MORPHOLOGICAL DATA CONCERNING THE INFLUENCE OF ATMOSPHERE POLLUTANTS ON SOME SPECIES OF CULTIVATED AND SPONTANEOUS LIGNEOUS PLANTS

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In this paper we have studied 19 species of ligneous plants – gymnosperms and angiosperms, spontaneous and cultivated ones – in areas adjacent to industrial platforms: **Borzeşti** (Bacău county), **Bicaz** and **Taşca** (Neamţ county). Our investigations have focused (during the period 1996–2005) on observations concerning species of ligneous plants in various phenophases and the state of the leaf surfaces under the influence of the atmosphere pollutants.

Key words: ligneous plants, atmosphere pollutants, defoliation, foliar surfaces (S.E.M.).

INTRODUCTION

It is not easy to find generalisations of a phenomenon connected with air pollution; the air pollutants, by their diversity, the particular atmosphere chemistry, the possible combinations with various compounds which result in new pollutants – often unknown ones – the multiple changes they undergo since the moment of their emission in the environment, the final state in which they interact with the vegetation, the way in which the species "respond" by metabolic and structural changes – whether morphologically phenotypised or not – are only a few of the aspects that we have tried to approach in this study (Ionescu, 1973; Ianculescu, 1973, 1977, 1978; Il'Kun, 1978; Smejkal, 1982; Toma *et al.*, 1993,1994; Toniuc *et al.*, 1993; Mănescu *et al.*, 1994; Yunus, Iqbal, 1996; Kerstiens, 1996; Zaharia, 1999; Ivănescu, Toma, 2003; Ivănescu and Zamfirache, 2005).

The present study continues the series of investigations concerning the ill-fated effects of air pollutants from the industrialized areas of Moldavia upon the protective vegetation, hoping to make the proper authorities well aware of the importance of keeping, in the investigated areas, the normal natural life conditions.

MATERIAL AND METHODS

We have studied 19 species of ligneous plants – gymnosperms and angiosperms, spontaneous and cultivated ones – in areas adjacent to industrial platforms: **Borzeşti** (Bacău county), **Bicaz** and **Taşca** (Neamţ 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).

Even if the pollutants involved are different from a chemical point of view, the fact that the three industrial centres are found in depression areas, the presence of the valley corridors and the air circulation along them, the thermic inversions, the precipitations, the considerable percentage of calmness contribute to the stability of the nucleus with the highest concentration over the vegetation along the valley corridors. That is the reason why the strongest impact between the noxious substances and the vegetation occurs in the areas adjacent to these industrial platforms.

Our investigations have focused (during the period 1996–2005) on observations concerning species of ligneous plants in various phenophases and the state of the leaf surfaces under the influence of the above-mentioned atmosphere pollutants.

The observations regarding the ligneous species have allowed us to notice the fact that, in most cases, they react to the pollutants no matter what their chemical nature is. Certainly, the "responses" are conditioned by a multitude of factors (genetic factors, pedo-climatic ones, natural habitat, degree of acclimatization, distance and position with regard to the source of pollution, age, physiological state, etc.) and therefore will not be seen as absolute, but will only be reunited as data which will complete the clinical picture of the leaf symptomatology.

To examine the micromorphology of the leaves we have used the scanning electron microscopy methods: the leaf samples were dehydrated using physical methods, metallised and analysed at the Tesla BS-340 scanning electron microscope. Microphotographs were taken from S.E.M.

RESULTS AND DISCUSSION

We shall now present a series of **common situations** of the investigated species, occurring as a general response to the action of (gaseous and solid) noxious industrial substances:

- (more or less adherent) **deposits of foreign substances** – lime and cement dust, carbon black, soot – on the surface of the leaves of most species found in the perimeter of industrial sources.

These deposits change the reactivity of the leaf surfaces, make important photosynthetically active areas inactive, and prevent breathing and perspiration by closing the ostiola of the stomata. The impossibility of performing the photosynthesis leads to serious metabolic disorders – the absence of nutritious and reserve organic substances from the bodies of plants, leading to a general "starvation" of the individuals, which stop growing, flowering and fructifying.

All these effects, cumulated over time, only weaken the general state of the trees, thus making them more sensitive, more vulnerable to other types of aggression (late spring frost, defoliating insects, or pathogenic fungi).

However, the most obvious solid deposits remain those in the Bicaz and Taşca industrial areas; we mention again the fact that, in the case of some ***Abies alba*** individuals in the Bicaz area, these deposits cover approximately 80–95% of the leaf surface and also lead to the lowering of the branches and/or the shadowing of the inferior ones; the adherent crusts on the ***Quercus robur*** leaves in the Bicaz area make them breakable; because of the excessive deposits, the leaves of some ***Populus nigra*** individuals close to the cement factory at Taşca seem to be "lying" along the branches, so that the non covered portions cannot get light and are therefore photosynthetically inactive; the individuals of ***Rosa canina*** observed in the Bicaz and Taşca areas are sometimes unrecognizable because of the quantity of lime and cement dust on their surface.

- **episodes of partial defoliation** (especially from June to August) noticed in the case of ***Pinus sylvestris***, ***Acer negundo***, ***A. pseudoplatanus***, ***Populus tremula***, ***Salix fragilis*** in the **Borzeşti** industrial area; ***Pinus sylvestris***, ***P. nigra***, ***Abies alba***, ***Picea abies***, ***Juniperus communis***, ***Quercus petraea***, ***Q. robur*** in the **Bicaz** and **Taşca** industrial areas;
- **total defoliation** in the case of ***Pinus sylvestris*** – Borzeşti and Bicaz; ***P. nigra*** – Borzeşti and Bicaz; ***Picea abies*** – Bicaz; ***Populus tremula*** – Borzeşti and Taşca; ***Aesculus hippocastanum*** – Bicaz; ***Salix fragilis*** – Borzeşti.

The total or partial defoliation phenomena observed in many species in the middle of the vegetation season are the main causes of the slowing down of the growth of the individuals and often of the impossibility of occurrence of viable fructifications.

We underline two important aspects connected to the general state of the leaves falling off branches: there are leaves which phenotypically present signs of "suffering" – necroses and/or chloroses of variable sizes – and there are leaves which do not present any morphological symptom which would constitute a warning sign for possible defoliations.

In other words, there are individuals which stop growing and fructifying due to the visible deterioration of the photo-assimilating apparatus and there are individuals which die without ever having shown signs of illness. Such individuals are encountered in areas where the noxious substances are gaseous, but also in those where they are predominantly solid; it is true that the defoliation phenomena are more widespread in the areas where the pollutants are mainly gaseous, where they afflict a larger number of species and individuals, while in areas with solid pollutants, the phenomenon afflicts a smaller number of individuals, but an equal number of species.

Sometimes, the defoliation phenomenon presents particular signs: some acicular leaves in ***Pinus sylvestris*** individuals in the Borzeşti area, covered in a black crust (carbon black) which is non adherent after rain fall off with the

microblast due to a simple mechanical touch; the heavy defoliations observed in some ***Juniperus communis*** individuals found close to the Bicaz-Chei quarry have also been accompanied by the complete detachment of the branches with leaves from the trunk due to the excessive deposits of solid particles. Sometimes, during not very heavy rain, the crusts are detached together with the leaves.

The electron microscopy researches (S.E.M) performed on the surfaces of leaves obtained from individuals which presented more or less important defoliations, as well as from individuals with leaf chloroses and/or necroses, have highlighted the definitely not negligible role the foreign deposits play in producing these phenomena.

The massive deposits of lime and cement dust on the surfaces of leaves in the gymnosperms (see Figs. 1–6), investigated in the Bicaz area close the ostiola of the stomata, change the characteristic cuticular relief by disorganising the model of cuticular striations, change the proportion between the crystallised wax and the amorphous one, in favour of the latter, which may contribute to a certain extent to the closing of the ostiola, and favour the development of a micro-flora (fungi and algae) which, once in place, covers photosynthetically active and may release a series of toxic substances influencing the general state of the leaf.

Although some authors consider the presence of a micro-flora on the leaf surfaces to be normal, there are also opinions according to which its presence is a warning sign for important physiological disorders, which are not expressed phenotypically in time; therefore, this is another possible answer to the controversial problem of the massive defoliations of trees.

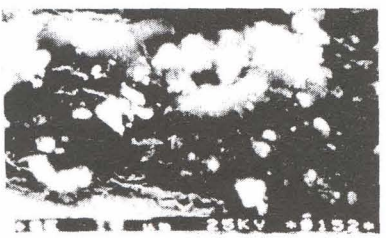

Fig. 1. *Pinus sylvestris* – adaxial surface of the polluted needle.

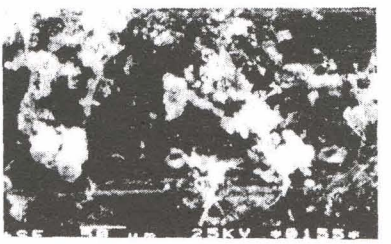

Fig. 2. *Pinus sylvestris* – adaxial surface of the polluted needle.

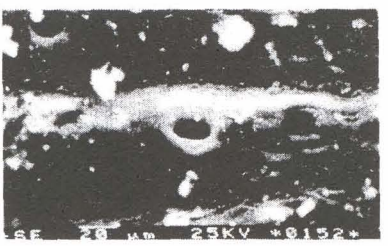

Fig. 3. *Pinus sylvestris* – adaxial surface of the polluted needle.

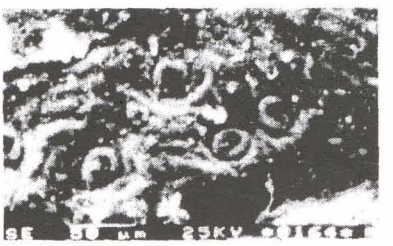

Fig. 4. *Pinus sylvestris* – abaxial surface of the polluted needle.

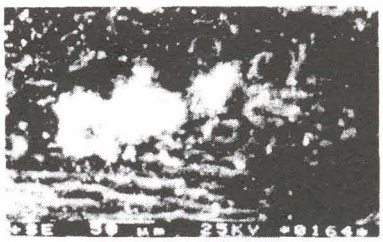

Fig. 5. *Pinus sylvestris* – abaxial surface of the polluted needle.

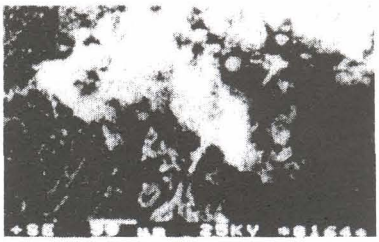

Fig. 6. *Pinus sylvestris* – abaxial surface of the polluted needle.

Similar, but less widespread phenomena have been observed on the leaves of ligneous gymnosperms (see Figs. 7–9) and angiosperms (see Figs. 10–14) in the Borzeşti industrial area; the soot and carbon black deposits are generally less adherent than those made of lime and cement, but they favour the settlement of a micro-flora made of "colonisers" in permanent competition among themselves.

Whatever the chemical nature of the noxious substance is, the settlement of this micro-flora is an indicator of the early senescence of the leaves and a possible cause of the defoliations occurring in the middle of the vegetation season. It is obvious that, the same as the leaf surfaces, this micro-flora is subject to the impact of industrial noxious substances, which may inhibit or, on the contrary, stimulate its expansion.

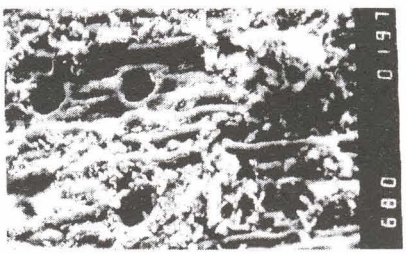

Fig. 7. *Pinus nigra* – adaxial surface of the polluted needle.

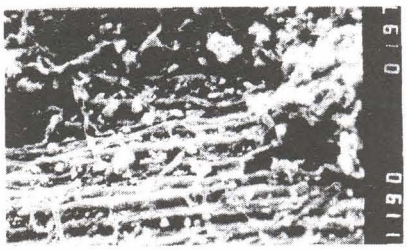

Fig. 8. *Pinus nigra* – abaxial surface of the polluted needle.

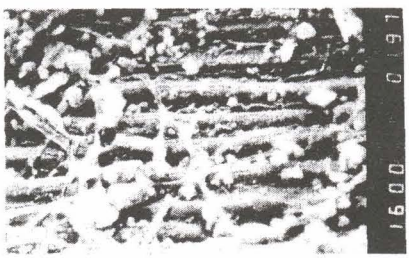

Fig. 9. *Pinus nigra* – abaxial surface of the polluted needle.

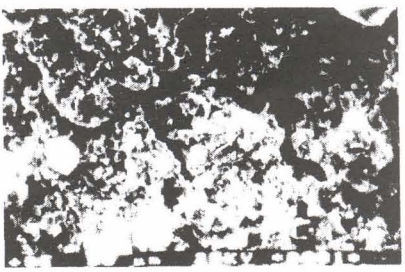

Fig. 10. *Populus tremula* – adaxial surface of the polluted lamina.

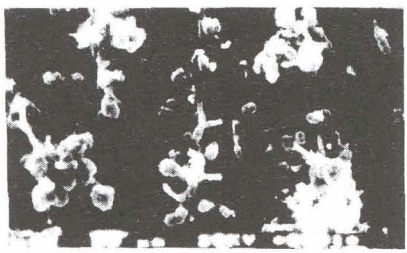

Fig. 11. *Populus tremula* – adaxial surface of the polluted lamina.

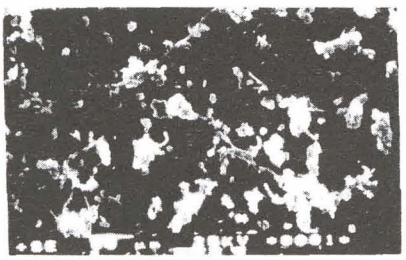

Fig. 12. *Populus tremula* – adaxial surface of the polluted lamina.

Fig. 13. *Populus tremula* – abaxial surface of the polluted lamina.

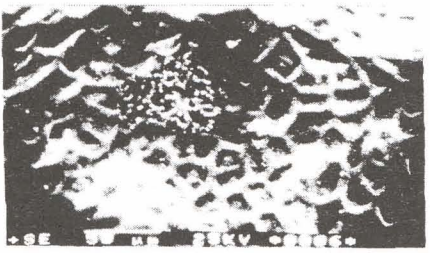

Fig. 14. *Populus tremula* – abaxial surface of the polluted lamina.

The fact that we have only observed the presence of such a micro-flora on the leaf surfaces of individuals with various symptoms of disorders definitely caused by atmosphere pollutants allows us to place our study in the category of those who consider that its settlement on the leaves of individuals on which the noxious substances act systematically is "normal."

♦ **partial withering phenomena** in ***Pinus sylvestris***, ***P. nigra***, ***Acer pseudoplatanus***, ***Salix fragilis*** – Borzeşti; ***Picea abies***, ***Aesculus hippocastanum***, ***Quercus petraea*** – Bicaz; ***Morus nigra*** – Taşca;

♦ **total withering phenomena** in ***Pinus sylvestris***, ***Populus tremula*** – Borzeşti; ***Abies alba***, ***Picea abies*** – Bicaz; ***Populus tremula***, ***P. nigra*** – Taşca.

The partial withering may affect the top of the crown (***Pinus sylvestris***, ***P. nigra***, ***Salix fragilis*** – Borzeşti; ***Picea abies***, ***Quercus petraea*** – Bicaz), the basis of the crown (***Pinus sylvestris***, ***Populus tremula***, ***Acer pseudoplatanus***, ***Salix fragilis*** – Borzeşti; ***Aesculus hippocastanum***, ***Quercus robur*** – Bicaz) or branches found at various levels of the crown (***Salix fragilis*** – Borzeşti; ***Quercus petraea***, ***Q. robur*** – Bicaz; ***Morus nigra*** – Taşca).

The withering phenomena may be preceded by episodes of defoliation or not; when there are visible signs of disorder (burns, necroses, chloroses) on the surfaces of leaves, we may expect withering to occur in the near future.

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

Conifers are regarded as the main victims of atmosphere pollution, as they are extremely specialised in extracting water from clouds and fog, out of which they absorb ions. Thus, it is not only acid rain, but also fog, dew and snow that are potential aggression factors.

♦ **smaller average length** of the 1-year-old and 2-year-old acicular leaves of some species of conifers found in the vicinity of the sources of pollution.

♦ **small average surface** of the leaves of some species of deciduous trees found in the vicinity of the sources of pollution.

The appearance of chloroses and necroses in the early stages of the development of leaves, the accumulation of physiological disorders due to nutrition deficiencies, the systematic aggression of the noxious substances, the existence of unfavourable stational factors have repercussions on the degree of development of the photo-assimilating apparatus of plants; the small average surface of the leaf (due to deposits and to necroses and/or chloroses) is the main cause of the "chronic starvation" of the individuals which stop growing, fructify more and more rarely and consume their own smaller and smaller organic reserves.

♦ **chloroses, necroses and leaf burns** of various sizes, with the aspect of dots, spots or bands, placed especially at the tip of the leaves (in the case of conifers, but also of some deciduous plants), on the brims of the lamina (more seldom at its basis), between the nervures (in the case of deciduous trees), on the adaxial or abaxial face (sometimes, bifacially).

On the 1-year-old leaves of ***Pinus sylvestris***, the afflicted areas occupy from 35% (Borzeşti) to 61% (Bicaz) of the average length; on the 2-year old leaves, they occupy up to 80% (Borzeşti) or 85% (Bicaz).

On the 1-year-old leaves of ***Pinus nigra***, the afflicted areas occupy up to 55% of the average length (Borzeşti) and are difficult to notice in individuals in Bicaz, due to the adherent deposits of lime and cement; on the 2-year-old leaves, the degree of occupation with chloroses and/or necroses may reach 90% (Borzeşti).

On the 2-year old leaves of ***Abies alba*** in the Bicaz area, the affected portions only occupy up to 16%; we suppose that the relatively low percentage found in fir-tree leaves as regards the presence of necroses and/or chloroses may be explained by the pectinated disposition of the leaves on the branches, which permits the rain to wash away the solid deposits better, thus preventing the adherence of the deposits or the formation of calcium hydroxide (by the reaction between lime dust and water), which usually produces severe burns.

The degree of occupation with chloroses and necroses of the leaf surfaces of the deciduous plants under investigation varies very much and reaches very high values both in the species in the Borzeşti area and in those in the areas of Bicaz and Taşca. For example, in the ***Populus tremula*** leaves in the Borzeşti area, the afflicted surface may reach 76.98%, while it is only of 27.06% in Bicaz. In the

Salix fragilis leaves in the Borzeşti area, the afflicted surface is of 24.48%, while in Bicaz it may reach 92.61%.

We have also found high values in the species ***Tilia tomentosa*** (67.16%) in Borzeşti, ***Quercus petraea*** (68.62%) and ***Q. robur*** (94%) in Bicaz.

In some cases, the presence of necroses in young leaves influences their symmetry (***Populus tremula – Bicaz*** and Taşca; ***P. nigra*** – Taşca; ***Prunus avium*** – Bicaz).

Sometimes, the leaf symptomatology reveals the presence of another pollutant than the one which is best-known in the area; for example, in the species ***Aesculus hippocastanum*** in the Borzeşti area, some leaflets present burns placed between the nervures, caused by the SO_2, while in others, the burns are found on the brims of the leaflets and along the median nervure, which reveals the presence of fluorine (apparently from an establishment producing chemical fertilisers). We mention that the two different symptomatologies generally characterise different individuals and it is only in a few cases that we have found indices of "synergic action" of the two noxious substances in leaflets.

The electron microscopy investigations (S.E.M) performed on the portions presenting necroses and/or chloroses (tip, median area, basis) of the acicular leaves of ***Pinus sylvestris*** and ***P. nigra*** have highlighted changes of the cuticular relief caused by the complete disappearance of the parallelism between the cuticular striae, their contortion, the disorderly disposition and smaller dimensions of the stomata, the presence of cuticular fissures, the closing of ostiola with amorphous wax, even the constant "absence" of the mycelial hyphae, which may be an indicator of the fact that the mesophyll of the leaf is generally "afflicted."

In the case of the species of deciduous plants with leaf burns and necroses (***Aesculus hippocastanum***, ***Acer negundo***, ***A. pseudoplatanus***, ***Populus tremula*** (see Figs. 10–14), ***Tilia tomentosa***), we have noticed changes of the general shape of the cells by their contraction due to the absence of the state of turgescence, the quantitative decrease and even the absence of the epicuticular wax, and the presence of algal and fungal "colonisers" in extremely large numbers on the surfaces of leaves showing premature signs of senescence (full yellowing in the month of June).

The existence of partial or total defoliation phenomena with no phenotypisation by necroses and/or chloroses doubtlessly constitutes a special aspect of the fact that, in the areas where the vegetation is subject to chronic aggressions from atmosphere pollutants, the responses are various, unexpected and cannot always be placed into clear categories, but rather in that of "possible responses." During our observations, it is not seldom that we contested the veracity of the chosen witness due to physiological or histo-anatomical changes observed on the investigated material, without the existence of any suspicion when the "phenological" observations were made.

♦ **rare foliage** in ***Pinus sylvestris***, **P. nigra** – Borzeşti, ***Aesculus hippocastanum*** – Bicaz, ***Prunus domestica*** – Taşca. The main causes are the defoliations, but also the general critical state of the individuals, which is worse every year, due to the cumulated effects of the action of the noxious substances over time.

♦ **the second sprouting** during the same vegetation season after serious defoliation episodes (***Acer pseudoplatanus***, ***Populus tremula*** – Borzeşti).

♦ **disorders of the inflorescences**, which become yellow-brown and fall shortly after they are formed (***Robinia pseudacacia*** – Borzeşti) or become brown before the proper flowering and have a mucilaginous consistency (***Syringa vulgaris*** – Bicaz).

♦ **disorders of the fructifications** made manifest as large necroses in the shape of dots present on the surfaces of disameres in ***Acer negundo*** – Borzeşti; small size, brown-reddish colour, rapid deterioration at the mechanical touch (***Rosa canina*** – Borzeşti); falling off the branches shortly after being formed (***Morus nigra*** – Taşca); the absence of fructifications in successive years (***Robinia pseudacacia*** – Borzeşti, ***Syringa vulgaris*** – Bicaz; most species of fruit-bearing trees).

CONCLUSIONS

Our investigations have focused (during the period 1996–2005) on observations concerning 19 species of ligneous plants in various phenophases and the state of the leaf surfaces under the influence of the atmosphere pollutants.

Certainly, the "responses" are conditioned by a multitude of factors (genetic factors, pedo-climatic ones, natural habitat, degree of acclimatization, distance and position with regard to the source of pollution, age, physiological state, etc.) and therefore will not be seen as absolute, but will only be reunited as data which will complete the clinical picture of the leaf symptomatology.

The common situations occurring as a general response to the action of (gaseous and solid) noxious industrial substances are: deposits of foreign substances – lime and cement dust, carbon black, soot – on the surface of the leaves; episodes of partial or total defoliation; partial or total withering phenomena; smaller average length and small average surface of the leaves; chloroses, necroses and leaf burns of various sizes; rare foliage; the second sprouting during the same vegetation season after serious defoliation episodes; disorders of the inflorescences and disorders of the fructifications.

The electron microscopy researches (S.E.M) performed on the surfaces of leaves obtained from individuals which presented more or less important

defoliations, as well as from individuals with leaf chloroses and/or necroses, have highlighted the definitely not negligible role the foreign deposits play in producing these phenomena.

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