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SHORT NOTE

Evidence for recrystallization of epicuticular wax on needles of *Pinus sylvestris*

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SUMMARY

Very young Scots pine needles were found to have a higher percentage of their peristomal rim surface area covered with amorphous wax than needles sampled 5 weeks later. This, in combination with the observation of wax crystalloids (rodlets) on top of amorphous wax crusts, is interpreted as evidence for wax recrystallization.

Key-words: air pollution, epicuticular wax, *Pinus sylvestris* L., recrystallization.

INTRODUCTION

The inherent instability of epicuticular wax, and continued recrystallization to maintain its three-dimensional structure have been hypothesized by Fox (1958), Schulze *et al.* (1989) and others, but conclusive evidence for recrystallization has to our knowledge never been presented. In a time-series of current and first year needles the amount of amorphous wax shows a strong increase, at the expense of 'crystalline' or 'structural' wax, irrespective of pollution stress (Thijsse & Baas 1990; Bačić *et al.* 1992). Others have interpreted the formation of amorphous wax crusts as an early biomarker for air pollution stress (e.g. Crossley & Fowler 1986).

In a pilot study on the effects of fumigation with air enriched with CO_2, O_3, NH_3 and various mixtures thereof on the epicuticular wax structure in young needles of *Pinus sylvestris*, we unexpectedly found strong evidence for recrystallization of amorphous wax.

MATERIALS AND METHODS

Five-year-old treelets of Scots pine were fumigated by various gas mixtures during a period of 5 weeks from 20 June until 25 July 1991. Controls were fumigated with purified ambient air. Young, still expanding needles were sampled 6 days before treatment, and—meanwhile fully expanded—needles were sampled 1 day after fumigation. All sampled needles were studied at high magnification with SEM (Fig. 1) following the procedures described by Bačić *et al.* 1992. Forty stomata were assessed in each of the 600 needles sampled for their structural epicuticular wax appearance.

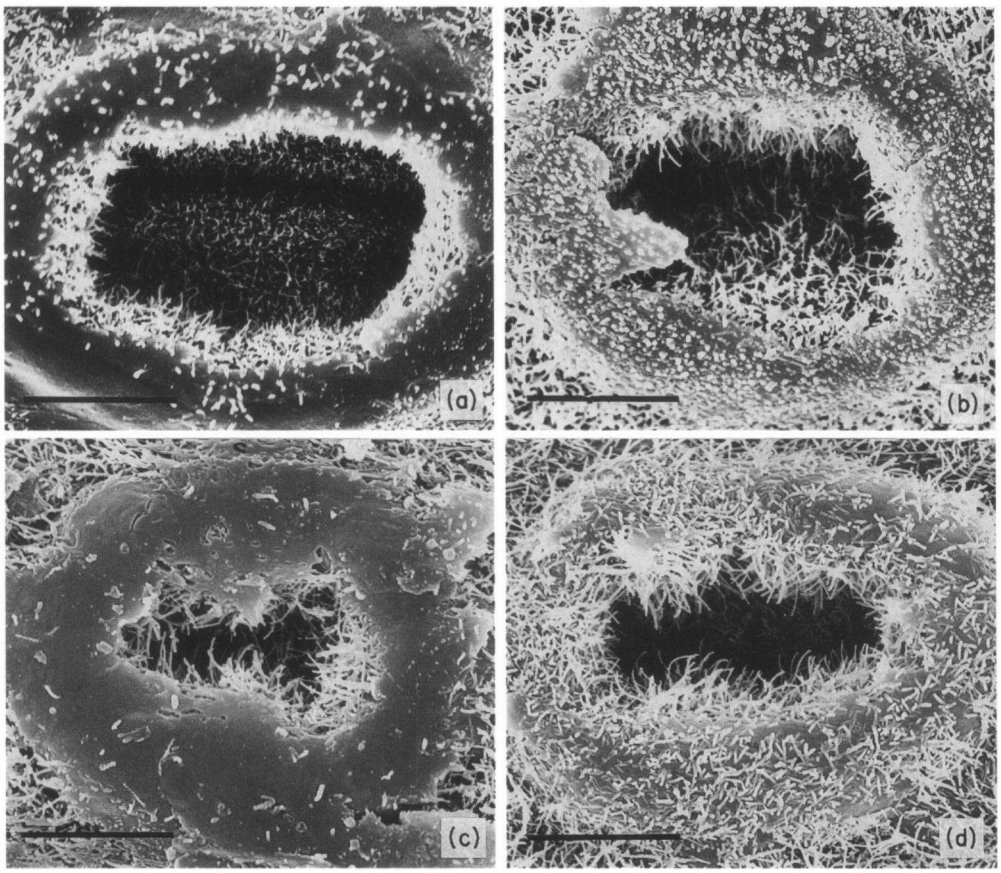

Fig. 1. Amorphous wax crusts on peristomal rims of *Pinus sylvestris* showing recrystallized wax rodlets on their surface. Scale bar = 10 μm. (a)–(b) An initial (a) and advanced (b) stage of recrystallization of the needles collected before fumigation. (c)–(d) An initial (c) and advanced (d) stage of recrystallization of the needles after fumigation with CO_2 and O_3.

Quantification of the wax appearance was according to the five stages recognized in our earlier study of complete time-series (Bačić *et al.* 1992).

Stage 1: amorphous wax absent.
Stage 2: 1–20% of rim surface covered with amorphous wax.
Stage 3: 21–50% of rim surface covered with amorphous wax.
Stage 4: 41–80% of rim surface covered with amorphous wax.
Stage 5: 81–100% of rim surface covered with amorphous wax.

These stages were averaged per needle and per treatment, and subjected to an analysis of variance, followed by the Student's *t*-test (Lindgren & McElrath 1969).

RESULTS

The results showed no significant differences between the treatments with various pollutants and the controls, but the surface area covered with amorphous wax crusts had decreased over the 5-week period in all treatments. The average wax stage according to the above classification had significantly declined from 3·0 to 2·2 in the overall sample.

In the SEM images of peristomal rims, both before and after treatment, we regularly observed wax rodlets on the surfaces of amorphous wax crusts (Fig. 1). These observations, in combination with the decreased surface area covered by amorphous wax crusts over time is interpreted as evidence for recrystallization of amorphous wax in young, expanding needles of *Pinus sylvestris*.

Occasional decreases in amorphous wax in current year needles observed in our previous study (Bačić *et al.* 1992), could also be interpreted as evidence of recrystallization, but the main tendency for amorphous wax stages to increase with needle age (Thijsse & Baas 1990; Bačić *et al.* 1992) has probably masked the occurrence of recrystallization in previous descriptive studies. The extent to which recrystallinization occurs in needles of different ages, and whether it is effected by air pollution stress and other environmental factors, requires further study.

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