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Interactions of ozone and pathogens on the surface structure of Norway spruce needles

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Introduction

The plant surface is at the interface between the plant and its atmospheric environment. The cuticle is covered by an inert layer of epicuticular wax which protects the plant from unfavorable conditions, such as frost, drought, radiation and pathogens. It also acts as a barrier to air pollutants (Jeffree, 1986).

The epicuticular wax of Norway spruce current needles consists of small tubes forming an evenly dispersed wax structure. As a result of natural erosion of the needle's surface, the wax tubes agglomerate, first forming a reticulate and then a plate-like wax structure (Sauter and Voss, 1986). The life span of healthy needles of Norway spruce varies from 7 to 17 yr (Gunthardt and Wanner, 1982).

Exposure to air pollutants is known to alter the structure of epicuticular wax, resulting in erosion and increased stomatal occlusion (Huttunen and Laine, 1981; 1983; Crossley and Fowler, 1986). This study was undertaken to assess whether or not ozone is also a factor which induces changes in the surface structure of Norway spruce needles.

Materials and Methods

The ozone-fumigated needles of Norway spruce were obtained from 3 different fumigation experiments carried out in summers 1985 and 1986 by Dr. Jürg Bucher at the Swiss Federal Institute of Forestry Research in Birmensdorf, Switzerland, (1985, 1986) and by Dr. Georg Krause at the Landesanstalt für Immissionschutz des Landes Nordrhein-Westfahlen in Essen, F.R.G. (1986).

In the Swiss experiments 4 yr old spruce graftings were fumigated in open top chambers with 0, 100, 200 or 300 μg of ozone/m^3 of filtered air during 109 or 114 weekdays using a different spruce clone each year. In the German experiment, 7 yr old spruce seedlings were fumigated continuously in open top chambers with 0, 100, 300 or 600 μg of ozone/m^3 of filtered air for 40 d.

Samples were sputter-coated with gold–palladium using a Polaron 5100 sputter coater. Coated samples were studied and photographed with a Jeol JSM-35 scanning electron microscope (15 kV accelerating voltage, sample current 10–11 A, exposure time 100 s). Stratified micrograph (2 groups: erosion observed/not observed) material was statistically analyzed with an IBM computer using a two way frequency table (BMDP P4F-program). Observed injury type was separately cross-tabulated with the ozone treatment. The statistical analysis used in the program was the non-parametrical likelihood-ratio chi-square test.

Results

When studying the effects of ozone fumigation, a slightly promoted surface erosion could be detected in the wax structure in epistomatal chambers. Tubular wax covering the stomata was more often flat and solid under ozone exposure with concentrations higher than 200 μg. The change was observable at the edges of the stomata ($P = 0.0346$). The apparently newly crystallized small wax tubes covering the eroded area were typical of this type of injury (Fig. 1A and B).

Apart from the erosion of wax within stomatal chambers, an overall erosion of the epicuticular wax could be observed that

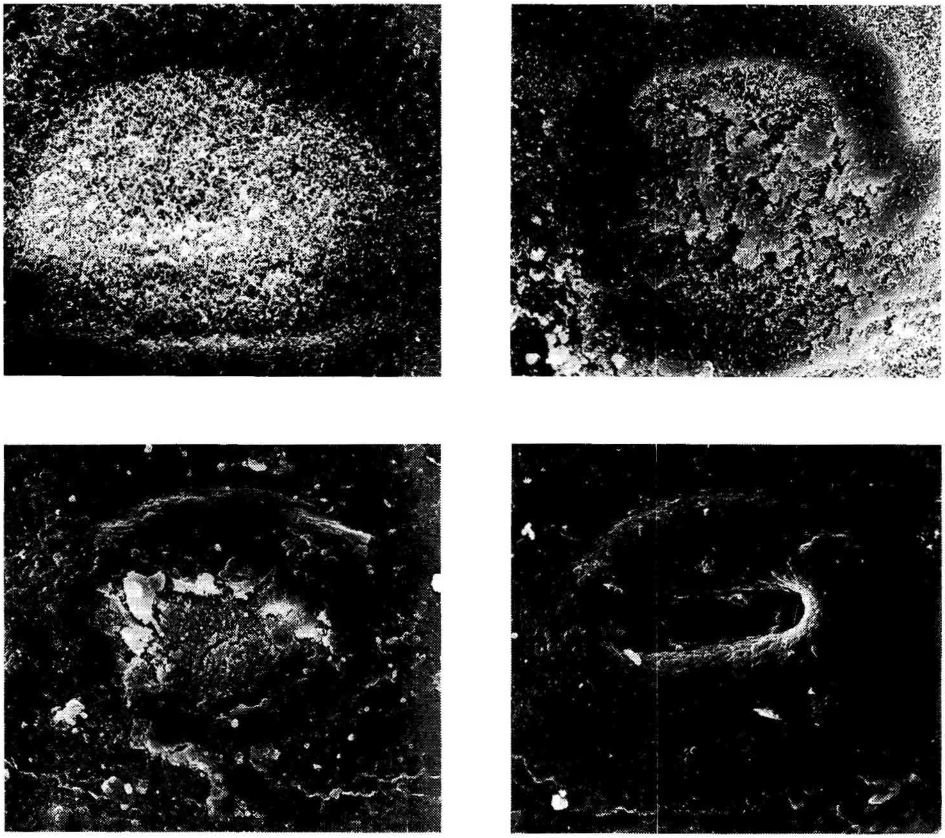

Fig. 1. A. Stoma of a healthy current yr needle is covered with an evenly dispersed wax structure. Clean air. **B.** Stoma of an ozone-fumigated spruce needle (300 μg/m^3). The tubular wax structure covering the stoma is flat and solid. Note the recrystallization of wax. **C.** Stoma of a fungus-infected control needle. **D.** Stoma of a fungus-infected needle that has been treated with ozone (600 μg/m^3 air). All magnifications 750x.

seemed to correspond to exposure to ozone. The healthy tubular wax structure, characteristic of the current yr needles, was less abundant in the ozone-fumigated needles. This can partly be associated with the fungal pathogens that were observed on the needles. The erosion-promoting effect of the fungal pathogens was observed to be faster and much more dramatic than that caused by air pollutants. Needles fumigated with ambient air and 100 μg of O_3/m^3 were the most infected, while only a few infected needles could be observed in the material that was fumigated with higher ozone concentrations. The surface structure of infected control samples was also more eroded than that of uninfected control needles (Fig. 1C and D).

Discussion

Air pollutants, especially S-compounds are known to alter the structure of epicuticular waxes of conifers (Cape and Fowler, 1981; Huttunen and Laine, 1981; 1983; Cape, 1983; Crossley and Fowler, 1986; Schmitt *et al.*, 1987). Also, natural erosion due to ageing causes chemical and morphological changes in the epicuticular waxes of the needles (Huttunen and Laine, 1983; Gunthardt-Goerg, 1986).

Erosion of the epicuticular wax, both natural and that caused by air pollution, probably increases cuticular transpiration (Cape and Fowler, 1981) and accelerates winter desiccation and needle shedding (Lewitt, 1980; Huttunen and Laine, 1983). Structural degradation of epistomatal wax tubes causes increased stomatal occlusion and potentially inhibits stomatal transpiration, which of course has far reaching physiological consequences on trees (Sauter and Voss, 1986).

In the long-term (2.5 yr) fumigation and simulated acid rain experiments carried out by Schmitt *et al.* (1987), ozone was not found to increase the erosion of the epicuticular wax of fir and spruce needles. Acid rain, however, caused severe surface erosion which was very much like that observed in our study. The ozone concentration used in the experiment carried out by Schmitt *et al.* (1987) was too low (100 μg) to cause significant surface erosion in the material analyzed in our study. Ozone concentrations higher than 200 $\mu g/m^3$ increased the surface erosion in the material analyzed in our study. Magel and Ziegler (1986) found wax plug disturbances in current needles of *Picea abies* after ozone and acid rain treatments. Since ozone concentrations of up to 180–190 $\mu g/m^3$ are known to be chronic in many areas and episodic in most densely populated areas (UBA, 1985; 1986), the fact that ozone can cause surface erosion in the needles is interesting at least as an intensifying factor in the erosion caused by other air pollutants.

In the current study, the surface changes were most severe in the needles with fungal infection in ambient air fumigation. The fumigation with higher ozone concentrations could be sterilizing. The effect of the ozone fumigation and fungal infection on the epicuticular wax structure seemed to be additive.

Ozone is known to affect the host–pathogen relationships between many plant and fungus species (Weidensaul and Darling, 1979; Dohmen, 1986). The interaction mechanisms between ozone and plant diseases are complicated and not very well understood, since the effects of ozone vary greatly with different plant and fungus species (Heagle, 1982). The fact that lower ozone concentrations might predispose conifers to fungal pathogens is also an important point with regard to stress factors.

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