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Studies on Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) needle cuticles

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Introduction

The structure of plant cuticular membranes and their permeability have been studied intensively (Holloway, 1982). Becker *et al.* (1986) studied the water permeability of plant cuticles and suggested that cuticles are primarily mobility barriers as far as water transport is concerned. They also showed that thin cuticles tend to be better and more efficient water barriers than thick cuticles. In dusted leaves, a naturally occurring clay significantly increased water loss when deposited onto cuticles of young leaves (Eveling and Bataille, 1984). However, the direct water, gas, ion and anion permeabilities of conifer needle cuticles are less known. Recently, Lendzian *et al.* (1986) and Heiska and Huttunen (1987) studied enzymatically isolated conifer needle cuticles.

Materials and Methods

Scots pine and Norway spruce needle cuticles were studied with SEM and TEM. For SEM studies, needles were covered with gold–palladium (45 nm) with sputter equipment (Polaron E 5100) and micrographed with a scanning electron microscope (Jeol JSM-35) at 15 kV and an exposure of 45 or 90 s. For TEM, the needles were prefixed in 2% glutaraldehyde in a 0.1 M phosphate buffer, then washed with the buffer and postfixed in 1% OsO_4 in the same buffer. Samples were dehydrated in an alcohol series and then embedded in Ladd's epon. The light microscope was used to evaluate the cuticles and to select areas suitable for electron microscopic evaluations with a Jeol JEM 100 CX II scanning–transmission electron microscope.

The enzymatic (4% pectinase, 0.4% cellulase) isolation of cuticles was made by a method adapted from Orgell (1955) and Schönherr and Schmidt (1979). The method (20% pectinase, 2% cellulase) developed by Lendzian *et al.* (1986) was also used.

The penetration of K^+ was studied using freshly isolated cuticles from young pine or spruce needles. The equipment used for penetration studies has been described by Heiska and Huttunen (1987).

The needle material studied so far comprised different populations of Finnish pines and spruces, some pine tree clones and seedlings following acid precipitation treatment, as well as some individual trees from polluted and clean forest environments.

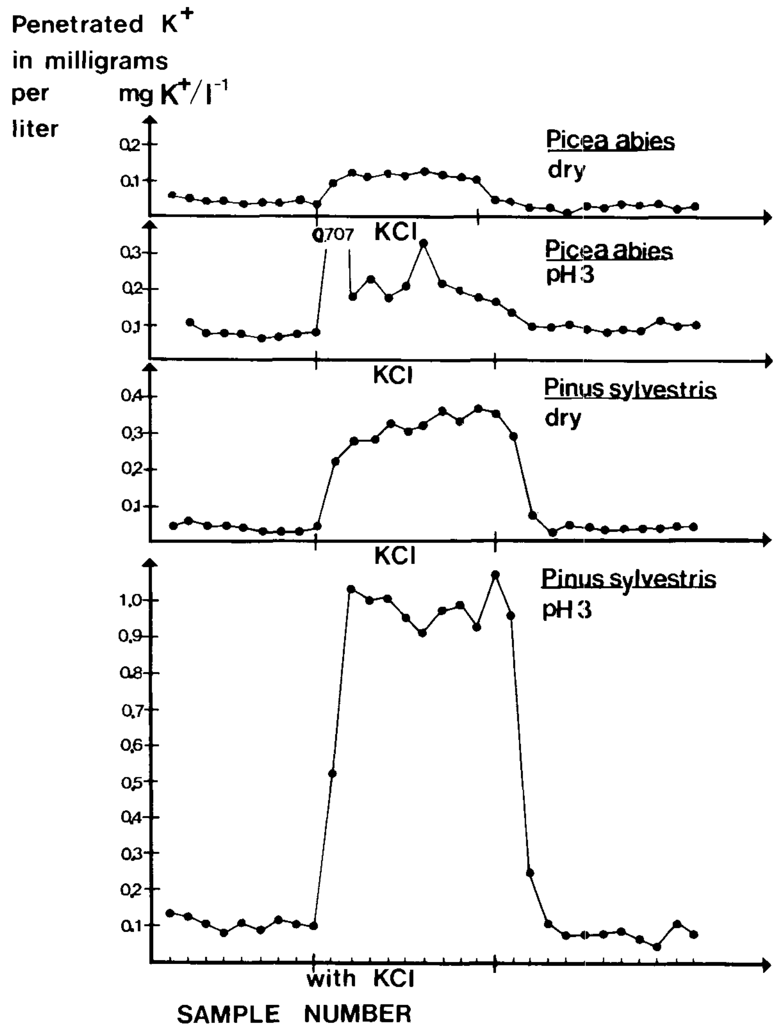

Fig. 1. The penetrated K^+ in mg/l (*y*-axis) through isolated needle cuticles. The *x*-axis indicates the sample number of the fraction with or without KCl. The cuticles from pH 3-treated needles have a many-fold penetration of KCl compared to untreated samples. The test was run with freshly isolated cuticles in July 1988. The needles were obtained from Lappish tree provenances, spruces from Sodankylä and pines from Rovaniemi and Kihlanki.

Results

The young needles of Norway spruce (*Picea abies* L. Karst.) develop in early June under northern Finnish conditions, the Scots pine (*Pinus sylvestris* L.) needles about 2 wk later. The development of a waxy cuticle takes a few weeks and the fully developed cuticle should be seen at the end of July. During the cold and rainy summer of 1987, the epistomatal waxes of *P. sylvestris* remained undeveloped. The young cuticles can be easily isolated from needles (*e.g.*, 3 d old pine needles, 8–15 d old spruce needles) with a short incubation period of 1–3 d. The young cuticles are very brittle and easily damaged. In young needles, the cutinization and lignification of epidermal cell walls are still incomplete and unstrengthened. In July, the incubation period needed for isolation of cuticles is about 7 d (20% pectinase, 2% cellulase) and these were used for penetration studies (Fig. 1). The KCl leaching from pH 3-treated spruce and pine needles was many-fold that of untreated dry spruce and pine needles when tested in early July. The cuticles used for penetration studies were also micrographed.

The needles of pine or spruce seedlings have less cutinized cells and the isolation of cuticle is easier than from adult trees. The needles in adult test trees around an industrial area have 'abnormal' cuticles, therefore causing many difficulties with respect to isolation studies. Structural changes and changes in water economy of needles have been observed.

On Lappish and northern Finnish pine needles, the extensive cutinization of both the anticlinal and inner periclinal walls of the epiderm was evident, which might be one reason for the poor isolation results. Over the central periclinal region, the thickness is about 1.3 μm and the cuticular layer traversed by greater cellulose microfibrils about 1.2 μm thick. The corresponding values for spruce cuticles varied from 0.5 to 1.5 μm and the layer with cellulose microfibrils was about 1 μm thick. The cuticles show a high degree of structural integrity.

Discussion and Conclusions

The foliar response of conifers to simulated acid rain has been ranked as less sensitive (Percy, 1987). However, the cuticular permeability and structural integrity have revealed a wide range of responses. The K^+ penetration through isolated cuticles of Norway spruce was lower than that of pine cuticles. This could be caused by needle age differences. The pine needles were about 20 d old, whereas the spruce needles were over 30 d old. An age difference of 10 d can be of importance in young needles. Percy (1987) found increased foliar uptake of ^{86}Rb at pH 2.6 in one clone of Sitka spruce. Ion mobility within leaves or needles was only affected at pH 2.6. Our observations of increased K^+ penetration after acid rain treatment at pH 3.0 are similar. Damage to forest trees in northern areas has been attributed to the acid deposition and cold climate. The young needle development seems to be one of the phases most sensitive to acid rain.

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