

EFFECTS OF AIR POLLUTION ON THE CHEMISTRY OF SURFACE WAXES OF
SCOTS PINE

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ABSTRACT. Surface waxes from Scots pine (*Pinus sylvestris*) trees from the same provenance, but growing at a polluted and an unpolluted site, were sampled for each year class of needles at least twice during the year. There was no change in the amount of wax per unit area over the lifetime of the needles, but less wax was obtained from trees at the polluted site. Quantitative thin-layer chromatography of the wax gave several components which decreased in amount with time. They were identified as long-chain alcohols and ketones, and comprised only a small proportion of the total wax. The rate of change was greater in polluted air than in clean air, and there was a strong correlation with measured contact angles for water droplets. The degradation in structure observed by scanning electron microscopy may be associated with particular chemical components of the surface wax.

1. INTRODUCTION

The surface waxes of leaves, and in particular the epicuticular waxes, form a barrier between the leaf and its atmospheric environment. The chemistry and morphology of these waxes have been studied for many plant species; a recent review by Baker (1982) summarizes the present state of knowledge. Both the formation and subsequent development of epicuticular waxes are sensitive to the external environment of the leaf. The physical form of the wax reflects both environment and chemical composition, and may be a good indicator of environmental change. Air pollutants form part of the external environment, and several studies have explicitly considered the influence of gaseous pollutants on wax morphology as observed by scanning electron microscopy (SEM) (Bystrom *et al.*, 1968; Grill, 1973; Percy and Riding, 1978; Huttunen and Laine, 1983; Riding and Percy, 1985; Crossley and Fowler, 1986).

There has been little or no published work on the effects of air pollution on wax chemistry. For Scots pine, the epicuticular waxes have been partially analyzed by Schütt and Schuck (1973) as a

function of needle age. They observed a greater proportion of longer-chain alkanes, alkyl esters and aliphatic alcohols in older needles, but it was not possible to show whether this was caused by increases in amounts of longer-chain components or by losses of the shorter-chain components. Indirect evidence of chemical changes has come from studies of the wettability of needle surfaces, as measured by the contact angle of small water droplets (Leyton and Juniper, 1963; Cape, 1983). The study reported here is a preliminary chemical analysis of the same material collected for the contact angle measurements described by Cape (1983).

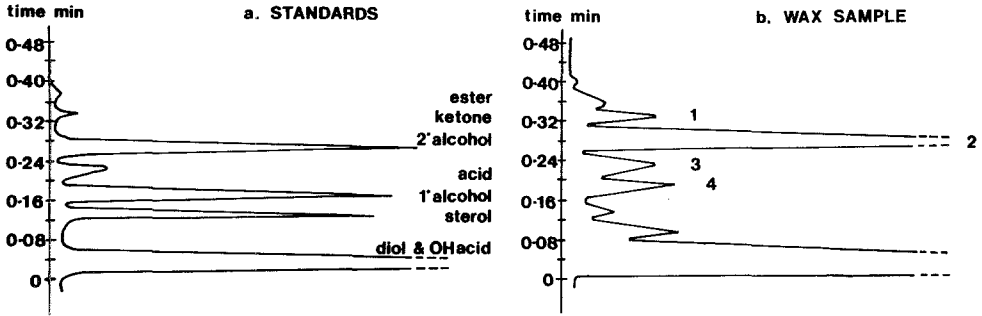
2. METHODS

Details of the 4 sites in the study are given in Cape (1983). Material from 2 of these sites was used for chemical analysis: Saltoun, a clean-air site in S.E. Scotland (annual mean SO_2 concentration $\sim 5 \mu\text{g m}^{-3}$) and Halifax (Gorple), a polluted site in W. Yorkshire, N. England (annual mean SO_2 concentration $\sim 40 \mu\text{g m}^{-3}$). The IUFRO standard for damage to conifers is in the range 25-50 $\mu\text{g SO}_2 \text{ m}^{-3}$, but at the polluted site, concentrations of NO_x and suspended particles were also much greater than at the clean-air site. The Scots pine trees at both sites were from the same provenance.

Foliage samples were taken towards the end of July and again at the end of November 1981 from each year class of needles. Shoots were taken from 5 trees at each site, from the fourth or fifth whorl. At the laboratory, batches of 1,000 needles from each sample (5 for each needle age) were shaken for 10s with 200 mL of A.R. chloroform, then rapidly filtered under vacuum and the solution reduced by vacuum distillation. Wash times between 5s and 2 min had no significant effect on the amount of wax removed from the needles. The surface area of each batch of 1,000 needles was estimated from the needle dry weight. An excellent linear relationship was found for sub-samples of 25 needles from each year class between needle dry weight and surface area (calculated from measurements of needle length, breadth and thickness).

Chemical analysis of waxes was performed using an Iatroscan. Examples of the use of this method in lipid analysis are given by Ackman (1981). In these experiments 0.5 μL drops of a chloroform solution ($100 \mu\text{g } \mu\text{L}^{-1}$) were developed in a solvent system of chloroform: toluene: acetic acid in ratios 60: 40: 0.3. Each wax sample was analyzed in triplicate, and quantitation was achieved by using standard solutions of model compounds, with multiple calibration points. Examples of a calibration analysis and a typical wax sample are shown in Figure 1.

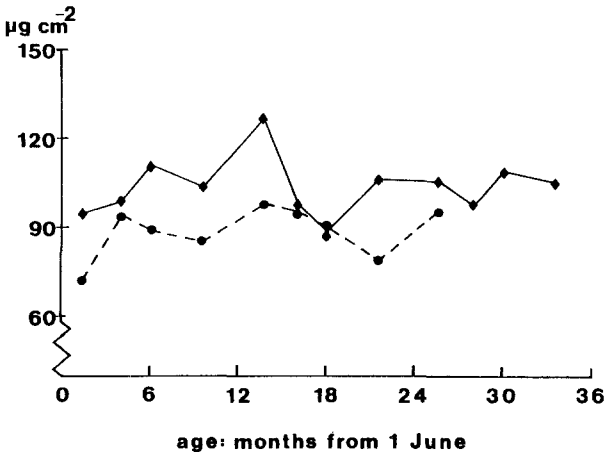
FIGURE 1. a) Calibration analysis (TLC/FID) of standards (0.5 μL of 100 $\mu\text{g } \mu\text{L}^{-1}$ solution).
 b) Typical analysis (TLC/FID) of wax (0.5 μL of 100 $\mu\text{g } \mu\text{L}^{-1}$ solution).



3. RESULTS

There was no significant change in the amount of surface wax removable by chloroform washing as the needles aged at either site. There was however a significant difference between the sites, with less wax at Halifax, the polluted site (Figure 2).

FIGURE 2. Variation in the amounts of wax per unit area ($\mu\text{g cm}^{-2}$) with time at Saltoun (clean air: \blacklozenge) and Halifax (polluted: \bullet).



In the subsequent chemical analyses at least 4 clear peaks were recognisable (Figure 1). These have been identified on the basis of their relative retention times as representing ketones, secondary alcohols, fatty acids and primary alcohols respectively. Peaks corresponding to alkanes were also sometimes observed, but were not easily quantifiable. The amounts of the individual components identified by chromatography form only a small proportion of the total, with initial values of around 2, 3, 4 and 15 $\mu\text{g cm}^{-2}$ for components 1-4, respectively, compared with average total wax of 104 $\mu\text{g cm}^{-2}$ at Saltoun and 91 $\mu\text{g cm}^{-2}$ at Halifax. However, only component 4 (primary alcohol) showed a significant difference between the 2 sites over the lifetime of the needles (Figure 3(c)). Of the groups of compounds all but the third (fatty acid) showed a significant decrease with time at each site (Figure 3). There was consequently a strong correlation (Table I) between the amounts of these components (expressed as $\mu\text{g cm}^{-2}$) and the contact angle of water droplets (Cape, 1983) on the needle surface.

TABLE I. Correlation between amounts of wax components and contact angle (Cape, 1983) for both sites together (11 samples)

COMPONENT	CORRELATION COEFFICIENT
1 (ketone)	0.879
2 (secondary alcohol)	0.755
4 (primary alcohol)	0.768

4. DISCUSSION

Washing with chloroform is an effective way of removing surface waxes from pine needles. The observation that there was no significant decrease in the amount removed as needles aged seems at variance with observations of marked structural changes in the form of the wax (Cape and Fowler, 1981; Huttunen and Laine, 1983; Crossley and Fowler, 1986). However, the mesh of fine rods observed by SEM covers only a small fraction of the needle area, around the stomata, and may be only a small proportion of the total wax removed by chloroform washing. It is not clear whether the differences between the 2 sites in amounts of surface wax are related to air pollution or to other factors such as climate or nutrient status. However, material from the other 2 sites in the original study (Cape 1983) showed a similar pattern of more wax at the unpolluted site.

The decrease with needle age in the amounts of components 1, 2 and 4 has been treated as a first-order decay process, following the earlier analysis of contact angle data (Cape, 1983). The straight lines in Figure 3 show the relationship of form:

$$\ln (\mu\text{g}/\text{cm}^2) = C - kt$$

where C is the (natural) logarithm of the amount of wax at time zero (1 June) and k is a first-order decay constant with units mo^{-1} . The coefficients of the lines are shown in Table II. Only for component 4 is the initial amount (C) significantly different between sites. However, there is a marked difference in the decay constants (k) for all 3 components between the polluted and unpolluted sites. This suggests that the 'natural' weathering of these components is accelerated in polluted air. The relative weathering rates for components 1 and 2 (ie a factor of 1.8 greater in polluted air) is similar to the factor of 2 observed for the rates of change in contact angle at these 2 sites (Cape, 1983).

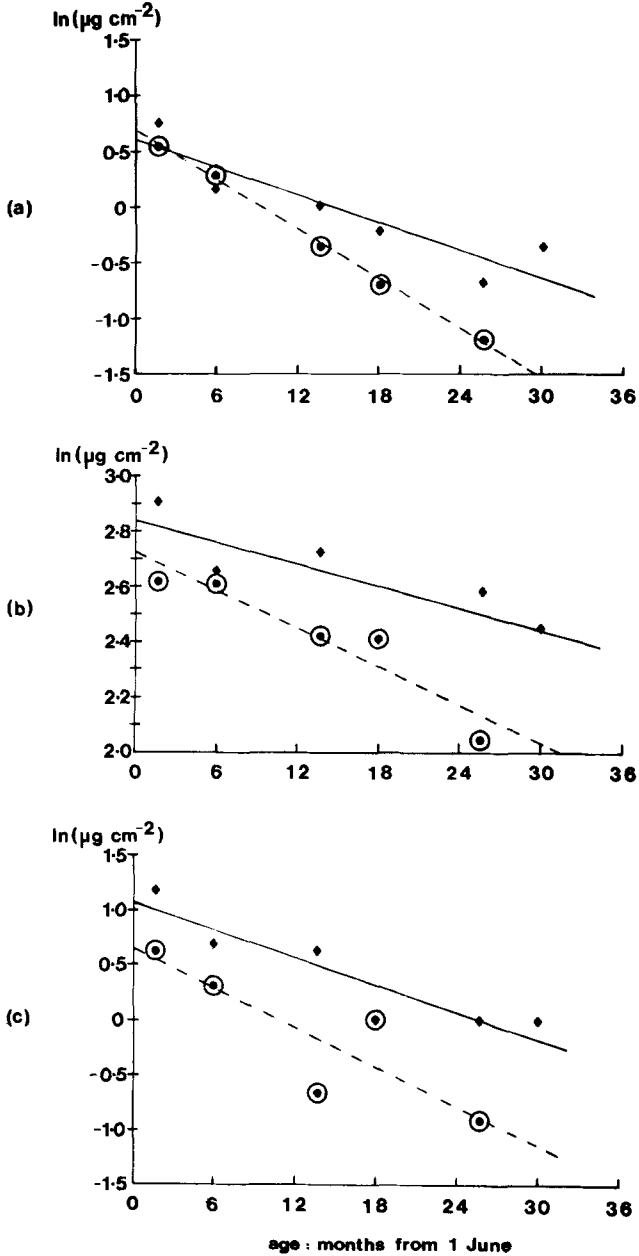
TABLE II. Equations of first-order decay process for components 1, 2 and 4 (Figure 3).

COMPONENT	SITE	INITIAL AMOUNT ($\mu\text{g cm}^{-2}$)	RATE ($\% \text{ mo}^{-1}$)
1 (ketone)	Saltoun	1.8	-4.1
	Halifax	2.0	-7.4
2 (secondary alcohol)	Saltoun	17.1	-1.3
	Halifax	15.2	-2.3
3 (primary alcohol)	Saltoun	3.0	-4.2
	Halifax	1.9	-6.0

It has been suggested (Baker, 1982) that rods or tubes which characterize the form of the wax around the stomata in Scots pine are associated with ketones and secondary alcohols. If this is so, the present observation of a decrease in amounts of these components fits well with the structural degradation observed by SEM. The effects of these changes on the measured contact angle of water droplets would also be expected, given the dependence of this parameter on both the chemistry and structural form of the surface.

Although there is now evidence that physical changes in wax structure may be related to the loss of certain (albeit minor) components of the surface wax there is no evidence of a specific chemical effect which would identify the causal agent(s). However, the present measurements suggest that the shift towards a greater proportion of longer-chain homologues observed by Schuck (1972) and Schütt and Schuck (1973) may have resulted from the preferential loss of the shorter-chain compounds, rather than from a shift towards biosynthesis of longer-chain homologues in older needles.

FIGURE 3. Variation in the amounts of wax components with time at Saltoun (clean air: \blacklozenge) and Halifax (polluted: \odot)
 a) component 1 (ketone)
 b) component 2 (secondary alcohol)
 c) component 4 (primary alcohol)



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