

LEAF - ANATOMICAL CHANGES IN PERISHING ACALUOUS OAKS

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Abstract

We examined the damaging effect of airpolluting substances emitted by the Cement and Lime Works in Vác on the leaves of *Quercus petraea* L. ssp. *petraea*. We found the foliage damage to be the greatest near the factory and at a distance of 5 and 10 km it gradually decreases. The surface of foliage-leaves significantly, 30-54%, decreases. The number of stomata on the abaxial epidermis also decreases in 6,5-36,5%, at the same time the closing cells are greatly distorted and there are frequent twin-stomata. On the sick leaves 1-2 radial cell hairs appear instead of the 3-5 cell trichomes on the healthy leaves.

We have concluded from the facts mentioned above that air-polluting substances have an inhibitory effect on leaf-forming meristems, as well as on the stomata and trichome initials, which results in changing the structure and distortion.

Key words: *Quercus*, damage of leaf, air-pollution.

Introduction

It is well-known that the rapid development in industry and agriculture in our times is full of contradictions. To protect the cleanness of water purity and air, or at least to decrease the rate of pollution is one of the basic tasks to be solved.

The air-pollution in the industrial regions is growing, which means a threat to our environment. When changes in pollution exceed the tolerance threshold of bioconosis, it is the living beings which indicate this threat in the shortest time. The most sensitive vegetative organ of a tree is foliage leaves the surfaces of which are greatly exposed to the air. This fact may explain the work of some scientists - among others - PAUL and HUYNH-LONG, 1975; DÄSSLER, 1976; EVANS and CURRY, 1978; JÄGER and KLEIN, 1980; JAKUCS, 1985, who dealt with the damage of foliage.

In our country, from the point of view of forestry, the perish of *Quercus petraea* is of the greatest degree due to antropogenic pollution. During the past years we have been trying to answer the question of what type of anatomical changes were caused in the epidermis of the leaves of acaluous oak by the air-pollution of the Cement and Lime Works in Vác, or by its damaging effect in a 5-10 km distance

from the factory. Since the leaves are continuously growing in the first phase of the growing period, the degree of damage could be detected continuously. Taking into consideration the fact that examinations of this kind are rare in Hungary, we would like to present data which gives a complex explanation of the causes of oak perish.

Literature survey

Air-pollution varies between different industrial regions, but is still increasing on a global scale. The characteristic feature of anthropogenic in regional pollution concentrations is that the highly industrialised countries of Western-Europe (England, Holland, Belgium, France, FRG) are situated on less than 1% of the Earth surface, their emission of sulphur makes up the 13% of the artificial emission of sulphur of the World (SASVÁRI, 1984).

In Hungary in the early 1970 there were some air-polluted regions which emerged in the wake of polluting sources, and these regions follow the pattern of industry and town agglomerations.

50–60% of air-pollution in Hungary is of foreign origin. 45% of Hungarian air-pollution comes from industrial activities, 35% from traffic, 20% from communal smoke. 1 million 200.000 metric tons of SO₂, 547.000 of dust and 370.000 tons of NO_x is released into the air in this country altogether by burning fuel and by industrial emission. The towns Ajka, Füzfő, Vác and their regions are considered to have most polluted air (HORVÁTH, 1986).

Atmospherical rain fall, and the small amount of nitrogen, sulphur, phosphorus, etc. dissolved in it are known to be important nutritional sources for plants. If these elements are in higher concentrations in the air, then when they get on the plants they form acids. The acids disturb the plant life cycle and upon reaching the soil change its pH. This phenomenon has become known as acid rain. The greatest damage to plants have been detected in forests (MÉSZÁROS, 1984).

The forest districts of the Earth decrease annually by 10 million hectares, mostly in the tropical areas and this loss cannot be counterbalanced by new plantations. The alarming news about the death of European forests is accumulating. About one third of FRG forests are attacked. The situation in Austria and Switzerland is not better at all. In Czechoslovakia the death of pine and oak forests causes the main concern. Data about the decay of acaluous oaks, *Quercus petraea* (MATT) LIEB. were first reported from Romania. The peak of this decay was between 1976–77. Several papers about the severe devastation of acaluous oaks in Slovakia have been published.

In Hungary the disease, known as withering of the tree, first of all devastates our acaluous oaks, has been reported by the foresters since 1978. The devastation of our acaluous oaks began first in the central range of mountains. Today, however, it

can be found in the Trans-Danubian central mountains, in the Mecsek mountains and in Somogy county, as well.

According to the survey made so far about 10% of the Hungarian forest regions has been damaged and the devastation of acaluous oaks makes up about 7,5% of the total (LÁNYI, 1986; BORHIDI, 1987; BERCZIK-BORHIDI, 1979.).

It is well known that the increase in the sulphur dioxide content of the air can slow down photosynthesis to such an extent that the degree of cell-division and cell-elongation in the meristem of the leaf will decrease too. This process results secondarily in deforming the cellular elements and forming chlorosis and necrosis.

JÄGER and KLEIN (1980) claim that the air-polluting substances enter the leaf, firstly, through the stomata where they exert a harmful influence, not only on the function of the mesophyll, but on that of the stomata cells, too, e.g. transpiration.

EVANS and CURRY (1979) write that the acid rain damages the upper epidermis, the gaseous substances do harm to the abaxial epidermis besides the mesophyll. Around the trichomes on the bottom of the leaves there is considerable damage.

PAUL and HUYNH-LONG (1975) in their article stated that the toxicity of sulphur dioxide causes the formation of abnormal cellulose cell walls.

BABOS (in his verbal statement) said that on the attacked leaves the stomata are smaller and the number of stomata in a unit area decreases.

On the basis of the facts mentioned above it is obvious that the growth of the trees with attacked foliage, and the production of organic substances are less than the average.

The Hungarian and foreign publications concerning this theme could fill a small library, so we are not going to give any more details about them. The opinions on the primary causes generating the disease differ, but every researcher agrees that they encounter a complex problem. This complexity may be explained by several factors: the soil pollution, the decrease in the number of root hair, bacteria, macro and micro fungus, insect pests, etc. directly or indirectly also may contribute to the devastation of oaks in the damaged environment.

The Cement and Lime Works in Vác, mentioned previously let 10 thousand tons of polluting substances (dust, SO₂, NO_x, etc.) into the air.

In our present survey we are trying to define the effect of damaging materials of the factory on the leaves of acaluous oaks.

Materials and Methods

In Hungary acaluous oak park comprises three subvarieties. We chose the leaves of *Quercus petraea* L. ssp. *petraea* to be examined. According to our hypothesis if the substances released by the factory in Vác damage the foliage-leaf, this harmful effect is the greatest near the factory and gradually decreases with distance. So the samples of leaf were taken from three places taking into account the prevailing wind:

1. From the trees near the factory
2. From the forest of the village Rád, 5 km from the factory, and
3. From the vicinity of the village Szendehely, 10 km from the factory

The control, healthy leaves were collected in the forest near the village Valkó. The samples of leaves were taken from the central third of the south-east foliage. The collected leaves were kept until examination in 40% ethanol. In comparison the following data were processed on the basis of epidermis skinning from the middle of the leaves:

- the number of stomata mm^2 /each leaf
- the state of the adaxial and abaxial epidermis cells
- the number and form of trichomes

Beside this we examined the formation of the healthy and damaged leaf area.

In order to be brief in this survey we are not going to deal with the gradual yellowing and withering of the foliage leaves with the damage of shoots etc. which were also detected.

To measure the leaf-areas we copied 100–100 pieces of leaves on tracing-paper, then they were cut and weighed on analytical scales and the differences were given in per cent. In order to examine the number of stomata, epidermis cells and trichomes, adaxial and abaxial skinning was prepared from 50 and 50 pieces of leaves, respectively. The obtained preparation was then purified in 5% sodium hypochlorite and then washed in 10% acetic acid. The cell walls were stained with methylene blue. Then with increasing ethanol concentrations – generally used in plant microtechnology the preparations were dehydrated, and after fixation in Canada balsam examined. The measurements were carried out in the field of view of a research microscope of NU-2 and NFPK types, magnified 160 times. The data concerning the leaf areas were processed on a Commodore computer.

Results

The gradual change of foliage indicates the beginning of the damaging effect of air-pollution. It is already in June that the light green leaves begin to turn into yellowish green. Later on, in August some of these turn into yellowish brown and the leaf totally dies by the middle of September. The withered leaves do not fall off the tree for a long time.

The blatant deformation in the leaf lamina exposed most to air-pollution may be observed, too. The number of the lobes of leaf lamina decreases. A lobe number of 4,5,6 is characteristic for the leaves of a sick tree, in contrast to the healthy ones which have 9–10 lobes. The decrease of the leaf lobe area – due to the increase of air-pollution – becomes greater and greater (Table 1.).

Table 1. Size of leaf lobe.

| area of samples | size of leaf lobe in% |
|---------------------------------------|-----------------------|
| VÁC near the air-polluting factory | 46,2 |
| RÁD 5 km distance from it | 58,8 |
| SZENDEHELY 10 km distance | 70,5 |
| CONTROL (VALKÓ) healthy leaves | 100 |

The differences at 0,1% level are significant.

Measuring the longitudinal growth of a one-year-old shoot we obtained similar results.

It can be stated that the proximity of the air-polluting source decisively influences the growth of the leaf.

If we take into consideration the fact that the large scale decrease of the leaf lobe is true for the whole foliage, it can be stated that the production of foliage by a forest, and at the same time the amount of dry fallen leaves, decrease.

In addition these sick leaves decay in a more difficult way, so humification in the soil will be poorer.

Examining the abaxial epidermis skinnings of the leaves we found that the number of stomata on a unit area is decreasing. The degree of decrease is indicated in Table 2.

Table 2. Number of stomata of leaves.

| Number of stomata/piece in 15.000 μm^2 | | |
|---|-------------------------|-----------|
| Area of samples | number of stomata/piece | value in% |
| VÁC near the air-polluting factory | 10,8 | 63,5 |
| RÁD 5 km distance from it | 12,7 | 74,7 |
| SZENDEHELY 10 km distance from it | 15,9 | 93,5 |
| CONTROL (VALKÓ) healthy leaves | 17,0 | 100 |

The results at the 0,1% level are significant.

The cause of the decrease in the number of stomata may be explained by the fact that the formation of stomata cells is inhibited by the air-polluting substances. This may lead to a smaller zone of inhibition around the stomata mother cells, since twin stomata often appear on the sick leaves (Plate I. photos 1-2.).

On the basis of 1-4 photos in Table I. we may conclude that the size of stomata becomes heterogenous under the influence of air-pollution and there is a frequent deformation of guard cells and air openings. On the other hand, the decrease in the number of stomata may result in less gas exchange ($\text{CO}_2\text{-O}_2$ exchange), as well as in less transpiration. The hairs formed on the leaves of acaluous oaks have a large area exposed to air, so air-pollution has an effect on the formation of trichomes of the abaxial epidermis (Plate II.). The 3 to 5 radial cells of the stellate hairs are to be found most frequently on the healthy leaves (Plate II. photos 1-2-3.). On the sick leaves these trichomes develop only 1-2 radial cells (Plate II. photos 4-5.).

The damaging substances may be supposed to exert an inhibitory effect, resulting in fewer numbers of radial cells. Some changes in the adaxial epidermis cells are also detectable which become more and more characteristic as the summer ends. The cells deform, shrivelling, their radial walls become uneven (Plate III.).

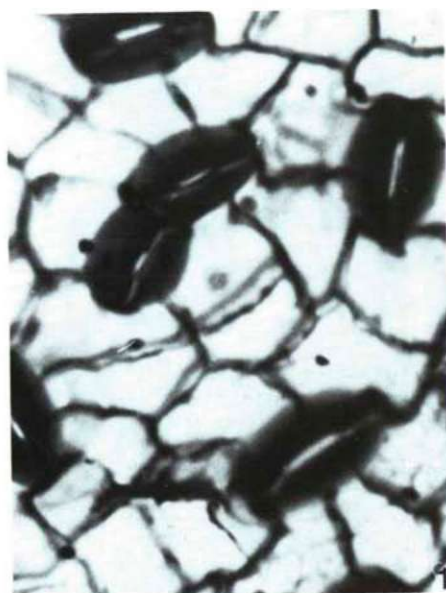
The results of the experiment verify that the distance from the cement factory, which is in direct proportion to the concentration of air-polluting substance plays a decisive role in the morphological and anatomical lesions of foliage-leaves of *Quercus petraea* L. ssp. *petraea*.

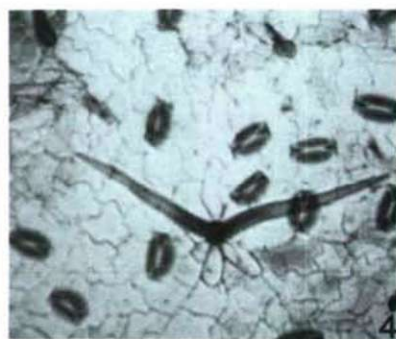
Therefore the damaging effects of air-polluting substances: cement dust, soot, SO_2 , NO_x of the cement factory gradually decreases moving away from the source of emission.

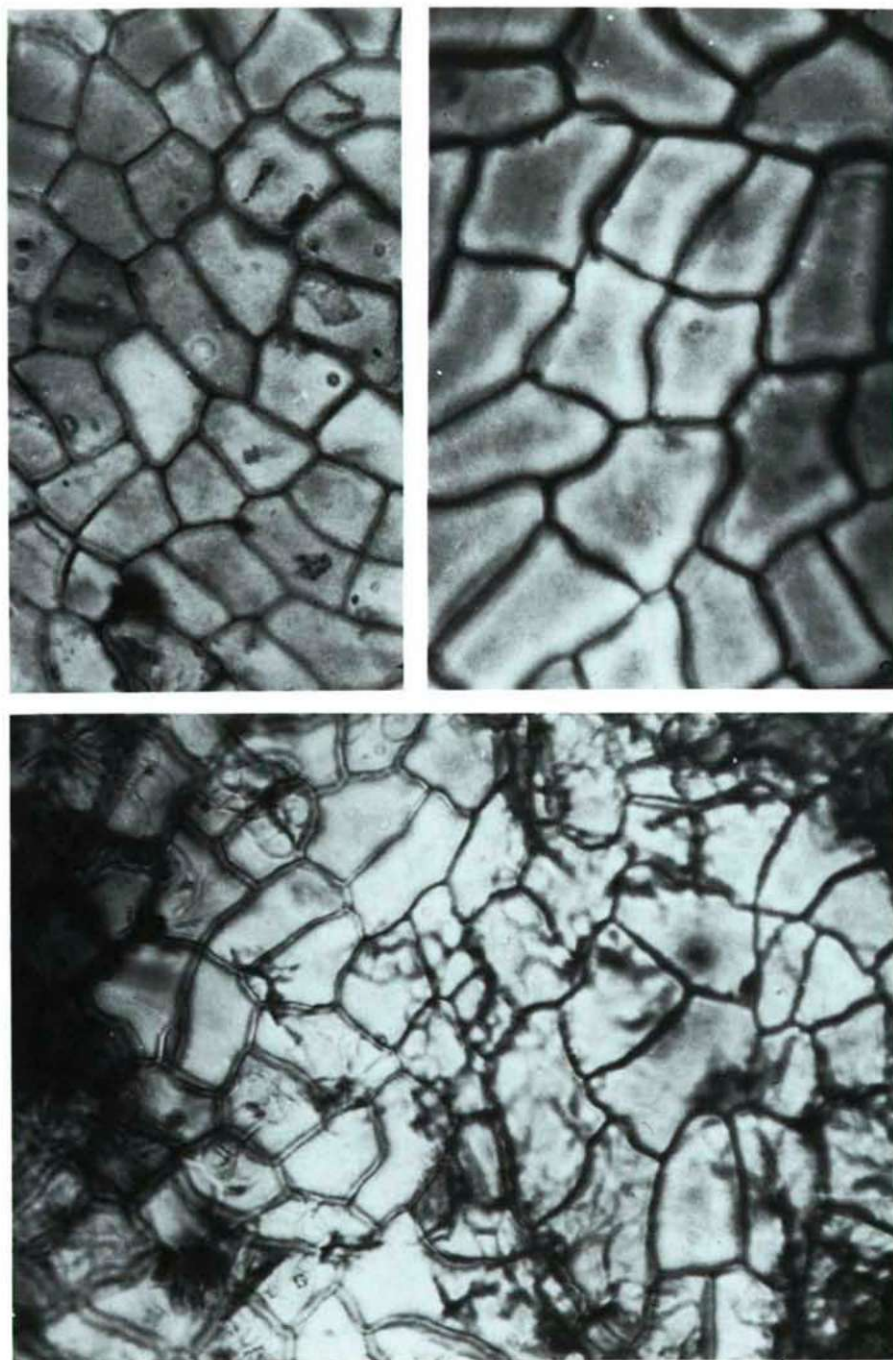
Plate I. *Quercus petraea* L. ssp. *petraea*, abaxial epidermis is alone and together with twin-stomata. Photos 1-2=less damaged epidermis in early July, magnified 310 times. 3 = severely damaged, disorganized epidermis at the end of August, magnified 250 times. 4 = healthy epidermis in early June, magnified 250 times.

Plate II. *Quercus petraea* L. ssp. *petraea* abaxial epidermis with trichomes. 1-2-3= healthy epidermis with multi-branch hair, magnified 120 times. 4-5 = damaged epidermis with stomata and trichomes, magnified 120 and 240 times.

Plate III. *Quercus petraea* L. ssp. *petraea* adaxial epidermis. 1-2= healthy, magnified 310 and 450 times, 3 = damaged epidermis, magnified 310 times.







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