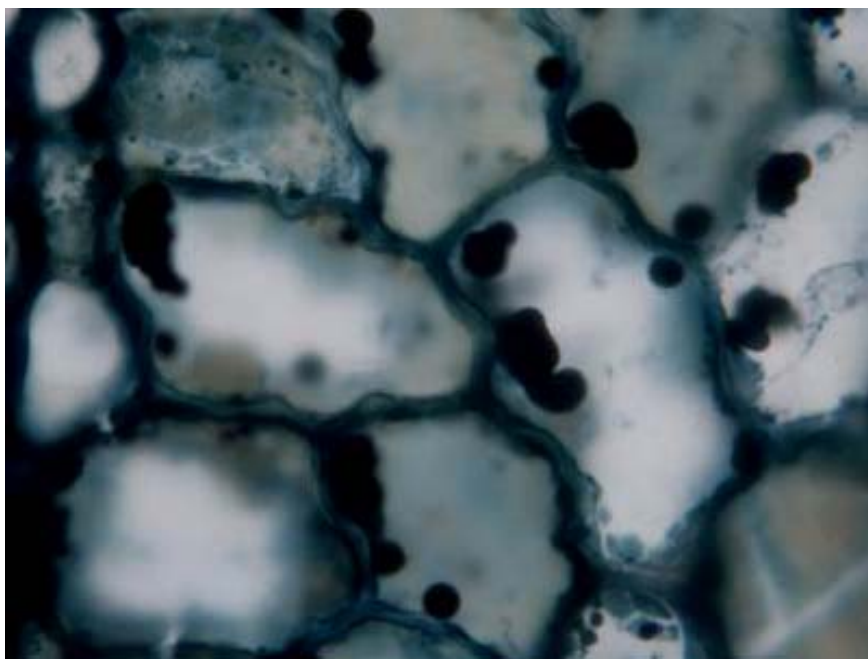


Photo 6. Semi thin cross section of *Picea abies* needle, Bicaz; we can see assimilatory cells with phenolic compounds.

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.

The cyto-histo-anatomical investigations performed on the leaves of *Picea abies* 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.

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