CYTOLOGICAL AND ANATOMICAL CHANGES IN PINE TREE XYLEM CAUSED BY SULPHUR COMPOUNDS

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ABSTRACT

In areas intensely contaminated with sulphur compounds some pine trees (*Pinus sylvestris* L.) have deformed branches with numerous short sprouts. In the branches lenticular areas with considerably changed xylem elements are found. In these greatly changed xylem areas (GCXAs) deformed tracheids are almost isodiametric, some form hooks and loops. The tracheids and ray parenchyma cells show a differentiated level of cell walls lignification. A significantly larger number of rays and the so-called pseudorings are observed.

KEY WORDS: *Pinus sylvestris* L., GCXAs-greatly changed xylem areas, environmental pollution, sulphur compounds.

INTRODUCTION

A destructive effect of pollution on morphology and physiology of trees has been often reported in the literature (Białobok, 1989). It has been observed that trees affected by pollution have bushy-like crowns with groundling branches (Gadzikowski, 1980; Kawecka, 1981). In the case of acute pollution, several damages of leaves such as: necrotic spots, necrosis, chlorosis, hyperchromatism, withering are found (Karolewski, 1990; Braniewski, 1983; Książek et al., 1989).

Many developmental abnormalities are of a physiological and a biochemical character (Malhorta and Hocking, 1976; Lorenc-Plucińska, 1982; Oleksyn and Białobok, 1986; Karolewski, 1989; Karolewski et al., 1994; Giertych and Karolewski, 1994).

There are relatively few data concerning pathological anatomy and cytology of pine wood caused by air pollution. As follows from the earlier studies (Grill et al., 1979; Bauch et al., 1979; de Kort, 1986) industrial emissions are responsible for short tracheids in gymnospermous trees. The diameter of tracheids and of other xylem elements decreases. The tracheids in trees growing under stress become shorter in comparison with the control (Spława-Neyman, 1994). Air pollution causes abnormalities in annual rings development (de Kort, 1986, Kozłowski and Constantinidou, 1986). The aim of the paper is to present the effect of environmental contamination with sulphur compounds on anatomical and cytological changes in pine xylem.

MATERIAL AND METHODS

Studies were carried out using pieces of the xylem taken from four-year-old branches of fifteen-year-old trees of *Pinus sylvestris* L. growing in the vicinity of the Sulphur Mine "Jeziórko" near Tarnobrzeg. The trees selected for the studies did not display outer damages of bark and they came from two localities situated 2 kilometers from a sulphur dump and its transport route. Measurements of air contamination showed sulphur compounds predominating (according to the data of the Reasearch and Development Centre for Sulphur Industry "Siarkopol"). For light microscopy collected wood samples were kept in 70% ethyl alcohol for a few months. Then they were softened by boiling in water with an addition of glycerol to be cut into transverse, tangential and radial sections of about 20 μm thick. For lignin detection the sections were stained with Fast Green and safranin (Johansen, 1940). After dehydration in absolute alcohol, the sections were twice washed with xylene. The slides were closed in Canada balsam.

A Zinc-chlor-iodine reaction was used for the detection of cellulose (Gerlach, 1972).

RESULTS

Morphology of sprouts

In the area of intense contamination with sulphur compounds a changed shape of the pine crown has been observed. The branches have short, horizontally growing sprouts (Fig. 1).

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Fig. 1. The horizontally growing branch of *Pinus sylvestris.*

Most of their top buds are dead or covered with resin. The needles turn yellow from the top downwards.

Anatomy of wood

Pine tree xylem is composed of tracheids arranged in regular ray tiers, one cell wide rays and resin ducts. The earlywood tracheids have thin walls with bordered pits distributed only in radial walls. In the latewood the thick walled tracheids do not possess so many bordered pits either in radial or in tangential walls (Hejnowicz, 1980). The rays are composed of tracheids and parenchymatic cells. The central part of the ray is occupied by prolate, live parenchymatic cells with cytoplasm and starch grains. The top parts are composed of tracheids with a roughly crenated inner layer of walls (Wodzicki and Braun, 1973). Traumatic resin ducts with thin-walled parenchyma cells run along spindle-shaped rays.

Cross sections of sprouts reveal lenticular, greatly changed xylem areas (GCXAs) located in the earlywood. The tracheids of this area are oval and very wide, exceeding several times those of normal wood (Fig. 2). GCXAs varied in size; from very small areas, composed of only a few cells to large ones, visible with naked eye. It is dificult to distinquish tracheids from the changed cells of rays in GCXAs due to the greatly changed shape and size of the cells. GCXAs are highly complex. They are composed of tracheids as well as changed parenchymatic cells. Moreover, they consist of living big cells with protoplasts and large, dead cells with lignified walls (Fig. 3). Some additional details can be seen in radial sections. Figure 4 presents changed xylem area. Usually, GCXAs appear in the earlywood and the first tracheids are more deformed (Figs 5, 6). They have a spherical or almost isodiametric shape. In further formation of xylem a gradual reconstruction of normal shape takes place and tracheids become thinner and longer, assuming later a spindle shape. A similar phenomenon can be observed in the case of parenchymatic cells. Ray cells lose their prolate shape, becoming extremely large, thus exceeding two-three times the size of normal ray cells (Figs 7, 8). With the flow of time the deformation is almost repaired. Figure 8 presents a changed ray. Its initial width is that of three cells, but when GCXAs appear this ray expands and undergoes a transformation, reaching the width of two cells.

In tangential GCXA sections some tracheids form hooks and loops (Fig. 9). The tracheids and parenchyma elements of the xylem are separated by additional, transverse walls.

Finally it is difficult to recognize the exact character of xylem cells, because of their variable shape and the level of cell walls lignification (Figs 10, 11).

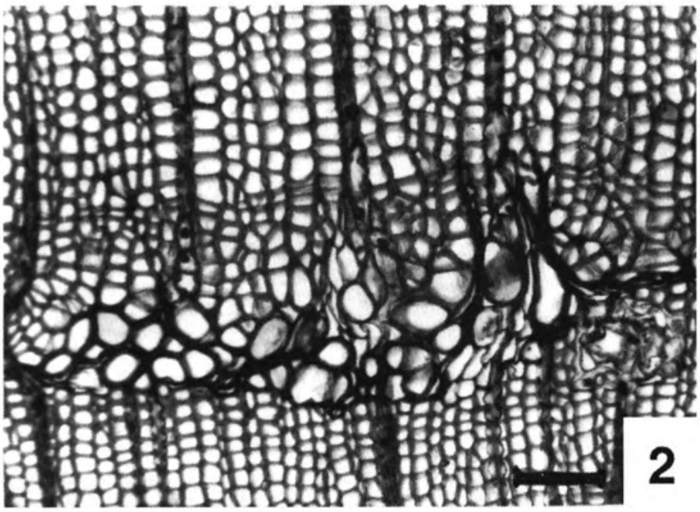
Cross sections of branches show that GCXAs has been formed in successive years from the same cambium area (Fig. 12). In such wood a significantly larger number of rays is found (Tabele 1). Sometimes trees growing in areas of highly polluted air and soil have xylem with the so-called pseudorings. These rings are narrower in comparison with annual rings of control trees and a few of them might be form during one vegetation season (Fig. 13). Outside the GCXAs other elements of xylem are normal.

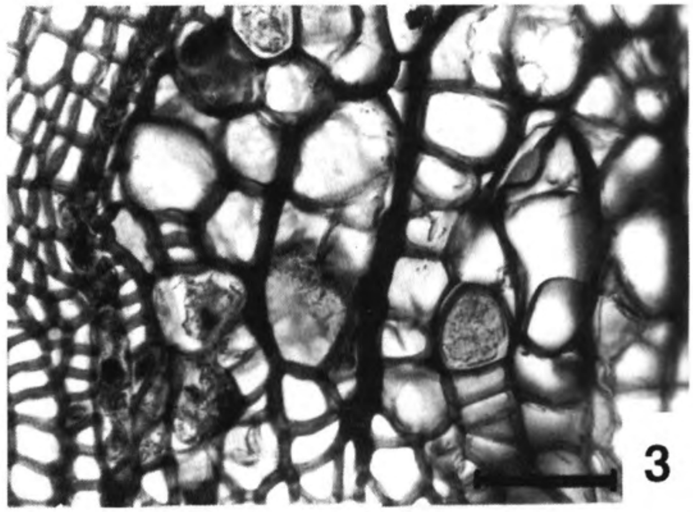
TABLE 1. The number of rays in xylem of *Pinus sylvestris* growing near the Sulphur Mine "Jeziórko".

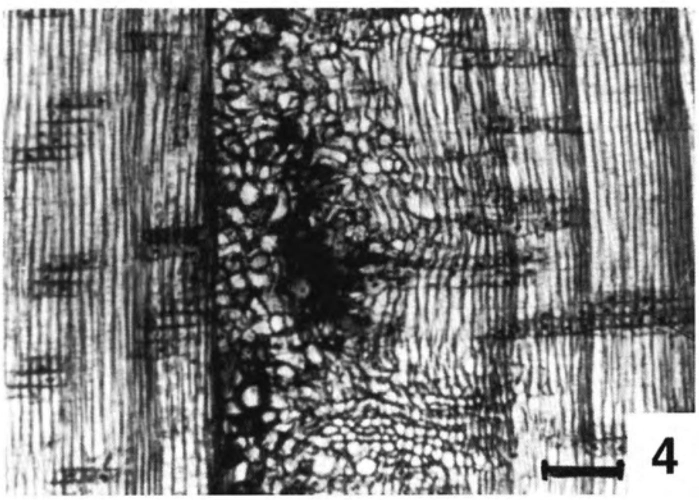
Number of Pine tree	Number of rays (in 400 μm tangential xylem segments)	
	in changed xylem	in normal xylem
1	4	2
2	5	2
3	6	2
4	4	3
5	4	2
Average of 5 trees	4.6	2.2

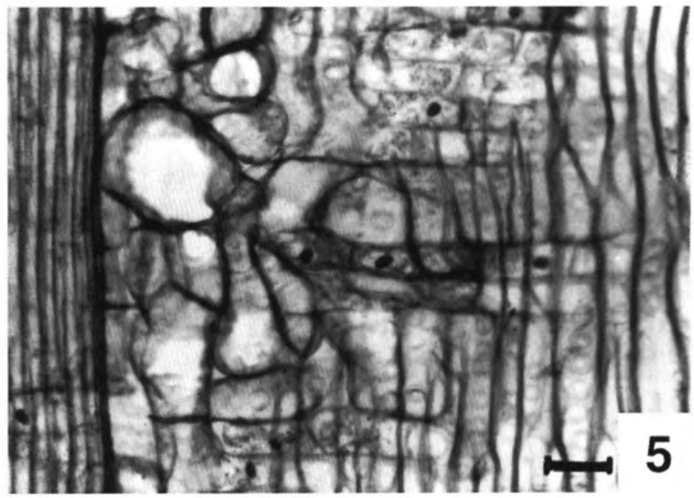
DISCUSSION

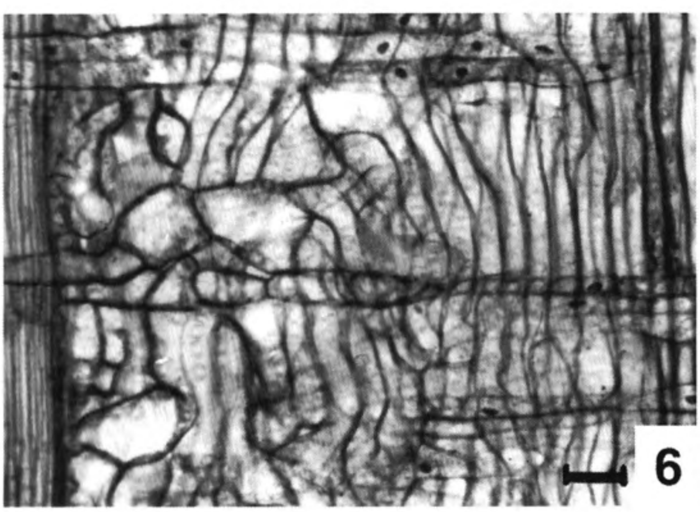
In areas where sulphur is exploited by the ("hot") open technique, e.g. in the Sulphur Mine "Jeziórko" near Tarnobrzeg, the atmosphere is highly contaminated with sulphur dust,

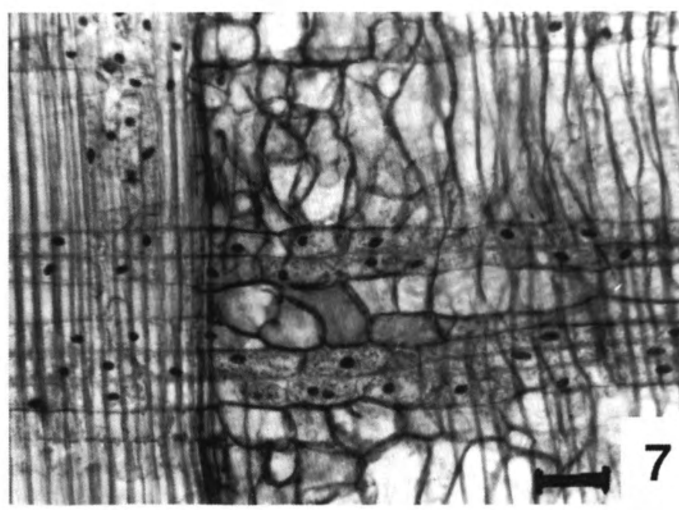




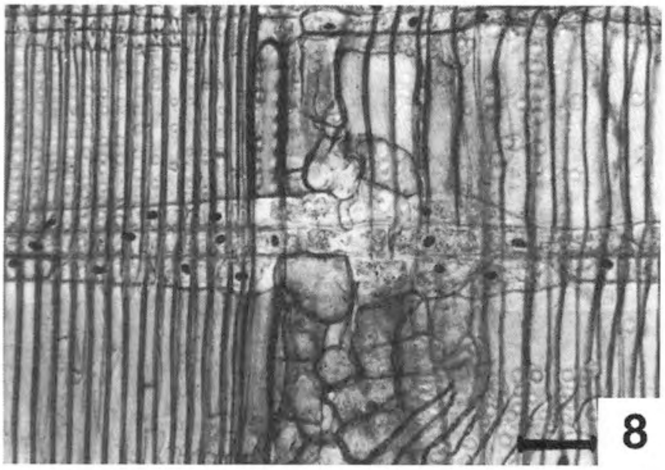


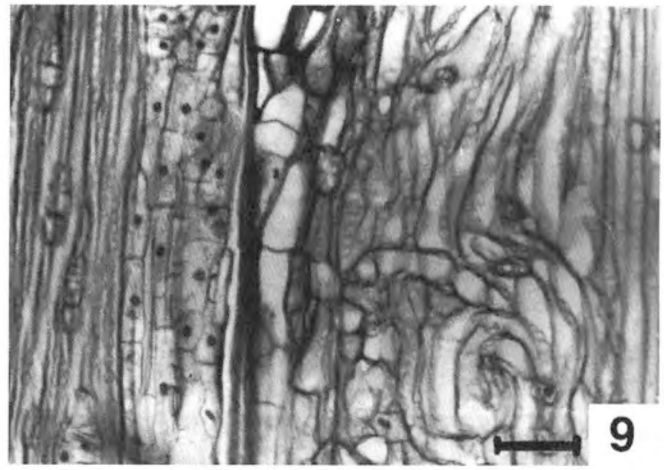


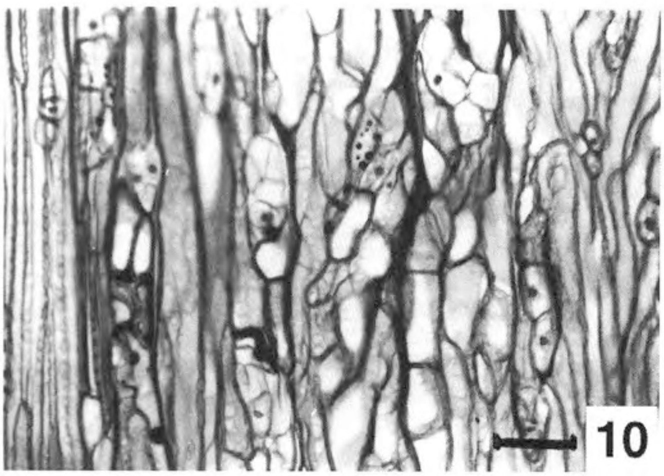


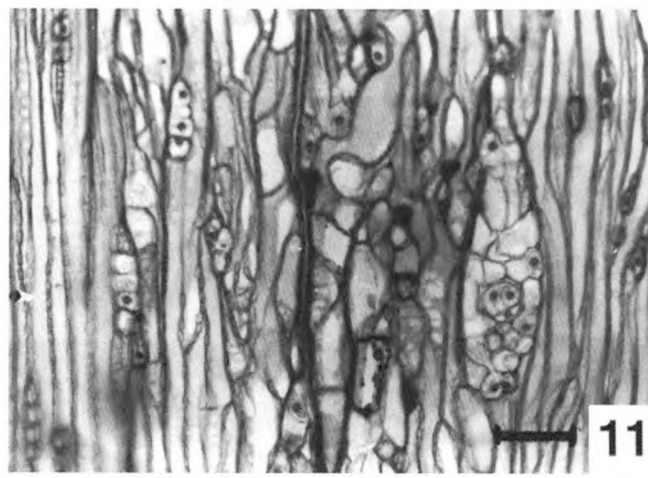


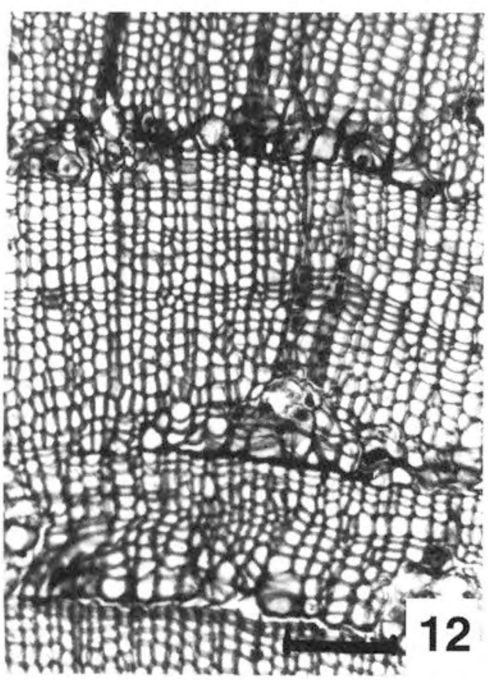
Figs 2, 3. Transverse section of greatly changed xylem area (GCXA) of pine tree. Bar length = 100 μm
Fig. 4. The lens of changed xylem in radial longitudinal section. Bar length = 200 μm
Figs 5, 6. Radial longitudinal section of GCXA. Large, spherical tracheids. Bar length = 100 μm
Fig. 7. Radial longitudinal section of GCXA. Deformed rays in the damaged area. Bar length = 100 μm











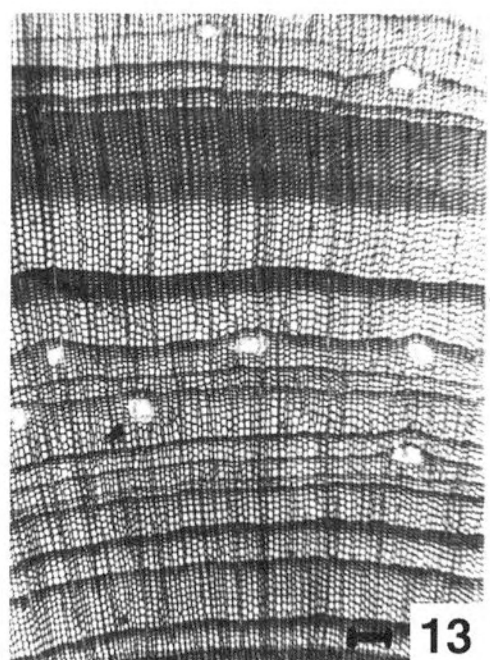


Fig. 8. Radial longitudinal section of GCXA. Deformed rays in the damaged area. Bar length = 100 μm

Fig. 9. Tangential longitudinal section of GCXA. The curved tracheids form loops. Bar length = 100 μm

Figs 10, 11. Tangential longitudinal section of GCXA. Numerous, transverse divisions of spindle-shaped cells. Bar length = 100 μm

Fig. 12. Transverse section of *Pinus sylvestris* xylem. The disturbances occurred in the some area of cambium in successive years. Bar length = 100 μm

Fig. 13. Transverse section of *Pinus sylvestris* xylem. The so-called pseudorings. Bar length = 200 μm

sulphuretted hydrogen and sulphur dioxide (Łącka-Pilaszek and Siuta, 1991). It is known that SO_2 and H_2S are the most dangerous of all gaseous pollutants of the atmosphere (Białobok, 1989). In 1990-1993, in the area of Jeziórko, the concentration of sulphur dioxide and sulphuretted hydrogen was about three times higher than the admissble norm (Kalisz et al., 1993). The sulphur contamination is here the most intense and has serious effect on vegetation. However, the other kinds of contaminations should not be neglected. The damages of trees result mostly from the action of many factors (Obmiński, 1970; Niemtur, 1977; Godzik, 1981; Karolewski and Lorenc-Plucińska, 1993).

Common pine trees are particularly sensitive to the action of industrial sulphur compounds (Białobok, 1989; Godzik, 1989). Growth inhibition and bushy forms are the first visible signals of tree reactions, but in some cases the normal shape of the crown can be found in highly polluted areas (pers. comm. W. Włoch). In the area of sulphur mines near Tarnobrzeg, only some pines exhibit deformed sprouts. A synchronomous phenomenon of deformed sprouts formation was not observed. This phenomenon consists in a simultaneous correlational reaction between individual sprouts. The evidence of which is, among others, a decrease in the number of needles and their thicker accumulation on the sprouts (pers. comm. W. Włoch). In the area of Sulphur Mine the pine needles were thinned out and some sprouts were almost completely deprived of them. Quite often but not always the pine needles turned yellow from the top; similar symptoms (turning yellow and withering) were observed in pines treated by sulphur dioxide in laboratory conditions (Karolewski, 1990). Abundant secretion of resin was observed on the buds and tops sprout needles.

There are some general data concerning the changes in anatomical structure of wood caused by pollutants. It has been reported that the shape and size of the xylem elements are different in comparison with control. The tracheids in pines growing in polluted areas are shorter and thinner (Grill et al., 1979; Spława-Neyman, 1994). I have observed local changes of xylem in the form of lenticular areas (GCXAs) with strongly deformed tracheids and xylem parenchyma, despite of the regular development of xylem beyond these areas. Such changes have not been described so far. Similar xylem deformations are known in the wood of trees attacked by insects (Liphschitz and Mendel, 1987). Such changes has been correlated with outer damages of the bark and easily seen under the microscope. Experimental wounding and scratching produces lignified parenchyma cells instead of tracheids (Kuroda and Shimaji, 1984; 1985; Lev-Yadun and Aloni, 1991; Fahn and Zamski, 1970). There is an irregular and spiral arrangement of tracheids and ray cells.

The early- and latewood of coniferous trees has a homogenous structure. The tracheids and ray parenchyma are arranged in tiers, because they originate from one cell in the cambial ring (Hejnowicz, 1980; Hejnowicz, 1993). Disturbances in the tier structure of tracheids in the damaged xylem area are caused by disturbed activity of the cambium. As follows from the recent studies (Kurczyńska et al., 1994) polluted environment causes: decreases in the number of cambial cells divisions and in the number of cells in the cambial zone and shortening of the period of cambial activity. In my investigations irregular elements have not been found outside GCXAs. In succesive years GCXAs appear from the same cambium area and more often occur in the earlywood. This may be caused by a penetration of contamination from the outside through the covering tissue. The penetration is particularly intensive in spring, when concentration of sulphur contamination is high. One might suggest, that GCXAs result from disturbances in the development of the xylem mother cells. However, after the disappearance of GCXAs a violent change in the structure showing the situation before GCXAs formation would take place and a gradual regeneration into a new changed arrangement of both tracheids and ray cells is observed. Probably GCXAs formation is associated with complete elimination of a large number of initial cells in cambium. To explain this problem it might be interesting to study cambium and phloem taken from such areas in spring. Smaller annual rings and formation of the so-called pseudorings has been found in trees growing in a polluted environment (Lise et al., 1975; Kozlowski and Constandtinidou, 1986). Such rings can be a result of a physiological drought i.e., a malfunction of water transport in the plant in spite of a sufficient amount of water in the soil (Czerwiński, 1976).

The presence of the so-called pseudorings in some trees in the area of the Sulphur Mine suggests that they have not been caused by soil drought but rather by disturbances in physiological processes. It can be supposed that irregular transport of water and growth factors are responsible for pseudorings and sudden changes. The physiological orientation of the polar transport of water and auxin can be changed in if mechanical blocking occurs. Such blocking can be caused by irregular tracheids loops (Gersani and Sachs, 1984). The above-mentioned substances can not reach all cells at the right time. The effect of this is the disorganization activity of cambium, conducting elements and whole plant.

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CYTOLOGICZNE I ANATOMICZNE ZMIANY W DREWNIE SOSNY (*PINUS SYLVESTRIS* L.) POD WPŁYWEM ZWIĄZKÓW SIARKI

STRESZCZENIE

W miejscach silnego skażenia związkami siarki, niektóre drzewa sosny (*Pinus sylvestris* L.) mają charakterystyczny pokrój. Ich gałęzie tworzą liczne, krótkie pędy rosnące poziomo. W drewnie gałęzi występują soczewkowate obszary o silnie zmienionym i zróżnicowanym kształcie elementów drewna. Zniekształcone cewki mają kształt prawie izodiametryczny. Niektóre tworzą zagięcia i pętle. W obszarze silnie zmienionego drewna ściany komórek są w różnym stopniu zdrewnieniałe. Żywe komórki miękiszowe promieni osiągają wymiary kilkakrotnie przekraczając objętość normalnych. Obserwuje się również dużo promieni rdzeniowych oraz tzw. pozorne słoje przyrostu.

SŁOWA KLUCZOWE: sosna, OSZD-obszary silnie zmienionego drewna, zanieczyszczenie środowiska, związki siarki.