Ann. Bot. Fennici 33: 265–273
Helsinki 13 December 1996 ISSN 0003-3847

Epicuticular wax structure of Norway spruce (*Picea abies*) needles in Estonia. Variability in naturally growing and cloned trees

Andres Koppel & Katrin Heinsoo

Koppel, A., Institute of Zoology and Botany, Riia 181, EE-2400 Tartu, Estonia
Heinsoo, K., Institute of Botany and Ecology, Tartu University, Lai 40, EE-2400 Tartu, Estonia

Received 5 June 1995, accepted 20 September 1996

The structure of epicuticular wax of Norway spruce (*Picea abies* (L.) Karsten) needles from 12 localities on the Estonian mainland was studied by SEM in 1988. Rapid degradation of tubular wax into flattened plate-like structures was observed in all sites. Even during the first growing season degradation was rapid in three localities, two of which are situated far from the local sources of air pollution. In 1993 epicuticular wax structure was studied in cloned young Norway spruces planted in 10 localities. Despite the different exposure of the study sites to air pollution, the rate of wax degradation from tubular into flattened structures in the sites was similar. However, the degradation speed in naturally growing trees was much slower in 1993 than in 1988. Great variability in wax structure within a sample and even within the same needle was found, despite the genetically identical material used for the analysis.

Key words: air pollution, cloned trees, epicuticular wax, *Picea abies,* variability

INTRODUCTION

All four sides of needles in Norway spruce (*Picea abies* (L.) Karsten) are covered with a layer of epicuticular wax of variable density and ultrastructure. The highest concentration of epicuticular wax can be found on the stomata (Huttunen 1994).

Wax ultrastructure changes with needle age and under the influence of various environmental factors. In young needles wax is of a tubular type (Jeffree *et al*. 1971, Huttunen 1994). During ageing the initially tubular wax undergoes both structural and quantitative changes. Fine-structured wax formations fuse, forming flakes and platelets. At the same time the chemical composition of wax changes while its amount decreases (Günthardt-Goerg 1986).

This naturally occurring wax degradation is accelerated in polluted air. Degradation can be caused by various pollutants: acid rain (Huttunen & Laine 1983, Riding & Percy 1985, Rinallo *et al*. 1986), nitrogen oxides (Friedland *et al*. 1984), ozone (Ojanperä & Huttunen 1989), traffic exhaust gases (Sauter *et al*. 1987), magnesium oxide (Bermadinger *et al*. 1988), fluor compounds (Blighy *et al*. 1973).

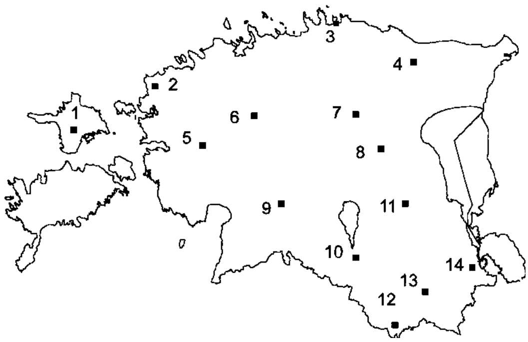

Fig. 1. Location of the study sites. — 1: Putkaste. 2: Nõva. 3: Käsmu. 4: Kiviõli. 5: Vigala. 6: Väätsa. 7: Rakke. 8: Voore. 9: Tipu. 10: Aakre. 11: Tartu. 12: Alska. 13: Haanja. 14: Värska.

Wax degradation can increase needle wettability (Cape 1983, Heinsoo 1994). Increased wettability, in turn, prolongs the contact time of water droplets on the needle surface, which can increase both wet deposition of pollutants (Lendzian 1984) and nutrient leaching from needles (Mengel *et al.* 1987).

The impairment of epicuticular wax can enhance cuticular transpiration. In coniferous trees, an increase in cuticular transpiration may be of crucial significance in winter and especially in early spring (Tranquillini 1976, Mengel *et al.* 1989, Koppel & Heinsoo 1994). If the soil and the conductive elements of the tree are frozen, transpirative water loss cannot be compensated, and the needle water content may drop below the level at which sublethal or even lethal damages occur. Winter desiccation may occur both near the alpine timberline (Baig & Tranquillini 1980) and in sites of polluted air (Sauter & Voß 1986).

The structure of epicuticular wax, revealed by SEM, has been used for the bioindication of atmosphere pollution (e.g. Huttunen 1994, Turunen *et al.* 1994). However, the results are controversial. Some authors have found good correlation between the speed of wax degradation and atmosphere pollution (e.g. Huttunen & Laine 1983, Sauter *et al.* 1987, Tuomisto 1988, Turunen & Huttunen 1990). Other authors have not detected the expected correlation between wax structure and the overall air pollution level (Hellqvist *et al.* 1992).

There might be two basic explanations of these contradictory results. One cause is that wax degradation depends not only on the pollution load but also on different climatic factors, e.g. on radiation (Baker 1982, Kim 1985), wind abrasion (Tranquillini 1976, Crossley & Fowler 1986), which may shade the effect of pollution. Besides climatic factors that may modify the speed of wax degradation, the wax structure has pronounced biological variability. Wax structure and the speed of its degradation differs significantly in different conifer species, subspecies and even in different individuals (Hanover & Reicosky 1971). Another reason for the lack of correlation between air pollution and epicuticular wax structure can be the natural variability of wax in naturally growing trees with a different genetic background.

The aim of the present study is to compare the structure of the epicuticular wax of Norway spruce in different parts of Estonia and to analyse the use of wax structure as an indicator of the air pollution level. Another objective is to evaluate wax degradation as a bioindicative parameter by using cloned Norway spruce seedlings, thus eliminating genetic variability in wax amount and structure.

MATERIAL AND METHODS

Naturally growing trees

The material was collected in the middle of November 1988 from 12 sites in Estonia (Fig. 1, Table 1). The samples were collected from the northern sides of open-grown trees of about the same age (40-year-old trees), at the height of 2 m from the ground. The samples were taken from needles of four age classes (current-year needles (C), previous-year needles (C+1), C+2 and C+4 needles). Since in Kiviõli and Aakre the oldest needle class was lacking, C+3 needles were studied.

Cloned trees

In 1988 four-year-old cloned spruces were planted in 10 different localities over Estonia (Fig. 1, Table 1). The goal was to represent areas of the country where different air pollution situations may occur. The plant material originates from the plant nursery of the Estonian Institute of Forestry and Nature Conservation. Cloning was done by grafting in 1984. Test trees were planted in open sites as close as possible to those used in 1988. In 1993 the sample trees had uniform and well-developed crowns, no grafting disturbances were detected. The trees had symmetrical crowns that did not differ from those of seed-grown trees of the same age. The height of the trees was 1.0–1.5 m.

Needle samples were collected at the end of November 1993. The samples were taken from the northern side of the crown from current-year to four-year-old needles. Three trees were sampled from each site except for the Värska site where two sampling trees are growing and only one of them had living C+3 needles. Two needles from the central part of the leading shoot were sampled from each year-class.

Thin epidermis samples were cut with a razor blade from the anatomically upper sides of fresh needles, mounted on sample stubs and dried for 24 h in a desiccator over $CaCl_2$.

In order to provide comparison for the Estonian material and to evaluate the resolution of the method used, needle samples were taken from a site in Northern Sweden devoid of any impact of local air pollution sources (Flakaliden Research Area, Vindeln Research Station, 64°07´N, 19°27´E, altitude 310 m a. s. l.) and treated in the same way in August 1993. Here the current-year needles of a 30-year-old tree were studied. As the experiment revealed, sample preparation and metal covering methods permitted to resolve the fine tubular structure of epicuticular wax (Fig. 4). The samples were covered with gold using JEOL Fine Coat Ion Sputter IFC-1100, the distance from the cathode being 20 mm in 1988. The samples were located directly under the cathode. In order to decrease the possible thermal effect on wax the distance between the cathode and sample stubs was increased to 40 mm in 1993, the stub surface being inclined at 60° in relation to the cathode surface. After covering with metal the samples were examined in the scanning electron microscope BS 300 (Tesla) with the acceleration voltage 15 kV and magnification × 2 500 in 1988, and with the acceleration voltage 17 kV and magnification × 3 000 in 1993.

In 1988 at least five typical microphotos were made from each sample. Wax structure was evaluated on the photos independently by both authors according to the 5-point scale, and the results were averaged. We constructed an arbitrary scale of typical sample photos which represented gradual degradation of epicuticular wax. Our scale was the same which was used by Huttunen and Laine (1983) for Scots pine (*Pinus sylvestris* L.).

In 1993 wax structures were examined in 10 stomatal antechambers from each needle. In each site/year-class about 60 antechambers were examined. One typical antechamber per needle was photographed for subsequent independent control evaluation by the authors.

The following wax status classes were defined: 1 — epicuticular wax has a well-defined fine-tubular structure, 2 — fine wax structures prevail, about 25% of the wax consisting of flakes or platelets, 3 — tubular and flattened structures have the same proportions, 4 — amorphous structures prevail, some coarse tubular structures are present, 5 — only amorphous wax is found in antechambers, or wax is mostly degraded (Fig. 2).

Table 1. Characterization of the study sites. In parentheses: A — Sites where naturally growing trees were studied; B — Sites of cloned trees. Data on air pollution were taken from Frey *et al.* (1991)[a] and Roots *et al.* (1992)[b]. Data marked with an asterisk originate from sites within the range of 20 km from sites of needle analysis. NA — Data not available. NAP — No local sources of air pollution.

Site	General description	Annual deposition of sulphur (S) and calcium (Ca^{2+}), kg $ha^{-1}yr^{-1}$
1. Putkaste (B)	Plant nursery in the middle of a forest, NAP	24 (S)[a]
2. Nõva (B)	Small opening in the centre of a forest, NAP	NA
3. Käsmu (A, B)	Village in the Lahemaa National Park, NAP, 60–80 km from Helsinki, Tallinn and the industrial area of North-East Estonia	17* (S)[b] 8* (Ca)[b] pH = 6.9*[b]
4. Kiviõli (A, B)	Industrial area, 2 km from a large oil-shale chemical plant	547 (S)[b] 3 820 (Ca)[b] pH = 8.3[b]
5. Vigala (A, B)	Agricultural area close to a local road, NAP	13 (S)[a]
6. Väätsa (A)	Opening in the centre of a forest, NAP	NA
7. Rakke (A)	Agricultural area, 3 km from a lime plant	NA
8. Voore (A, B)	Open area, 3 km from a village, NAP	18 (S)[a]
9. Tipu (A, B)	Open field in the Soomaa National Park, NAP	13 (S)[a]
10. Aakre (A)	Opening in the centre of a forest, NAP	NA
11. Tartu (A, B)	Centre of the town, air pollution of complex nature (traffic, communal heating)	64 (S)[a]
12. Alska (A, B)	Small village in the Paganamaa Landscape Reserve, NAP	22 (S)[a]
13. Haanja (A)	Sparsely populated agricultural area, NAP	22 (S)[a]
14. Värska (A, B)	Opening in the centre of a forest, NAP	13 (S)[a]
Average for Estonia		17 (S)[a]

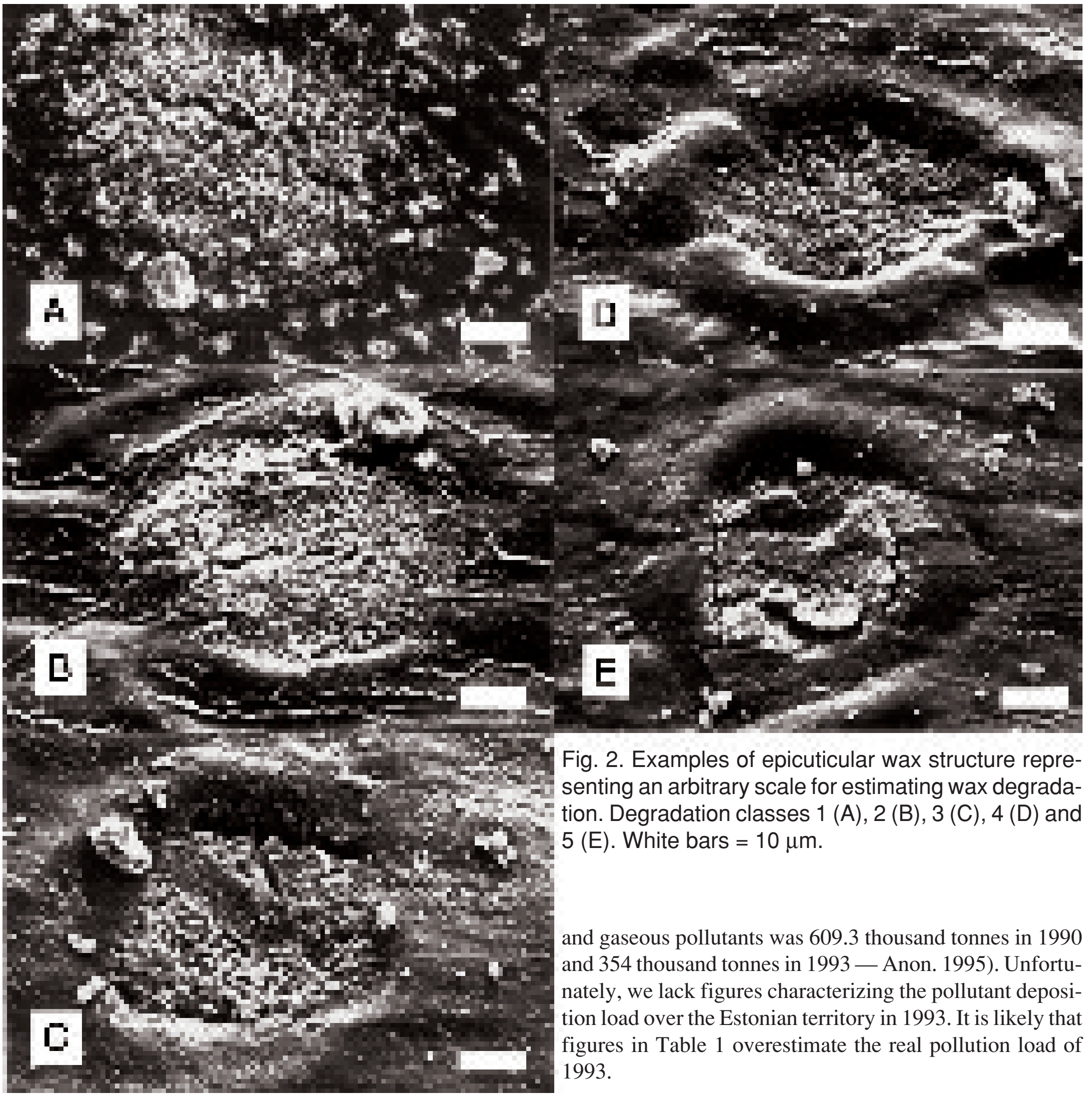

Fig. 2. Examples of epicuticular wax structure representing an arbitrary scale for estimating wax degradation. Degradation classes 1 (A), 2 (B), 3 (C), 4 (D) and 5 (E). White bars = 10 µm.

The data on air pollution in Estonia were collected from different sources (Table 1), which illustrates the highly variable situation over the whole territory of the country. While the majority of the sites represent the close-to-average deposition of sulphur (for 1986–1989 the average sulphur deposition for Estonia was 17 kg ha^{-1}yr^{-1} according to Frey *et al.* 1991), deposition in the industrial region and in the town of Tartu was much higher. The most conspicuous site was Kiviõli where 2 km from the oil-shale processing plant the annual deposition of both sulphur and calcium was very high, 547 and 3 820 kg ha^{-1}yr^{-1}, respectively. High alkaline deposition resulted in the alkaline reaction of precipitation. The consumption of both energy and motor fuel decreased almost twofold at the beginning of the nineties, compared with the second half of the eighties. This resulted in a decline of emitted air pollutants (total amount of emitted dust and gaseous pollutants was 609.3 thousand tonnes in 1990 and 354 thousand tonnes in 1993 — Anon. 1995). Unfortunately, we lack figures characterizing the pollutant deposition load over the Estonian territory in 1993. It is likely that figures in Table 1 overestimate the real pollution load of 1993.

RESULTS

Naturally growing trees

The results of our study indicate a rapid degradation of epicuticular wax in all studied sites in 1988. We did not find needles with undegraded epicuticular wax even among current-year samples in any of the sites (Table 2). In half of the sites epicuticular wax was entirely degraded even in C-needles. The biggest differences between the sites could be detected in young (C and C+1) needles, while in older needles uniformly strong wax degradation occurred in all sites.

Cloned trees

With a few exceptions (Tartu, Tipu), notable wax degradation occurred in the course of needle ageing (Tables 2 and 4). After three vegetation periods most of the tubular wax structures had changed into flattened structures. The degradation speed did not differ significantly in different sites.

The variability of epicuticular wax structure was significant even within a single needle. Typically, neighbouring antechambers had a similar or slightly different wax cover, however, in some samples, wax structure may differ drastically even between the closest antechambers (Fig. 3). Differences usually occurred between neighbouring stomata rows. In the same site within-shoot wax structure variability was similar to variability between trees (Table 3). The highest variability occurred in C-needles where both fine tubular and flattened amorphous wax structures could be found. The surface structures of older needles, usually lacking fine wax structures, are more uniform. In 1993 fine tubular wax structures, being

Table 2. The status of epicuticular wax of naturally growing trees in 1988 on a 5-point arbitrary scale.

	Needle age class				
Site	C	C+1	C+2	C+3	C+4
Käsmu	5	5	5		5
Kiviõli	4	4	5	5	
Vigala	2	4	4		5
Väätsa	2	3	4		5
Rakke	5	5	5		5
Voore	5	5	5		5
Tipu	2	4	4		4
Aakre	5	4	5	4	
Tartu	5	4	5		5
Alska	4	5	5		5
Haanja	4	3	5		5
Värska	2	4	5		5

Table 4. The status of epicuticular wax of cloned trees in 1993 expressed in points on an arbitrary 5-point scale. Average values of more than 60 estimates.

	Needle age class			
Site	C	C+1	C+2	C+3
Putkaste	3	4	4	5
Nõva	3	3	4	4
Käsmu	2	3	4	4
Kiviõli	2	2	5	5
Vigala	2	4	4	4
Voore	2	3	4	4
Tipu	3	3	4	3
Tartu	3	3	3	3
Alska	2	3	4	3
Värska	3	3	4	4

Table 3. Variability of epicuticular wax within the shoot and between trees from the same site (Käsmu) and clone, expressed in points on an arbitrary 5-point scale (± *S.E.*, the number of examined antechambers). The estimations were made in 1993.

Needle average age	Sample tree no.	1st needle	2nd needle	Average
C	1	2.0 (0.7, 12)	1.9 (0.2, 10)	2.0 (0.7, 22)
C	2	1.9 (0.6, 8)	1.6 (0.8, 11)	1.7 (0.7, 19)
C	3	1.7 (0.7, 9)	4.0 (0.0, 12)	2.9 (1.2, 21)
Average				2.2 (1.2, 62)
C+1	1	1.9 (0.5, 10)	2.4 (0.6, 13)	2.2 (0.6, 23)
C+1	2	2.8 (0.4, 8)	3.3 (0.6, 11)	3.1 (0.6, 19)
C+1	3	3.3 (0.8, 10)	3.4 (0.8, 15)	3.4 (0.8, 25)
Average				2.9 (0.9, 67)
C+2	1	3.7 (1.1, 17)	5.0 (0.0, 8)	4.2 (1.1, 25)
C+2	2	3.6 (0.5, 10)	4.7 (0.5, 9)	4.2 (0.7, 23)
C+2	3	4.0 (0.8, 10)	4.4 (0.6, 13)	4.2 (0.7, 23)
Average				4.1 (0.9, 71)
C+3	1	4.2 (0.8, 13)	3.5 (0.9, 12)	4.2 (1.1, 25)
C+3	2	3.5 (1.0, 10)	3.8 (1.1, 10)	3.7 (1.1, 20)
C+3	3	4.6 (0.6, 11)	4.4 (0.5, 10)	4.5 (0.6, 21)
Average				4.0 (0.9, 66)

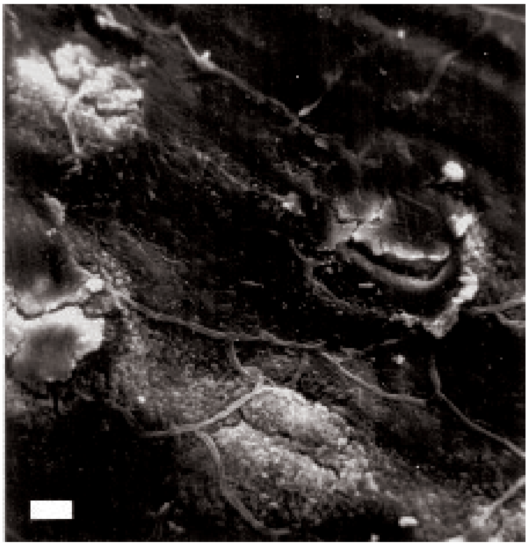

Fig. 3. Variability of wax structure in neighbouring stomatal antechambers. The degradation classes of wax cover on the stomatal antechambers: upper left — 3, upper right — 5, lower left — 4, lower right — 2. White bar = 10 µm.

very rare in 1988, were relatively widespread. However, they were not so fine-structured as in samples taken from Northern Sweden (Fig. 4).

The evaluation of wax structures involves a certain degree of subjectivity. Some antechambers are covered with almost uniform plate-like amorphous wax. When wax plates were damaged or removed during sample preparation, finer structures were revealed under removed flakes (Fig. 5). However, differences in wax structure as evaluated by two authors were rather small: only in 5% of the photos were points different.

On an average, wax structures were quite similar in different sites (Table 4). The biggest differences occurred in C-needles. At the same time, this age class displayed the highest within-shoot variability.

DISCUSSION

In 1985 we made the first pilot study of epicuticular wax structure on spruce from the Vooremaa site and found fine-structured tubular wax. Similar wax structures were lacking in the samples of 1988 originating from all parts of the country. This phenomenon may indicate an overall increase in atmospheric pollution in Estonia during the second half of the 1980s.

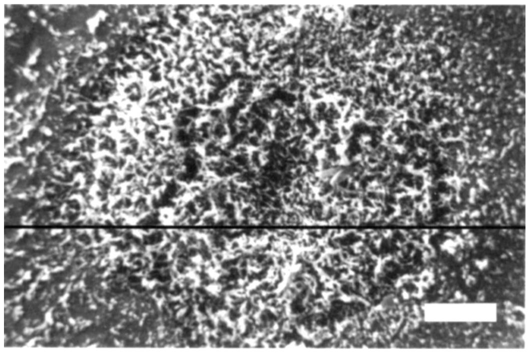

Fig. 4. Epicuticular wax in the stomatal antechamber of a current-year needle of spruce from Northern Sweden. White bar = 10 µm.

In three sites wax degradation occurred in needles of all year classes. Only one of these sites, Rakke, is situated close to a local industrial enterprise (a lime plant emitting alkaline lime dust). The other two sites are located far from local pollution sources. Voore is a typical East-Estonian village with sparsely lying farmhouses, Käsmu is a resort area in the Lahemaa National Park. Surprisingly, slightly less damaged needles were found in the Kiviõli site which is situated in the north-eastern part of the country. This is the area where numerous oil-shale industrial enterprises, including thermal power plants and chemical plants, are concentrated. The study site is located 2 km from a major oil-shale chemical plant. Here the high concentration of acid sulphur emission is neutralized by alkaline dust. Excess of calcium ions causes alkalinization of precipitation. Rapid wax degradation in the Tartu site was expected, since this locality is exposed to intensive air pollution caused by traffic and emission from local heating plants.

Unexpectedly rapid wax degradation occurred in the Alska and Aakre sites. Both these sites are situated far from any significant sources of atmospheric pollution. Sites with a relatively slower rate of wax degradation are situated in central (Vigala, Tipu, Väätsa) or Southern Estonia (Värska).

Since the overall rate of wax degradation was high all over the Estonian territory, changes in wax structure can be employed as bioindicators of air quality by using only young (C or C+1) needles. Due to the rapid degradation of wax, this method has a limited bioindicative value in our conditions. Moreover, since the method, based on the visual evaluation of wax structures is rather subjective, it is impossible to apply statistical methods to the results. One possibility to improve the method would be the use of computerized image analysis techniques.

Our study shows that even when using genetically identical plant material, thus excluding possible genetical variability, natural variability in the epicuticular wax of single Norway spruce needles is remarkable. Therefore, the method can be used only for the discrimination of drastically different pollution situations, whereas it can be too rough for the bioindication of smaller differences in air pollution. A possible reason for the controversial results obtained from studying wax degradation can be the different response of epicuticular wax structure in different species and the complex nature of environmental impact on wax formation and degradation. Huttunen and Laine (1983) analysed changes in wax structure in *Pinus sylvestris*, Sauter *et al*. (1987) followed the impact of a particular type of air pollution (pollution caused by motor transport) in *Picea abies*. In the present study trees were exposed to the impact of pollutants of various origin: heavy sulphur load combined with alkaline dust (Kiviõli site), traffic pollution (Tartu), long-distance transport of air pollutants in areas without any local sources of air pollution (all other sites).

The occurrence of fine tubular wax in 1993, which was not found in 1988, can be explained either by slightly different methods used in the metal coating of samples, by the different natural structure of epicuticular wax on cloned spruce or, more likely, by a decrease in air pollution in Estonia during the study period. After regaining independence, the Estonian economy has undergone dramatic changes. The use of fossil fuels, especially oil shale, has diminished, sulphate emission has dropped by 40% in 1992 as compared with 1980 (Anon. 1993), the amount of dust and gaseous emissions from stationary sources decreased almost twofold in 1993 compared with 1990 (Anon. 1995).

Since epicuticular wax structure is influenced by numerous environmental factors, including particle pollution, acid deposition and weather conditions during needle development and exposition (e.g. Huttunen 1994), a possible cause for different wax erosion rates in different regions of the country may lie in climatic differences. However, these differences are rather small over the Estonian territory, e.g. the length of the vegetation period does not differ by more than 10 days, climatic differences between different parts of the country are smaller than variability between years (Kivi 1976).

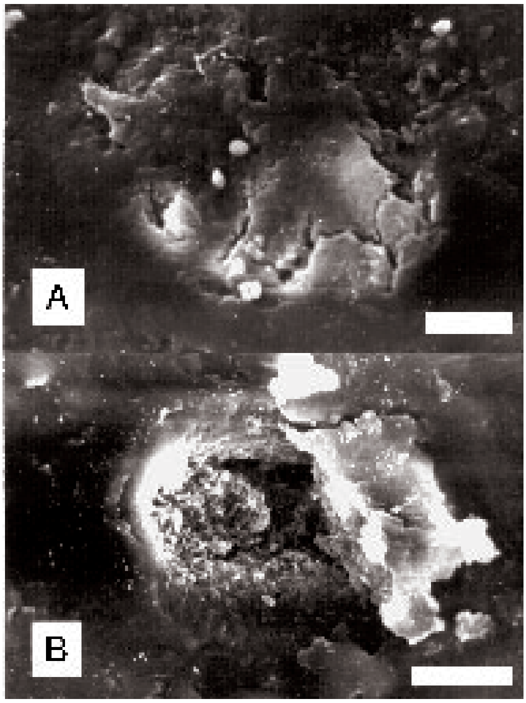

Fig. 5. Under the amorphous wax plate (A) fine tubular wax structures can be found (B). White bars = 10 µm.

One explanation for the lack of correlation between wax degradation and the overall air pollution level may be the relatively young age of the trees studied in 1993. The sample trees were 9 years old. We have not found any study dealing with changes in wax structure in relation to tree age. Another cause for the lack of correlation between wax structure and air pollution may be the small dimensions of the trees (the samples were collected at the height of about 0.5 m from the ground). And last but not least — long-distance transport of air pollutants may cause a uniform pollution background in Estonia so that spruce needles are almost equally damaged all over the Estonian territory.

Acknowledgements. We thank Dr. Tõnu Terasmaa (Estonian Institute of Forestry and Nature Conservation) for providing us with cloned spruce material, Dr. Märt Rahi (Institute of Zoology and Botany) for making SEM photos, Prof. Satu Huttunen (University of Oulu) for valuable comments on the early version of the manuscript and Mrs. Ester Jaigma for linguistic help. This project was partly supported by an individual grant of the Open Estonia Foundation to K. Heinsoo.

REFERENCES

Anon. 1993: Eesti energeetika. — Eesti Riiklik Energiaamet, Tallinn. 46 pp.

Anon. 1995: Estonian Environment. — Ministry of the Environment of Estonia, Environmental Information Centre, Tallinn. 112 pp.

Baig, M. N. & Tranquillini, W. 1980: The effects of wind and temperature on cuticular transpiration of Picea abies and Pinus cembra and their significance in desiccation damage at the alpine treeline. — Oecologia 47: 252–256.

Baker, E. A. 1982: Chemistry and morphology of plant epicuticular waxes. — In: Cutter, F., Alvin, K. L. & Price, C. E. (eds.), The plant cuticle: 139–165. Acad. Press, London.

Bermadinger, E., Grill, D. & Golob, P. 1988: The different influence of magnesite emissions on the surface waxes of Norway spruce and silver fir. — Canadian J. Bot. 66: 125–129.

Blighy, R., Bisch, A. M., Garrec, J. P. & Fourcy, A. 1973: Observations morphologiques et structurales des effects du fluor sur les eires epicutiaelaires et sur les chloroplastes des aiguilles se sapin (Abies alba Mill.). — J. Microscopie 17: 207–214.

Cape, J. N. 1983: Contact angle of water droplets of Scots pine (Pinus sylvestris) growing in polluted atmospheres. — New Phytol. 93: 293–299.

Crossley, A. & Fowler, D. 1986: The weathering of Scots pine epicuticular wax in polluted and clear air. — New Phytol. 101: 207–218.

Frey, J., Frey, T. & Rästa, E. 1991: Sademete saastatusest Eestis 1986–1989. — In: Frey, T. (ed.), Problems of contemporary ecology. Abstracts of the Estonian 5th Conference in Ecology: 25–29. Tartu.

Friedland, A. J., Gregory, R. A., Kärenlampi, L. & Johnson, A. H. 1984: Winter damage to foliage as a factor in red spruce decline. — Canadian J. For. Res. 14: 693–695.

Günthardt-Goerg, M. S. 1986: Epicuticular wax of needles of Pinus cembra, Pinus sylvestris and Picea abies. — European J. For. Path. 16: 400–408.

Hanover, J. W. & Reicosky, D. A. 1971: Surface wax deposits on foliage of Picea pungens and other conifers. — American J. Bot. 58: 681–687.

Heinsoo, K. (Хейнсоо, К. Т.) 1994: (Wettability of the needles as an indicator of air pollution). — Lesovedenie 3–4: 71–77. (In Russian.)

Hellqvist, S., Nyman, B. & Stadenberg, I. 1992: Epicuticular waxes, peroxidase activity and contents of chlorophyll, phaeophytin and carotenoids of needles from Pinus sylvestris and Picea abies as indicators of air pollution in the region of Stockholm. — Scandinavian J. For. Res. 7: 39–51.

Huttunen, S. 1994: Effects of air pollutants on epicuticular wax structure. — In: Percy, K. E., Cape, J. N., Jagels, R. & Simpson, C. J. (eds.), Air pollutants and the leaf cuticle. NATO ASI Series, Vol. G36: 81–96. Springer-Verlag, Berlin.

Huttunen, S. & Laine, K. 1983: Effects of airborne pollutants on the surface wax structure of Pinus sylvestris needles. — Ann. Bot. Fennici 20: 79–86.

Jeffree, C. E., Johnson, R. P. C. & Jarvis, P. G. 1971: Epicuticular wax in the stomatal antechambers of Sitka spruce and its effects on the diffusion of water vapour and carbon dioxide. — Planta 98: 1–10.

Kim, Y. S. 1985: REM-Beobachtungen immissionsbeschädigter Fichtennadeln. — Cbl. Ges. Forstwesen. 102: 96–105.

Kivi, K. 1976: Eesti NSV agrokliima ressursid. — Valgus, Tallinn. 142 pp.

Koppel, A. & Heinsoo, K. 1994: Variability in cuticular resistance of Picea abies (L.) Karst. and its significance in winter desiccation. — Proc. Estonian Acad. Sci. Ecol. 4: 56–63.

Lendzian, K. J. 1984: Permeability of plant cuticles to gaseous air pollutants: 77–81. — In: Koziol, M. J. & Whatley, F. R. (eds.), Gaseous air pollutants and plant metabolism: 77–81. Butterworths, London.

Mengel, K., Hogrebe, A. M. R. & Esch, A. 1989: Effect of acidic fog on needle surface and water relations of Picea abies. — Physiol. Plant. 75: 201–207.

Mengel, K., Lutz, H.-J. & Breininger, M. Th. 1987: Auswaschung von Nahrstoffen durch sauren Nebel aus jungen intakten Fichten (Picea abies). — Pflanzenernähr. Bodenk. 150: 61–68.

Ojanperä, K. & Huttunen, S. 1989: Ozone fumigation accelerates surface erosion on Norway spruce needle. — In: Bucher, J. B. & Bucher-Wallin, I. (eds.), Air pollution and forest decline. Proc. 14th Int. meeting for specialists in air pollution effects on forest ecosystems 2: 490–492. Switzerland, IUFRO.

Riding, R. T. & Percy, K. E. 1985: Effects of SO_2 and other pollutants on the morphology of epicuticular waxes on needles of Pinus strobus and Pinus banksiana. — New Phytol. 99: 555–563.

Rinallo, C., Raddi, P., Gellini, R. & Lonardo, V. 1986: Effects of simulated acid deposition on the surface structure of Norway spruce and silver fir needles. — European J. For. Path. 16: 440–446.

Roots, O., Saar, J. & Saare, L. 1992: Air quality above the territory of Estonia. — In: Air pollution in Estonia 1985–1990. Env. Rep. 3: 5–27. Helsinki.

Sauter, J. J. & Voß, J.- U. 1986: SEM-observations on the structural degradation of epistomatal waxes in Picea abies (L.) Karst. and its possible role in "Fichtensterben". — European J. For. Path. 16: 408–423.

Sauter, J. J., Kammerbauer, H., Pambor, L. & Hock, B. 1987: Evidence for the accelerated micromorphological degradation of epistomatal waxes in Norway spruce by motor vehicle emissions. — European J. For. Path. 17: 444–448.

Tranquillini, W. 1976: Water relations and alpine timberline. — In: Lange, O. L., Kappen, L. & Schulze, E.-D. (eds.), Water and plant life. Ecological Studies 19: 473–491. Springer-Verlag, Berlin.

Tuomisto, H. 1988: Use of Picea abies needles as indicators of air pollution: epicuticular wax morphology. — Ann. Bot. Fennici 25: 351–364.

Turunen, M. & Huttunen, S. 1990: A review of the response of epicuticular wax of conifer needles to air pollution. — J. Env. Quality 19: 35–45.

Turunen, M., Huttunen, S., Lamppu, J. & Huhtala, P. 1994: Characteristics and geographical distribution of the changes in Scots pine needle surfaces in Finnish Lapland and the Kola Peninsula. — In: Percy, K. E., Cape, J. N., Jagels, R. & Simpson, C. J. (eds.), Air pollutants and the leaf cuticle. NATO ASI Series, Vol. G36: 360–369. Springer-Verlag, Berlin.