

The Effect of SO₂ Pollution on Pine Needle Structure

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Abstract.—Fall and winter needles from pines growing near the Kostomuksha ore-dressing mill (KODM) were collected and studied by light microscopy. Fall needles showed symptoms of SO₂ influence and no specific seasonal changes in mesophyll. The injury rates of needle surface and mesophyll showed that pollutants penetrate into the needles through stomata and cuticle. Compared to fall needles winter needles exhibited a greater number of destroyed mesophyll cells and heavy cuticle damage. The effect of the KODM pollutants is thought to reduce the tolerance of mesophyll to low temperatures. It is obvious that pine needles near the KODM suffer both from acid rain and SO₂.

FALL NEEDLES

0.5 - 2 Kilometers From Mill

First-Year Needles

High vesiculation rate is characteristic of mesophyll cells. Tannin appears as a thick ribbon, in a granular form, or as a droplike substance. Some cells seem to be "empty" because the vacuole outlined with tannin fills in the whole cell. It is impossible to distinguish cytoplasm between tonoplast and plasmalemma. The study of serial sections shows that the next stage in these "empty" cells' structure can be their full disintegration and cell wall destruction. This would increase the intracellular space.

Chloroplasts of the living cells are big and rounded, and sometimes concave. Their dark coloring indicates the presence of plastoglobuli. Occasionally, starch grains can be detected in plastids.

It should be noted that "empty" and semidestroyed cells are situated in inner mesophyll layers closer to endodermis or resin canals. Both the abaxial and adaxial needle sides demonstrate destroyed cells.

Parenchyma cells of vascular bundles show tannin and lipid accumulation. Needle cuticle and surface tissues are strongly injured. Sometimes the epidermis splits off from the hypodermis.

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Second-Year Needles

The difference between the first- and second-year needles is that the latter have more "empty" and semidestroyed mesophyll cells and larger intracellular spaces. In some cells, tannin occurs as large lumps, which are not seen in first-year needles.

25 Kilometers From Mill

First-Year Needles

In inner mesophyll layers, the needle cells have more varied conditions. Thus, the cells with granular tannin in their vacuoles are adjacent to cells containing granular and ribbonlike tannin in their vacuoles. Chloroplasts are occasionally small and dark, or in adjacent cells they can be larger, blurred, and of pale pink color. In some sections, large intracellular spaces are located close to endodermis and resin canals. The total necrosis of needle angles can often be observed: epidermis and hypodermis are destroyed, and only remnants of mesophyll cell walls can be distinguished.

Second-Year Needles

The needles seem to be heavily injured. Only some cells have the average rate of damage. The protoplast and walls of mesophyll cells are being destroyed. In some needle sections, the adaxial side shows total necrosis; on the abaxial side of the needle, half of the mesophyll cells are at different stages of destruction. Tannin lumps can be found in some cells.

WINTER NEEDLES

Winter needles show a greater rate of injury. In general, the needle sections were found to have more destroyed mesophyll cells as compared to those of fall needles.

Chloroplasts are very large; this is likely due to their strong swelling. Needle surface also seems to be greatly destroyed. Ruptures run through the epidermal and hypodermal layers towards the mesophyll. Such needles can hardly survive winter frosts.

CONCLUSIONS

Cytological analysis revealed the disturbance of normal seasonal changes in the mesophyll of pine needles in the vicinity of the Kostomuksha ore-dressing mill (KODM). Healthy cells were found to have the normal structure characteristic of the fall-winter season with large tannin granules and light cytoplasm.

The first stage of damage is characterized by darkened cytoplasm, large tannin granules, along with a thin ribbon along the margin of the central vacuole. At the second stage of damage, the cells contain tannin in the form of a thick ribbon and very large scattered granules, or large lumps (in the second-year needles). "Empty" cells show the third stage of damage. Tannin is seen as a thick ribbon or often completely disappears. Cytoplasm also disappears or is difficult to detect. This stage appears to be final and is followed by complete cell destruction.

During winter dormancy, mesophyll cell structure is characterized by the growing amount and condensation of tannin to large granules (Anttonen and Karenlampi 1996; Palomaki and Ratio 1995; Soikkeli 1978, 1980). The ribbonlike state of tannin goes along with cellular membrane disturbances and is a sign of inadequate hardening. An increased amount of tannin is thought to influence the frost resistance of cells: tannins may significantly affect the vacuole water potential by changing its osmotic potential. Experiments with the use of different molarity of fixative buffer on pine needles showed the dependence of tannin structure on cellular osmotic potential (Soikkeli 1980).

Seasonal changes in tannin condition during fall-winter and spring-summer are thought to be connected with fluctuations in osmotic pressure in the needles in the summer and winter (Huttunen *et al.* 1981). Osmotic potential is determined by the tonoplast condition; active transport of ions and molecules into the vacuole is taken as its function. Changes in the tannin structure can indicate the structural and functional alterations in tonoplast.

Sulphur dioxide affects the structure of cellular membranes and their functional activity (Malhotra and Hocking 1976). SO₂ results in the condensation of tannin to large granules as well as ribbon on the vacuole margin (Soikkeli and Tuovinen 1979, Sutinen and Koivisto 1992).

Air pollution with SO₂ around the KODM is thought to cause the abnormal tannin accumulation in pine needle mesophyll cells by altering tonoplast properties, which is followed by changes in vacuole osmotic potential.

Chloroplasts in injured mesophyll cells are very large, light in color, and blurred; in other cells, they are small and dark. Obviously, there is swelling of plastid membranes and accumulation of light plastoglobuli in stroma of chloroplasts in some cells, which is typical for needles affected by sulphur dioxide (Soikkeli and Tuovinen 1979, Sutinen and Koivisto 1992). In these cells, large plastids are situated along cell walls, as is their summer arrangement. However, grouping of plastids and their translocation into the cell corners is typical for intact autumn-winter needles (Anttonen and Karenlampi 1996; Palomaki and Ratio 1995; Soikkeli 1978, 1980).

Structural analysis of the winter needles revealed increased damage as compared to the fall needles. Our results showed that the impact of the KODM pollutants on pines reduces the tolerance of needle tissues to low temperatures by winter hardening disturbance.

Heavy damage of the needle cuticle and disturbance rates of surface and internal needle tissues show that pollutants penetrate into the needles through stomata and cuticles. Tannin and lipid deposits in parenchyma cells of vascular bundle indicate that vascular tissues participate in the removal of excessive pollutants from needles. Pine needles collected near the KODM are thought to suffer from SO₂ impact along with acid rain and natural stress factors such as winter frosts.

ACKNOWLEDGMENT

The author is grateful to: Valery A. Isidorov, Department of Analytical Chemistry, and Vsevolod V. Polevoi, Department of Plant Physiology, St. Petersburg University.

LITERATURE CITED

- Anttonen, S.; Karenlampi, L. 1996. Slightly elevated ozone exposure causes cell structural changes in needles and roots of Scots pine. *Trees*. 10: 207-217.
- Huttunen, S.; Karenlampi, L.; Kolari, K. 1981. Changes in osmotic potential and some related physiological variables in needles of polluted Norway spruce (*Picea abies*). *Annals of Botany Fennici*. 18(1): 63-71.
- Malhotra, S.S.; Hocking, D. 1976. Biochemical and cytological effects of sulphur dioxide on plant metabolism. *New Phytology*. 76: 227-237.

- Palomaki, V.; Ratio, H. 1995. Chemical composition and ultrastructural changes in Scots pine needles in a forest decline area in southwestern Finland. *Trees*. 9: 311-317.
- Soikkeli, S. 1978. Seasonal changes in mesophyll ultrastructure of needles of Norway spruce (*Picea abies*). *Canadian Journal of Botany*. 56(16): 1932-1940.
- Soikkeli, S. 1980. Ultrastructure of mesophyll in Scots pine and Norway spruce: seasonal variation and molarity of fixative buffer. *Protoplasma*. 103: 241-252.
- Soikkeli, S.; Tuovinen, T. 1979. Damage on mesophyll ultrastructure of needles of Norway spruce in two industrial environments in central Finland. *Annals of Botany Fennici*. 16(1): 50-64.
- Sutinen, S.; Koivisto, L. 1992. Cell injuries in the needles of Scots pine (*Pinus sylvestris*) in Finnish Lapland. A light and electron microscopic approach. Proceedings, symposium on the state of the Environment and Environmental Fennoscandia and the Kola Peninsula; 1992 October 6-8; In: Arctic Center Publications. 4: 232-234.

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