CUTICULAR AND STOMATAL ANTECHAMBER CONDUCTANCE TO WATER VAPOUR DIFFUSION IN *PICEA ABIES* (L.) KARST.

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CONTENTS

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are further referred to by their Roman numerals

I Koppel AT, Vainikko KH. 1988. Variability and ecological significance of stomatal antechambers in Norway spruce. Lesovedenie. 4: 11–16. (in Russian).

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

III Heinsoo KH. 1994 Wettability of needles as an indicator of air pollution. Lesovedenie 4: 71–77. (in Russian).

IV Koppel A, Heinsoo K. 1996. Epicuticular wax structure of Norway spruce (*Picea abies*) needles in Estonia. Variability in naturally growing and cloned trees. Ann. Bot. Fennici 33: 265–273.

V Heinsoo K, Koppel A. 1998. Minimum epidermal conductance of Norway spruce (*Picea abies* (L.) Karst.) needles: influence of age and shoot position in the tree crown. Ann. Bot. Fennici (accepted).

VI Heinsoo K, Koppel A. 199X. Minimum epidermal conductance in Norway spruce varies within the branch (manuscript).

PREFACE

When water loss from leaves is discussed, usually water loss through opened stomata is considered. The regulation mechanisms of water movement through stomata are widely investigated. However, stomata represent the largest but not the only pathway of water vapour diffusion from leaves. In Norway spruce, stomata are sunken deep into the epidermis. The resistance of the space between guard cells and the epidermis may affect transpiration in case of fully opened stomata. However, its variability and influence on transpiration have not been adequately studied.

Even in case of closed stomata, there occurs continuous cuticular water loss from leaves. Cuticular transpiration is much lower than stomatal transpiration, but it may become dangerous for plants which have to withstand long periods of water deficit. Conifers are such kind of plants in our climate. In winter their needles lose continuously water and their roots are not capable of water uptake from the frozen soil. Air pollution has been supposed to affect needle surface and to enhance the danger of tree desiccation. Considering the above said, it appeared necessary to establish the variability of minimum water loss from spruce needles in the absence of air pollution and to evaluate the impact of different air pollution levels occurring in Estonia.

I started my research in 1984 studying the role of the cuticle in water economy. Within the past years a vast amount of new information has been published on this topic (Table 1). In several cases new results have pointed to shortcomings in the methods that were used earlier. During recent years more detailed research has been possible thanks to progress in technical equipment. In the present thesis, the latest information has been taken into account in the analysis of older results. Therefore, the conclusions drawn in the thesis need not always coincide with those published in earlier papers.

The thesis was completed during postgraduate studies at the University of Tartu, the only Estonian *alma mater*. I feel much obliged for the opportunity to study in my native language in a native university. Therefore, the major part of the thesis is published in Estonian as well. I feel deep gratitude to Gustav Vilbaste, who is the author of the bulk of botanical terms in Estonian, as well as my teachers.

LIST OF SYMBOLS

β	—	angle between the needle surface and the water droplet on it
ψ	—	water potential
A	—	ellipse length
A_1	—	length of the upper ellipsoid of the stomatal antechamber
A_2	—	length of the lower ellipsoid of the stomatal antechamber
B	—	ellipse width
B_1	—	width of the upper ellipsoid of the stomatal antechamber
B_2	—	width of the lower ellipsoid of the stomatal antechamber
C	—	current year needles
C+1	—	previous year needles
C+2	—	two-year-old needles
C+3	—	three-year-old needles
C+4	—	four-year-old needles
C+5	—	five-year-old needles
d	—	pore diameter
D	—	gas diffusion coefficient
DW	—	dry weight
g	—	needles conductance to water vapour diffusion
g(min)	—	minimum epidermal conductance to water vapour diffusion
G_s	—	stomatal conductance of a single pore to water vapour diffusion
1	—	tube depth
L_1	—	depth of the upper ellipsoid of the stomatal antechamber
L_2	—	depth of the upper ellipsoid of the stomatal antechamber
r_a	—	boundary layer resistance to water vapour diffusion
r_c	—	cuticular resistance to water vapour diffusion
r_{ch}	—	resistance of stomatal antechambers to water vapour diffusion per unit area
r_s	—	stomatal resistance to water vapour diffusion
R_{p1}	—	resistance of the upper elliptic pore of the stomatal antechamber to water vapour diffusion
R_{p2}	—	resistance of the lower elliptic pore of the stomatal antechamber to water vapour diffusion
R_{ch}	—	resistance of the stomatal antechamber to water vapour diffusion
R_{t1}	—	resistance of the upper truncated cone of the stomatal antechamber to water vapour diffusion
R_{t2}	—	resistance of the lower truncated cone of the stomatal antechamber to water vapour diffusion
RH	—	relative air humidity
RWC	—	relative water content (% of water at the beginning of experiment)

S — needle area
S_m — needle specific area (area per dry weight)
WC — water content (% per dry weight)

1. INTRODUCTION

Starting from the 1970s, a number of reports described extensive desiccation of conifers in several European countries where older needles dropped and the average age of needles decreased. This phenomenon was common in industrial areas and was associated with air pollution. Changes in needle water economy was presumed to be one of the causes of desiccation (e.g. Roberts, Blank 1984). It therefore became necessary to investigate diffusion of water vapour from needles and to study the value and variability of diffusion resistance components. The main diffusion pathway through the stomata of needles has been widely investigated. However, less is known about the resistance of stomatal antechambers. Braun (1977) demonstrated that the pollution-resistant grafts of *Picea abies* had longer, wider and heavier needles than the grafts influenced by air pollution. Consequently, various grafts may also reveal differences in the volume and diffusion resistance of stomatal antechambers. The variability of stomatal antechambers has not been studied so far.

The surface of needles is affected by external factors over many years. At the same time, it is a source of continuous water loss. According to its absolute value, cuticular transpiration in conifers is more than ten times smaller than stomatal transpiration (Larcher 1995). However, the plant loses its water through the cuticle continuously. In case of increased cuticular transpiration (e.g. due to air pollutants) or under unfavourable climatic conditions (e.g. during long winter when the trunk and roots are frozen) water loss may become lethal. Cuticular water loss has been studied in mountainous regions (e.g. Tranquillini 1979). Less attention has been paid to the natural variability of cuticular transpiration in forest conditions. Not much information is available about the influence of pollutants, occurring in the air, on the water loss of needles. Therefore, the current thesis is focused primarily on cuticular water loss from the needles of *Picea abies* with the purpose to find out its dependence both on various natural factors and air pollution. Besides, the possibility of tree desiccation due to winter water loss in Estonia has been analysed.

The rate of water vapour diffusion along the pathway of diffusion depends on the driving force (difference in vapour pressure) and on the resistance of diffusion. Resistance to water diffusion from leaves can be divided into several components according to the mechanism of water vapour diffusion and its regulation (Fig. 1). Since the flux of water vapour is linearly correlated with pressure differences, an analogy of Ohm's law can be used when calculating the values of whole resistance (Passioura 1984).

Boundary layer resistance (r_a) is caused by a stationary layer of air on the leaf surface. At wind velocities higher than 3...5 m s^{-1} the value of r_a is very low in most leaves (Larcher 1995). At lower wind velocities the value of r_a

depends on leaf morphology. The value of r_a is higher in large, hairy or grooved leaves. Due to the small size of conifer needles their r_a is small. In Norway spruce (*Picea abies*) the values of r_a about 1 s cm^{-1} at a wind velocity of 0.25 m s^{-1} and 0.3 s cm^{-1} at a wind velocity of 1 m s^{-1} have been measured (Koppel 1981). For *Picea pungens*, r_a value of 0.46 s cm^{-1} and for *Picea pungens*, 0.12 s cm^{-1}, have been found at a wind velocity of 0.35...1.5 m s^{-1} (Jarvis *et al.* 1976). Low values of r_a have only a limited influence on water diffusion from needles. Many grass species are able to control r_a by folding their leaves (Rahi 1971). Conifer shoots are unable to change r_a actively. However, sun-type conifer shoots with densely packed needles have higher r_a compared with shade-type shoots with sparsely packed needles.
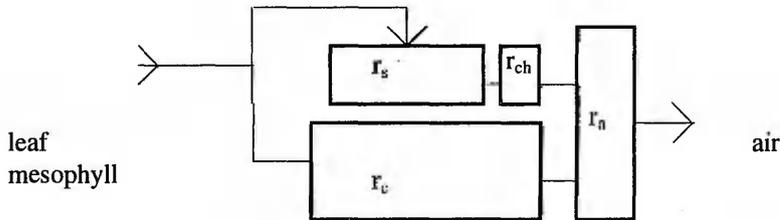
Figure 1. Resistance to water diffusion from leaves: r_a — boundary layer resistance, r_c — cuticular resistance, r_s — stomatal resistance, r_{ch} — resistance of stomatal antechambers.

Joonis 1. Lehest auruva vee difusioonitakistus: r_a — piirkihi takistus, r_c — kutikulaartakistus, r_s — õhulõhede takistus, r_{ch} — õhulõhe eeskambrite takistus.

Stomatal resistance (r_s) depends on the degree of stomatal opening as well as on the size of stomata and density of leaf surface area. This is the only part of transpiration resistance that can be actively controlled by the plant. Not all the stomata on a leaf are opened equally (Laisk *et al.* 1980; Larcher 1995). Most stomata are only partially opened even in optimal conditions. Relatively small size of the stomatal aperture is the most successful compromise between water loss and CO_2 uptake because of additional mesophyll resistance to the latter (Larcher 1995). Under severe water deficit stomata close to a maximum extent. However, in some cases the stomatal pores may remain incompletely closed due to dysfunction of closure mechanisms and the presence of algae, fungal hyphae or dust particles between guard cells (Grace 1990; Donnelly, Dowding 1994).

In several plant species (including most conifers) guard cells are sunken deep into the epidermis. The space between guard cells and the epidermis is called the stomatal antechamber. Antechambers form an additional component of resistance to water vapour diffusion (r_{ch}). The value of r_{ch} depends on the size of antechambers and their number per leaf area. In conifer species, stomatal antechambers are filled with epicuticular wax. It is difficult to estimate the resistance of wax formations in antechambers. Jeffree *et al.* (1971) suggest

that in *Picea sitchensis,* epicuticular wax increases the value of r_{ch} about twice. Since epidermis thickness in the sun and shade needles of conifers varies, the size of stomatal antechambers too may be different (Koppel 1981).

Cuticular resistance (r_c) is related to the cutinized layer of the outer wall of the epidermis, which in the majority of vascular plants reduces efficiently evaporation. Depending on the growth conditions of different species there are marked differences in the thickness and chemical composition of the cuticle. Evergreen leaves and conifer needles, which have to withstand periods of limited water uptake, have a thick cuticle and high values of r_c. The cuticle consists of hydrophobic cuticular lipids (SCL), which are soluble in a wide range of organic solvents, and an insoluble polymer, cutin (Riederer 1989; Holloway 1994). In most plant species, cutin includes a matrix reticulum of fine polysaccharide fibrillae. These polysaccharides often appear to be continuations of the structural elements of the underlying cell wall (Tenberge 1992) and are suggested to be the major pathway of water vapour diffusion (Kerstiens 1994; Riederer *et al.* 1994). In some species (including *Picea abies*) the reticulum does not reach the outer surface of the cuticle. This makes r_c of Norway spruce needles higher compared with that of the needles of some other conifers with a different cuticle structure (e.g. *Picea sitchensis*) (Kerstiens 1994).

In *Picea abies,* as in most other conifers, needle surface is covered with epicuticular wax. The highest concentration of wax can be found in and around stomatal antechambers. The quantity and chemical composition of epicuticular wax on *Picea abies* needles have been thoroughly studied (Günthardt-Goerg 1986). The quantity of epicuticular wax on a plant is genetically determined. Wax compounds are chemically very stable and are unlikely to react with air pollutants (Günthardt-Goerg 1994). Epicuticular wax is a final product of biochemical synthesis (Thair, Lister 1975). Tubular wax forms the rims of guard cell mother cells during the period of bud flush in spring (Günthardt-Goerg 1994). In the course of needle ageing the amount of epicuticular wax decreases. At the same time, the relative proportions of different compounds of epicuticular wax change (Günthardt-Goerg 1986). In *Picea abies* needles, the relative amount of the main component, nonacosan-10-ol, generally increases, whereas that of free T-hydroxy fatty acids decreases. The existence of hydrophobic components in the plant cuticle reduces the amount of water loss from needles.

The effect of the wax layer on cuticular permeability depends also on wax structure. Epicuticular wax structure changes with needle ageing and under the influence of various environmental factors. A large number of wax types have been distinguished using the scanning electron microscope (Tuomisto 1988; Trimbacher, Eckmullner, 1997). The newly formed amorphous wax layer in the stomatal antechamber of *Picea abies* needles consists mainly of long wax tubes (Tuomisto 1988). These tubes are assumed to be hollow (Jeffree *et al.* 1971; Riederer 1989). Wax tubules in *Pseudotsuga menziesii* are 2.3 µm long, with an

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outer diameter of 124...148 nm and wall thickness of 35...40 nm (Thair, Lister 1975). In *Picea sitchensis*, the length of the tubes is 1 µm and the diameter, 150 nm (Jeffree *et al.* 1971). During needle ageing, wax tubes fuse and form a net-like structure. This is followed by the formation of granules and crusts. Such a naturally occurring wax degradation is accelerated 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 (Bermandinger *et al.* 1988), and fluor compounds (Blighy *et al.* 1973). Epicuticular wax of fine structure prolongs the pathway of water vapour diffusion and has a greater influence on water loss compared with fused crusts with cracks.

Besides using electron microscopy, the status of the epicuticular wax layer has been studied also by an indirect method — measurement of the droplet contact angle (Fogg 1947). The contact angle of a water droplet on leaf surface depends on the chemical composition and roughness of the surface. Newly formed epicuticular wax structures are suggested to be rougher than the degraded ones. The contact angle of different epicuticular wax fractions may vary in a large range (Holloway 1970). The value of the contact angle can be measured from the enlarged image of the water droplet on the leaf (Fogg 1948; Holloway 1970). This method is easy to apply even in field conditions and is widely used in biomonitoring (e.g. Cape 1983; Turunen *et al.* 1994). The published values of the contact angle in case of several *Picea* species have been reviewed by Cape and Percy (1993). The value of the contact angle on needles may be different in various parts of the tree crown (Boyce 1994). Needle wettability increases with ageing (Boyce *et al.* 1991; Donnelly, Dowding 1994) and in polluted air (Cape, Percy 1993; Percy *et al.* 1993). Fumigation of needles with ozone and/or acid mist may also increase their wettability (Barnes, Brown 1990; Esch, Mengel 1994). Increased leaf wettability may in turn enhance the uptake of water soluble pollutants (Fuentes *et al.* 1994). However, the results of contact angle measurements should be interpreted with care. If needle surface below the wax layer is rough, wettability of the needle may decrease after wax removal (Jagels 1994). The physico-chemical properties of the particles deposited on needle surface may also affect the contact angle (Kisser-Priesack *et al.* 1987; Staszewski *et al.* 1994).

Direct measurement of cuticular water loss can be easily carried out on hypostomatous leaves. The abaxial side of a detached leaf is covered with water impermeable substance, which allows estimation of water loss from only the adaxial side of the leaf (e.g. Hoad *et al.* 1996). At the same time, isolated cuticles can be used to measure cuticular permeability to water vapour and other gases (e.g. Schönherr, Schmidt 1979; Scherbatskoy 1994). The latter method is less suited to amphistomatous leaves, since it is difficult to prepare their samples excluding stomata. Use of this method in case of needles is impossible due to their small dimensions. In amphistomatal leaves the value of cuticular permeability is usually estimated from the mass loss of detached and

desiccating leaves at some fixed temperature and air humidity (RH). Since in this case it is impossible to separate cuticular and stomatal components of water loss during desiccation, the terms "surface conductance" or "minimum epidermal conductance" have been proposed instead of "cuticular conductance" (Grace 1990; van Gardingen et $al.$ 1991). Calculation of minimum epidermal conductance — g(min) instead of resistance (g(min)=1/r$_c$) is employed owing to its linear relationship with transpiration intensity.

Mass loss curves of various conifer species have been studied by several authors in order to predict water loss from needles under various growth or air pollution conditions. Published values of minimum epidermal conductance for $Picea$ species are presented in Table 1. The values of g(min) vary to a great extent in different studies and depend on the method used. Most studies on conifers have been carried out by measuring the water loss of a detached shoot, while various periods have been used for the calculation of g(min). Moreover, during desiccation shoots have been kept at different air temperatures and RH.

Cuticle permeability may be affected by several environmental factors and pollutants. Unfavourable climatic conditions may impede cuticle maturation. For example, in mountains, the cuticle thickness of $Picea$ $abies$ needles in the wind-exposed treeline made up only 30% of that measured in trees in valley bottom (Baig, Tranquillini 1976). Incomplete maturing of the cuticle and/or exposure to the wind (van Gardingen et $al.$ 1991) increase g(min). Increased water loss may be a major factor determining the altitudinal treeline. In "Kampfzone" (zone of dwarfed and deformed trees between the timberline and the treeline) the soil is frozen deep and thaws late in spring. Plants cannot absorb soil water during a long period in winter, and their survival depends upon tissue water reserves and drought resistance. Trees with a poorly developed cuticle are unable to control imposed transpiration stress and may desiccate (Tranquillini 1979; 1982).

Huttunen et $al.$ (1981) demonstrated that in late winter the water potential of $Pinus$ $sylvestris$ needles was the lower the more sulphur had been deposited on its needles. According to Barnes and Davison (1988), O_3 fumigation enhanced minimum epidermal conductance. Mengel et $al.$ (1989) showed that acid mist (pH=3) affected significantly the water status of $Picea$ $abies$ trees in a greenhouse. The water potential of treated shoots was found to be lower, while the difference between treated and control trees was especially marked during periods of sunshine. At high temperature, the water vapour deficit gradient between needles and air increases. Heating of needles in early spring, when the soil is still frozen, proves to be an additional cause of desiccation in natural conditions as well (Baig, Tranquillini 1980).

Table 1. Published data of minimum water loss in shoots of these species. When possible original data have been recalculated to primary ... $10^{-5} \times 10.5^{-1}$. Abbreviations: n.c. — normal conditions, which are most likely to occur in nature; e.c. — extreme conditions during which water loss was estimated; ** — the same comments as given above; PCP — ... ; 1/d — previous-year needles; C — current-year needles; C+1 — previous-year needles; C+2 — two-year-old needles and C+3 — three-year-old needles.

Tabel 1. ... PCP — pen-klorofenool; 1/d — RWC ... C — ... C+1 — ... C+2 — ... C+3 — ...

Spec...	Age	Measured value	Calculated i (min)	Measured value	Calculated g(min)	Condition of experiment	Note	Reference
P. ...	C+1	2.3 mg H_2O/ g h dry wt.		2 ... mg H_2O/ g h dry wt.		climated wind tunnel; e.c.: treeline and "Kampfzone"	In December transpiration was up to 10% higher than in October	1
... ab...	C	2.5 mg H_2O/ g h dry wt.		5 ... m H_2O/ g h dry wt.		** P = 25 – 115 h	transpiration declined throughout the experiment	2
	C+1	4.5 mg H_2O/ g h dry wt		... 6 mg H_2O/ g h dry wt				
... abie	C	3200 ... d/cm	0.2 ...	...	0.5 ...	**	g(min) depends on temperature and/or RH	3
... es	C+1	0.60...1.20% water loss/h		0.70...1.04% water loss/h		dark P = 25...70 h; e.c. O_3 fumigation	water loss varied between the clones	...
... abi	C	0 % water loss/24 h		55 water loss/24 h		desiccator P = 48 h; e.c.: acid mist	stiffness of acid mist was significant	**
P. ab...	C	... × 10^{-9} m/s	0.2...7	6.5 × 10^{-9} m/s	0.0...5	scale leaf from PCP uptake — damaged needles	cuticular permeability of older needles to PCP was significantly lower	...
	C+1	0 × 10^{-9} m/s	0.0...0	9.5 × 10^{-9} m/s	0.0...6			
	C+2	1 × 10^{-9} m/s	0.0...1	4 × 10^{-9} m/s	0.0...0			

1	2	3	4	5	6	7	8	9
P. abies	C	0.45 1/d	*	0.44...0.65 1/d	*	detached needles; P > 3 h; e.c.: O3 fumigation	variability statistically described	7
P. engelmannii	C+1	$8.2 * 10^{-6}$ m/s	0.82			kept in cold before experiment; P = 12...24 h		8
P. engelmannii	C	195 ks/m	0.51	90...195 ks/m	0.51...1.11	P = 4...24 h; e.c.: higher elevations	C needles had significantly lower values of g(min) compared with older needles	9
	C+1	125 ks/m	0.80	60...140 ks/m	0.71...1.67			
	C+2	125 ks/m	0.80	70...155 ks/m	0.65...1.43			
	C+3	100 ks/m	1.00	50...150 ks/m	0.67...2.00			
P. engelmannii	C+1	$8.6...12.4 * 10^{-6}$ m/s	0.86...1.24			dark; P < 60 h	g(min) decreased continuously	10
P. glauca	C and C+1	0.0013...0.088 cm/s	1.3...8.8			P < 15 h	g(min) increased with ageing; shade needles had higher g(min) than sun needles	11
P. rubens	C	85 ks/m	1.17	71 ks/m	1.41	dark and cold; in 24 to 72 h; e.c.: trees with more than 25% foliage brown in spring	g(min) increased with elevation	12
P. rubens	C+1	79 ks/m	1.3			dark and cold; in 3...4 days	g(min) varied during winter	13
P. sitchensis	C	0.57 mmol/m²s	1.27	1.25...1.32 mmol/m²s	2.6...2.8	dark; e.c.: exposure to high wind	values of g(min) were lognormally distributed; standard error of ln(g (min)) was more than 10% (n=76)	14
P. rubens	C	0.95 1/d	*	0.63...1.12 1/d	*	detached needles; P > 3 h; e.c.: O₃ fumigation		7

References: 1 — Platter 1976 (ref. by Tranquillini 1982); 2 — Baig et al. 1974; 3 — Balg, Tranquillini 1980; 4 — Barnes, Davison 1988; 5 — Mengel et al. 1989; 6 — Schreiber 1994; 7 — Cape, Percy 1996; 8 — Hadley, Smith 1990; 9 — Hadley, Smith 1994; 10 — Sowell et al. 1996; 11 — Cowling, Kedrowski 1980; 12 — Herrick, Friedland 1991; 13 — Boyce et al. 1992; 14 — von Gardingen et al. 1991.

The data of g(min) have been used for modelling wintertime water economy of conifers in order to estimate possible water loss. The results of modelling allow to predict needle survival in case water transport into needles is hindered by the frozen soil or stem (Sowell 1985; Hadley, Smith 1990; Boyce *et al.* 1992; Sowell *et al.* 1996). However, in the last few years the use of laboratory-measured water loss parameters in predictions of water loss from the whole tree has been placed under suspicion. Sowell *et al.* (1996) demonstrated that the use of laboratory-measured g(min) of *Picea engelmannii* in the desiccation model yielded considerably higher predicted values of needle desiccation in winter compared with real values observed in naturally growing shoots. This may be caused by errors in the values of the climate parameters employed in the development of the model (incorrect estimation of real RH, neglecting of differences in air and needle temperatures etc.). At the same time, the authors supposed that the value of g(min) decreased due to wintertime low temperature and dry air. The value of g(min) may be affected also by cuticle moisture content (Kerstiens 1996). Another problem arising in the modelling of wintertime needle water economy is the possibility of water uptake. Uptake of water from the stem may begin when air temperature is above –4°C (Boyce *et al.* 1992). Direct water uptake by needle and twig surface is more unpredictable. Water uptake from melting snow, hoar-frost or dew has been suggested impossible and has not received much attention. However, Katz *et al.* (1989) demonstrated with a laboratory experiment that twigs of *Picea abies* absorbed 80 mm^3 of sprayed water per 1 g dry weight during 200 min (at the same time their xylem water potential recovered from –1.00 MPa to –0.15 MPa). Dye was found to penetrate through twig bark rays and parenchymal cells.

In the current thesis emphasis has been laid on the role of the stomatal antechambers and the cuticle (including epicuticular wax) of *Picea abies* needles as pathways for water vapour diffusion. Neither of them is controlled by the plant in a short term. Both depend on long-term growth conditions and can influence plant water loss in extreme conditions: stomatal antechambers become involved in case stomata are fully opened, while the role of the cuticle and epicuticular wax is revealed in case of winter water deficit and/or polluted air.

The following problems have been studied:
1. Variability of the dimensions of stomatal antechambers on needles from various crown positions (sun and shade needles), the effect of antechamber dimensions on water vapour diffusion resistance (Paper I).
2. Suitability of contact angle method for monitoring air pollution in Estonia (Paper III).
3. Variability of epicuticular wax structure in different crown positions and the effect of air pollution on wax (partly Paper IV).
4. Variability of minimum epidermal water loss in the course of needle ageing, in various crown positions and in different situations of air pollution (Papers II and V).
5. Mechanisms causing variability of minimum epidermal conductance (Paper VI).

2. MATERIAL AND METHODS

2.1. Experimental sites

The material was collected from *Picea abies* trees growing at various sites of Estonia (Fig. 2A, Table 2). For more detailed description of the study sites see Table 2 in Paper IV. Most of the studies were carried out on naturally growing adult trees. To eliminate possible differences caused by the plant genotype, 4-year-old cloned spruces were planted in 1988 at 10 sites in Estonia representing areas where different air pollution situations may occur. Monoclonal young trees were used in the study of the variability of epicuticular wax structure (Paper IV).

Table 2. Description of the studied trees. Location of sites is shown in Fig. 2A. Needle age: C — current-year needles; C+1 — previous-year needles; C+2...C+5 — two- to five-year-old needles.

Tabel 2. Uuritud puude kirjeldus. Kasvukoht on näidatud joonisel 2A. Okaste vanus: C — käesoleva aasta okkad; C+1 — eelmise aasta okkad; C+2...C+5 — vastavalt kahe kuni viie aasta vanused okkad.

Studied value	No. of paper	Study site No.	No of trees	Sampling height (m)	Needle age	Notes
1	2	3	4	5	6	7
size of stomatal antechambers	I	8	7	2; 12; 34	C+1	one tree from closed canopy, the others from open site, tree age 9...100 years
droplet contact angle	III	4; 8; 11; 12; 15	8	2; 10; 18	C...C+5	adult trees from open site
epicuticular wax structure	IV	3...14	12	2	C...C+4	adult trees from open site
epicuticular wax structure	IV	1...5; 8; 9; 11; 12; 14	29	0.5	C...C+3	9-year-old monoclonal trees
epicuticular wax structure	unpublished	8	2	12; 34	C...C+3	one tree from closed canopy, the other from open site
minimum epidermal conductance (g(min))	II	4; 8; 11	8	1; 2; 12; 30	C+1...C+4	one tree from closed canopy, the others from open site, tree age 12...100 years
g(min)	V	8; 16	3	2; 14; 30	C; C+2	adult trees from open site
g(min)	VI	11	1	2	C	adult tree from open site
g(min)	unpublished	3...14	12	2	C...C+3	adult trees from open site
stomata opening	VI	11	1	2	C...C+5	the same tree used for g(min) studies in Paper VI
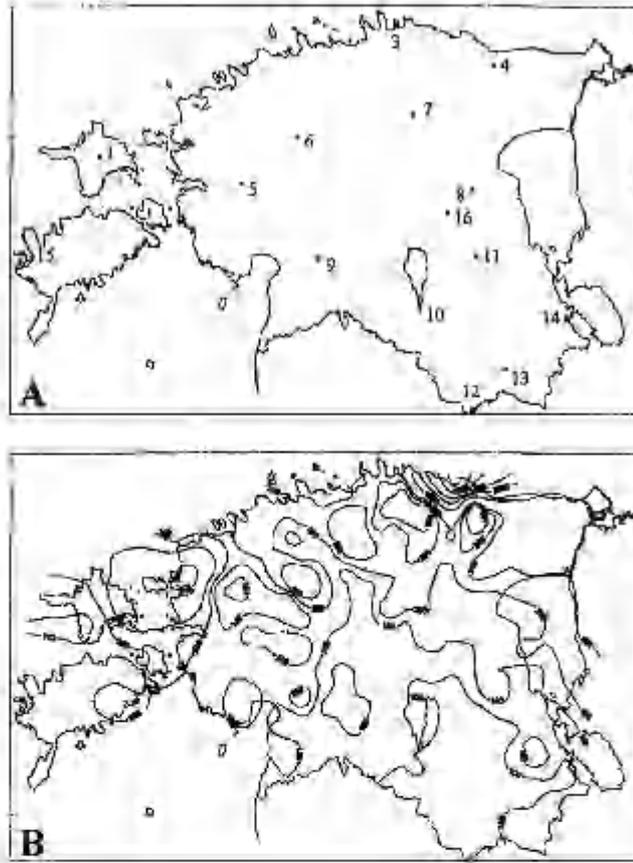
Figure 2. A. Location of the study sites: 1 — Putkaste; 2 — Nõva; 3 — Käsmu; 4 — Kiviõli; 5 — Vigala; 6 — Väätsa; 7 — Rakke; 8 — Voore; 9 — Tipu; 10 — Aakre; 11 — Tartu; 12 — Alska; 13 — Haanja; 14 — Värska; 15 — Vilsandi; 16 — Värska. **B.** Load of sulphate deposition in snow cover ($\times\ 10^{-3}$ g m^{-2}) in 1996 (Roots *et al.* 1997).

Joonis 2. A. Kasvukohtade paiknemine. **B.** 1996 aasta lume sulfaatioonide sisaldus ($\times\ 10^{-3}$ g m^{-2}).

2.2. Methods

2.2.1. Antechamber size measurements (Paper I)

Stomatal antechambers from the cross and longitudinal sections of the epidermis were studied. In order to remove epicuticular wax, needles were kept in ethyl ether for 3 minutes. One shoot per sample was used. Antechamber measurements were made from microphotos. At least 38 photos of either section per sample were employed.

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The stomatal antechamber of *Picea abies* needles can be divided into two parts each having a different shape (Paper I, Fig. 1). The diffusion resistance of the antechamber (R_{ch}) can be divided into four components: resistance of the upper elliptic pore (R_{p1}), resistance of the upper truncated cone (R_{t1}), resistance of the lower elliptic pore (R_{p2}) and resistance of the lower truncated cone (R_{t2}). Total resistance of the antechamber was calculated according to the formulas derived for small pores and tubes (Bange 1953):

$$R_p = 0.5 \times D \times d;$$

$$R_t = \frac{4l}{\pi \times D \times d^2};$$

$$R_{ch} = R_{p1} + R_{t1} + R_{p2} + R_{t2};$$

where D is the diffusion coefficient (for water vapour at t = 14°C D = 0.24 cm^2 s^{-1}); d — diameter of the pore (cm) and 1 — depth of the tube (cm). For the elliptic pore the effective diameter (d) was calculated using its length (A) and width (B):

$$d = \frac{\sqrt{A \times B}}{4}.$$

In order to calculate the resistance of antechambers per needle surface area (r_{ch}) stomatal density was estimated on five needles per sample. Stomata were counted under the microscope on 1 mm sections taken from all four sides of the needle. Needle area was calculated from the dimensions of needle cross-section diagonals assuming that needles had a rhomboid cross section.

2.2.2. Droplet contact angle measurements (Paper III)

Twenty needles per sample were positioned horizontally in a protractor. A droplet of distilled water with a volume of 1...3 µl was dropped on the central part of the needle. In order to estimate wettability of the cuticle, the contact angle was measured in the needles from which epicuticular wax had been removed with ethyl ether. All studies were carried out on dry needles at room temperature.

2.2.3. Epicuticular wax structure and stomatal opening (Paper IV; VI)

Thin epidermis samples were cut with a razor blade from the anatomically upper sides of fresh needles, mounted on sample tubes and dried. The samples were covered with gold. For this purpose the JEOL Fine Coat Ion Sputter IFC-1100 was used. The samples from adult trees originating from different

parts of Estonia were placed directly under the cathode at a distance of 20 mm from it. Our study showed that temperature directly under the cathode may rise up to 100°C and melt some epicuticular wax structures. In order to diminish the possible thermal effect on wax during sputtering the distance between the cathode and the sample stubs was increased to 40 mm and the stub surface was inclined at 127° in relation to cathode surface when studying samples from monoclonal trees and needles taken from various heights of a tree. In all these cases the scanning electron microscope BS 300 (Tesla) was used. Acceleration voltage was 15...17 kV.

In order to study the wax structure of adult trees, at least five typical micro-photos were made of epicuticular wax present in stomatal antechambers from several lateral shoot needles. An arbitrary scale of typical sample photos, which represented gradual degradation of epicuticular wax, was constructed (for details see Paper II, Fig. 2). The scale coincided with the one used by Huttunen and Laine (1983) for *Pinus sylvestris* needles. The wax structures on the photos were evaluated independently by two persons and the results were averaged. On mono-clonal trees, two needles from the central part of the leading shoot from the northern side of a tree were collected. About 60 antechambers per site/year were evaluated directly from the microscope screen. One typical antechamber per needle was photographed for subsequent independent control evaluation by two persons. Out of the sun and shade needles of a tree, 3...5 needles were used per height/year, of which about 5 microphotos were made.

The degree of stomatal opening was studied on detached needles kept for 24 hours in dark and at room temperature. Epicuticular wax was removed with ethyl ether and the specimens were coated with the JEOL Fine Coat Ion Sputter IFC-1000. Stomata were examined under the scanning electron microscope JEOL JSM 840 A at accelerating voltage 7 kV and current 0.3×10^{-15} A. Sto-matal openings were investigated on several needles per age-class.

2.2.4. Measurements of minimum epidermal conductance — g(min) (Papers II, V, VI)

Eight to ten shoots were collected per sample. Weighing of shoots within 4 to 14 hours after the beginning of the experiment was used to estimate g(min) in needles of various age, crown position and site (for details see Paper II or V). Room temperature and RH were measured and their values were used in the calculation of g(min). The role of epicuticular wax was estimated in an experiment with needles whose wax layer had been removed by keeping them in ethyl ether for some minutes. A cohort of needles which flushed in 1985 were observed throughout two and a half years. Most of the other g(min) studies were performed in autumn 1985...1995, except for the one carried out in spring before budbreak, in which the needles of an adult forest tree were used.

Variability of g(min) within one branch (Paper VI) was studied by weighing shoots at every 10 minutes after cutting in order to find out water loss curves. The experiment lasted for 24...48 hours in a climatic chamber (t = 22°C, RH = 45%).

Needle area was estimated either by measuring needle dimensions (details given in Paper VI) or by using glass bead technique (Thompson, Leyton 1971).

3. RESULTS

3.1. Resistance of stomatal antechambers (Paper I)

Antechamber dimensions did not vary significantly on needles within the shoot and branch ($P > 0.05$; Student's t-test). Nor were there differences in antechamber dimensions in young trees growing at one site. However, antechamber dimensions differed both between sun and shade needles and between the trees of different age (Table 3). The highest variation was observed in antechamber depth, which depended directly on epidermis thickness. Stomatal antechambers on the sun needles of a tree were 50...60% deeper compared with those on the shade needles of the same tree. Antechamber dimensions of needles in young trees were similar to those of shade needles.

The resistance of a single antechamber (R_{ch}) of different needle types did not vary significantly, since the width and length of deeper antechambers were larger (Fig. 3). The values of R_{ch} varied between $2.67...3.75 \times 10^9$ s m^{-3}. Variability of stomatal density caused larger than twofold differences in the values of r_{ch} between sun and shade needles as well as between needles from different individual trees (the range of r_{ch} varied between 63 and 144 s m^{-1}).

Table 3. Dimensions of antechambers. Tree No 1 — about a 100-year-old forest tree; trees No 2 and 3 — open site trees aged 66 and 10 years, respectively; a — significantly larger values and b — significantly lower values among at least half of the other sample series (in both cases $P < 0.05$; Scheffe test); $n \geq 27$. For symbols see Paper I, Fig. 1.

Tabel 3. Eeskambrite mõõtmed. Puu nr. 1 — umbes 100-aastane metsapuu; 2 ja 3 — üksikud lagedal kasvavad puud, vastavalt 66- ja 10- aastased. a — oluliselt suuremad; b — oluliselt väiksemad mõõtmed vähemalt poolest uuritud võrsetest.

Tree No; height	Length ($\times 10^{-6}$ m)		Width ($\times 10^{-6}$ m)		Depth ($\times 10^{-6}$ m)		Stomatal density ($\times 10^6$ m^{-2})
	upper part A_1	lower part A_2	upper part B_1	lower part B_2	upper part L_1	lower part L_2	
No 1; 34 m	62.0 [a]	33.0	41.3	16.3 [a]	12.2 [a]	15.8 [a]	47.5
No 1; 12 m	56.6	28.4	34.8	15.4	8.6	12.6	26.9
No 2; 2 m	56.8	27.5	39.2	12.4 [b]	11.1	11.2	27.0
No 3; 2 m	55.8	31.6	34.7	13.9	7.1	10.3	37.7
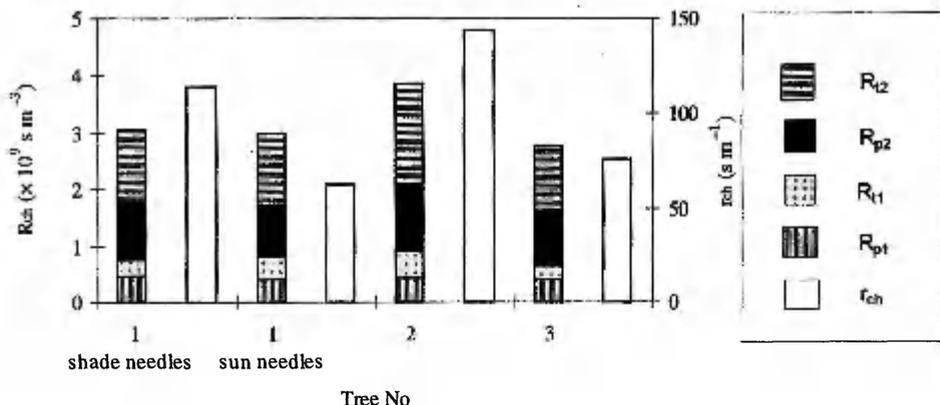
Figure 3. Resistance of stomatal antechambers to water vapour diffusion. Left-hand columns — resistance (R_{ch}) of a single stomatal antechamber divided into four components (for symbols see 2.2.1); right-hand columns — resistance of antechambers expressed per unit of needle area (r_{ch}).

Joonis 3. Õhulõhe eeskambrite takistus veeauru difusioonile. Vasakpoolsed tulbad — üksiku eeskambri takistuse (R_{ch}) neli osa (tähistused vt. 2.2.1); parempoolsed tulbad — eeskambrite takistus pindalaühiku kohta (r_{ch}).

3.2. Droplet contact angle (Paper III)

Wettability of different sides of *Picea abies* needles did not vary significantly (P > 0.1; Student's t-test). Standard deviation of the droplet contact angle (β) of twenty investigated needles was about 3% of the average. Needle wettability increased during ageing (Fig. 4) while increase was faster in the lower part of the tree compared with the upper crown (P < 0.01; Two Factor Anova test).

The contact angle of the needles of spruce growing in different parts of Estonia varied in a broad range (Fig. 5). The smallest contact angle was observed on the needles of one tree in Vilsandi (study site No 15), whereas very high values of β occurred in another tree from the same site. Needles from these two neighbouring trees showed larger differences in their wettability compared with needles from trees growing at sites with different air pollution load. Differences in needle wettability between various study sites were not significant (P > 0.1; One Factor Anova test). After removal of epicuticular wax wettability of needles decreased (P < 0.1; Student's t-test) (Fig. 6).
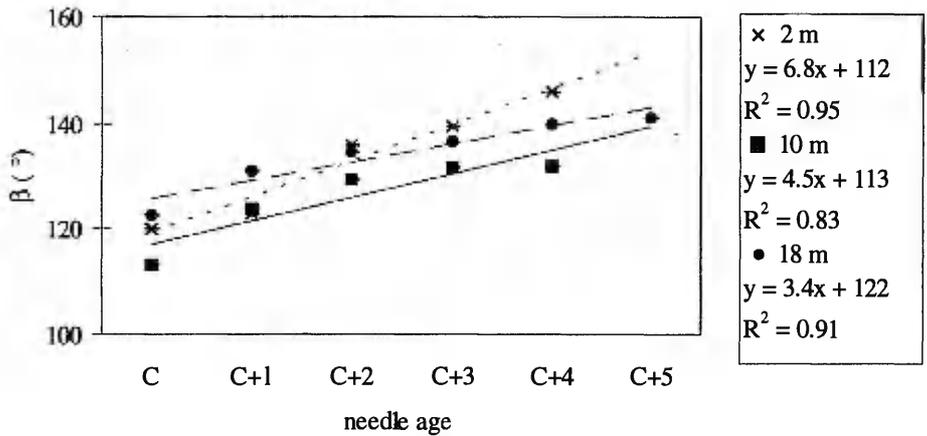
Figure 4. Droplet contact angle (β) in relation with needle age in an open site tree. Height of sample shoots from the ground: dotted line — 2 m; broken line — 10 m; continuous line — 18 m. Each point represents the average of 20 samples.

Joonis 4. Kontaktnurga (β) suurus lageda kasvukoha puu eri kõrgustel kasvavatel erivanuselistel okastel. Punktiirjoon — 2 m; katkendlik joon — 10 m; pidev joon — 18 m. Iga punkt joonisel on 20 mõõdetud kontaktnurga aritmeetiline keskmine
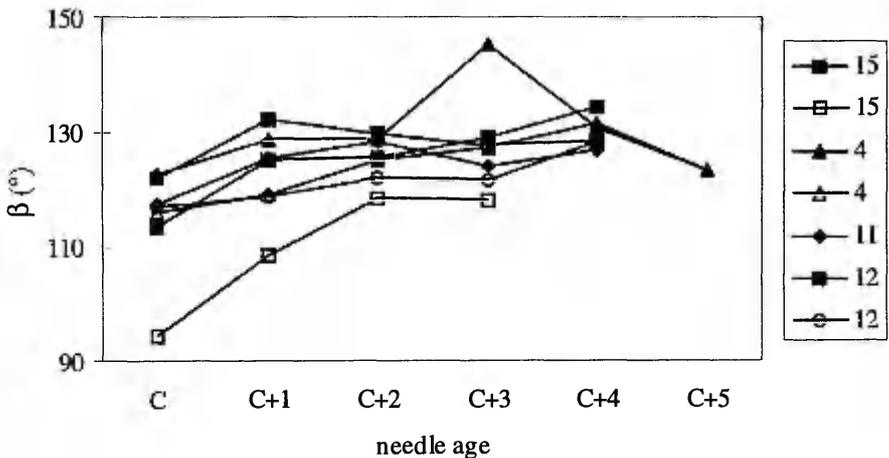
Figure 5. Droplet contact angle (β) of different trees from various sites of Estonia. Numbers show the location of a study site (given in Fig. 2A). Each point represents the average of 20 samples.

Joonis 5. Eri kasvukohtades ning eri puudel uuritud kontaktnurk (β). Arvud legendis näitavad puu kasvukohta joonisel 2A. Iga punkt joonisel on 20 mõõdetud kontaktnurga aritmeetiline keskmine.

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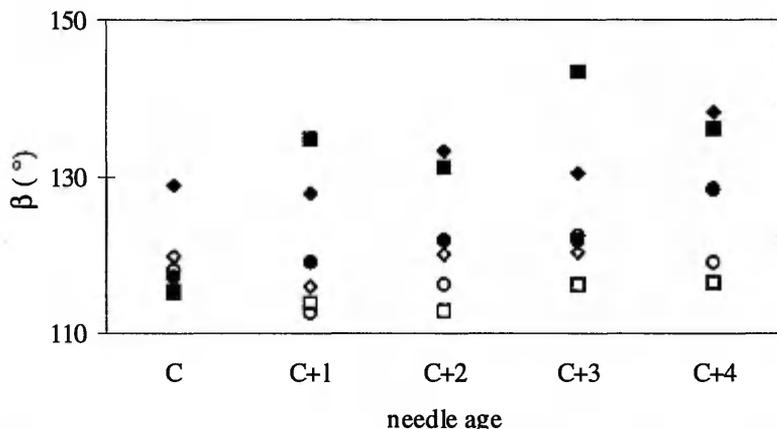
Figure 6. The effect of removal of epicuticular wax on the needle contact angle (β). Filled symbols — wettability of needles from three branches of a tree from study site No 12; opened symbols — wettability of needles from the same branches after wax removal. Each point represents the average of 20 samples.

Joonis 6. Epikutikulaarse vahakihi mõju okaste kontaktnurgale (β). Mustad tähised — prooivialal nr. 12 kasvava puu kolme oksa okaste märguvus; valged tähised — samadelt okstelt korjatud okaste märguvus peale vaha eemaldamist. Iga punkt joonisel on 20 mõõdetud kontaktnurga aritmeetiline keskmine.

3.3. Epicuticular wax structure
(Paper IV, partly unpublished)

The current year sun needles of a forest tree had more "crystalloid" wax structures in their antechambers than the shade needles of the same tree (Fig. 7 A; B). Wax tubes fused during needle ageing, and wax structure was uniform in older sun and shade needles. However, we were not able to prove the result statistically. A number of authors have used statistical analysis to compare the degree of wax degradation (Tuomisto, Neuvonen 1993; Trimbacher, Eckmullner 1997). After evaluating wax structures in more than 3000 stomatal antechambers it appeared that the application of statistics was impossible. Evaluation of wax structures involved a certain degree of subjectivity. Sometimes wax structure was found to differ drastically even between the closest antechambers (Paper III, Fig. 3). The highest variability occurred in C-needles where both fine tubular and flattened wax structures were observed. In some cases variability of wax structure within a shoot was similar to variability between trees. (Paper III, Table 3). Therefore, only the average values of the estimated degradation levels were used to compare the wax structures of needles from different sites.
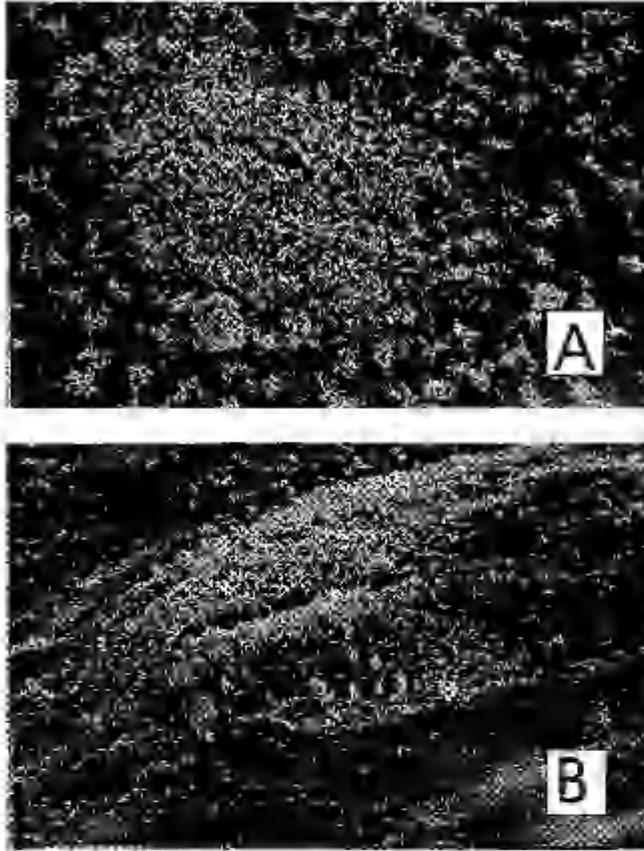
Figure 7. Typical epicuticular wax structure on sun (A) and shade (B) needles of a forest tree.

Joonis 7. Tüüpiline epikutikulaarne vahakiht metsapuu valgus- (A) ja varjuokastel (B).

In adult trees non-degraded epicuticular wax structures were not found even among C-needles at any studied site in Estonia. The surface structure of C- and C+1 needles on young monoclonal trees was finer compared with that of needles on adult trees. In both cases the largest differences in epicuticular wax structures between the sites were detected in C- and C+1 needles. In older needles uniformly high wax degradation occurred at all sites (Paper III, Tables 2 and 4).

3.4. Minimum epidermal conductance g(min)

3.4.1. How to calculate g(min) (Paper V, VI)

During the first hours of desiccation, water loss from a detached shoot was rapid. After 3 hours, decline of shoot mass attained an almost linear character, indicating the onset of the classical "cuticular phase" of transpiration, i.e. closure of the major part of stomata (Fig. 8). However, in 48 hours of the experiment, conductance (g) was constant in no period. Continuous decline of g raises the question which value of g (and which period of its calculation) is suitable to describe minimum epidermal conductance. In our studies g(min) was calculated, when possible, from water loss 4 to 14 hours after shoot detachment. This period of the experiment can be considered suitable, as by this time the first fast decline in mass, related to stomatal transpiration, is over. At the same time, RWC of needles is still high to avoid re-opening of stomata.
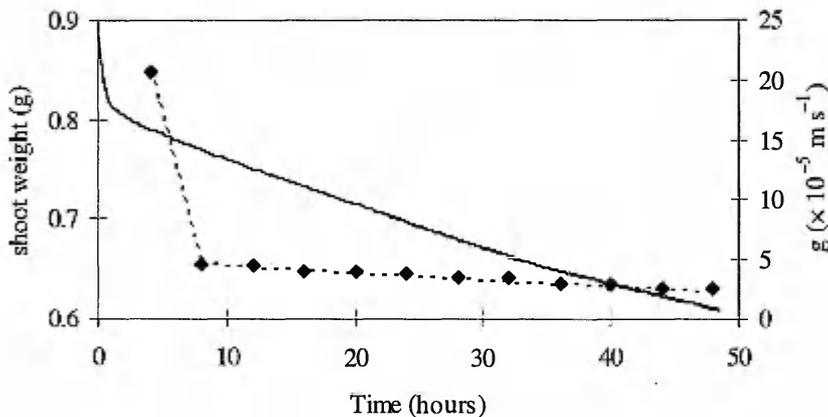
Figure 8. Changes in shoot RWC (solid line) and g (dotted line) during desiccation.

Joonis 8. Võrse kuivamisega kaasnevad RWC (pidev joon) ja g (punktiirjoon) muutused.

Analysis of the g(min) values of 40 shoots from the same tree/age class did not confirm the normal distribution of the data (Paper V, Fig. 3). Kolmogorov's test of distribution showed the validity of lognormal distribution ($P > 0.15$). Therefore, for statistical analysis the data were transformed by log-transformation. In the figures and text of the thesis the geometric mean of non-transformed data was used to describe the value of g(min).

3.4.2. Variability of minimum epidermal conductance within the branch. Stomatal opening (Paper VI)

Individual shoots from the same branch of a tree displayed considerable variation of g (Paper VI, Fig. 3). The values of g(min) calculated on the basis of weight loss within 4...14 hours of desiccation differed threefold, ranging from 1.3 to 4.3×10^{-5} m s^{-1}. The geometric mean of g(min) of 10 shoots was 2.26×10^{-5} m s^{-1}. No significant correlation of g(min) with different shoot characteristics was found (Table 4).

Table 4. Coefficients of linear correlation between shoot characteristics and g(min) of ten shoots from the same branch.
Tabel 4. Mitmesuguste võrset iseloomustavate suuruste ning g(min) vahelise lineaarse korrelatsiooni kordajad.

Factor	Correlation coefficient
shoot dry weight (DW)	0.52
twig DW	0.50
needle DW	0.53
RWC	−0.11
water content/DW	−0.36
needle area (S)	0.36
needle specific area ($S_m = S/DW$)	−0.60

Observations of stomata without epicuticular wax in antechambers under the SEM showed that after desiccation most of the stomata were completely closed. However, several stomata did not close completely, leaving a slit of 0.2...0.3 μm between the guard cells (Paper VI, Fig. 4). The proportion of incompletely closed stomata was impossible to estimate, since a number of stomata were inclined in relation to the viewing direction.

3.4.3. Needle ageing and spatial variability of minimum epidermal conductance (Paper II, V, partly unpublished)

Minimum epidermal conductance increased with needle ageing (Paper V, Fig. 2). The results of the investigation of a cohort of needles over two and a half years demonstrated that g(min) increased steadily during this period (Paper V, Fig. 4). Large fluctuations of g(min) occurred against the background of overall increase in g(min). For example, in April 1986, g(min) was about twice as large as the values recorded in March 1986. In May 1986, g(min) dropped to the March level. Laboratory experiments showed that the variability of g(min) did not correlate with water vapour deficit.

Minimum epidermal conductance of sun needles from a forest tree was higher than that of the shade needles from the same tree. This was the case with all investigated needle age-classes (for C, C+1 and C+4 needles $P < 0.05$, for C+3 needles $P < 0.1$; Student's t-test) (Fig. 9). There was no difference ($P > 0.1$; Two-Factor Anova test) in increase in g(min) with ageing between sun and shade needles.
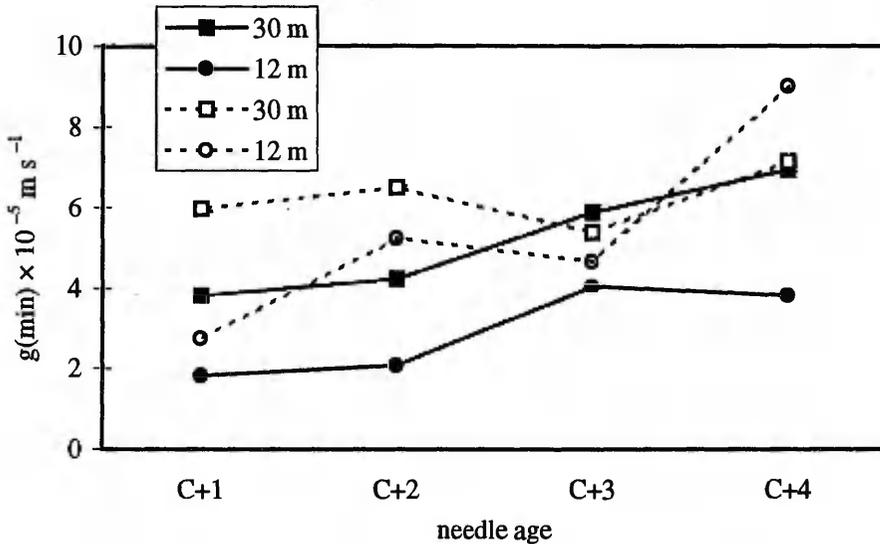
Figure 9. Changes in g(min) during needle ageing at various crown heights of a forest tree. Filled symbols — needles with the layer of epicuticular wax; opened symbols — needles with epicuticular wax removed. Each point represents the geometric mean of ten samples.

Joonis 9. Metsapuu eri kõrgustel kasvavate okaste g(min) muutused okaste vananedes. Mustad tähised — okkad koos epikutikulaarse vahakihiga; valged tähised — okkad, mille epikutikulaarne vahakiht on eemaldatud. Iga punkt vastab kümnele uuritud võrsele

After removal of epicuticular wax, g(min) increased in all needle age-classes. In younger needles (C and C+1) this difference was significant for sun as well as for shade needles ($P < 0.1$ in both cases; Student's t-test). In case of older needles, only C+4 needles collected from a height of 12 m had a significantly higher ($P < 0.01$; Student's t-test) value of g(min) among needles without wax. The minimum epidermal conductance of needles without epicuticular wax increased steadily with ageing. However, this increase was statistically significant only for shade needles ($P < 0.05$; Single Factor Anova test).

No universal trends were found in correlation between shoot position in the crown and g(min) in open-site trees (Paper V, Fig. 1). The role of the quadrant was significant for C-needles of both studied trees, whereas either tree revealed opposite trends.

No correlation was found between g(min) and stomatal density, which indicates that there occurred no additional water loss through the upper surface of stomata guard cells (Fig. 10).
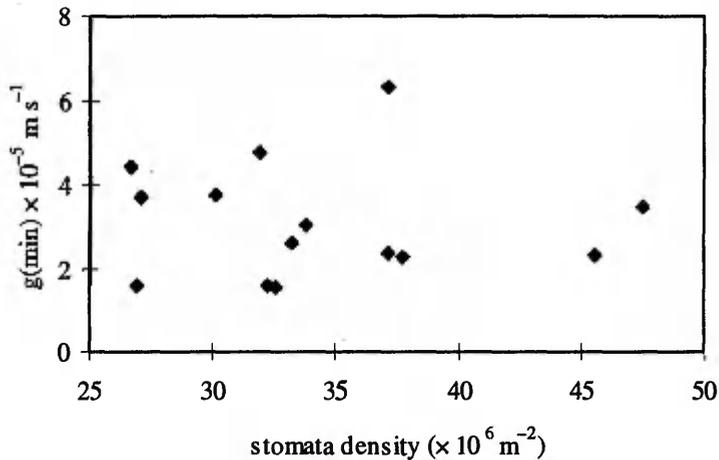
Figure 10. Relationship between g(min) and stomatal density of C-needles in different trees.

Joonis 10. Erinevate puude C-okaste g(min) ja õhulõhede pindtiheduse vaheline seos.

3.4.4. Variability of minimum epidermal conductance in different study sites (Paper II, V, partly unpublished)

The values of g(min) in adult trees from various sites in Estonia are given in Table 4. The highest values of g(min) for C-needles were found in Alska (site No 12), those for C+3 needles, in Tipu (site No 9). Increase in g(min) with needle ageing (calculated as the ratio of the g(min) values for C+3 needles to those for C-needles) was the highest in Tipu (site No 9) and the lowest in Vigala and in Alska (sites No 5 and 12). All these study sites are far from major sources of air pollution and without any local industry. In 1996 deposition of sulphate ions in the snow cover at these sites was between 0.10 and 0.15 g m^{-2} (Fig. 2B) (Roots *et al.* 1997). The sites assumed to represent polluted areas, Rakke (site No 7, 3 km from a lime plant) and Kiviõli (No 4, an industrial area, 2 km from a large oil-shale chemical plant), were characterized by the values of g(min) that were not significantly higher compared with those at sites without any local sources of air pollution (Table 6).

Table 5. Values of g(min) ($\times 10^{-5}$ m s^{-1}) for needles at various study sites. Location of the study sites is shown in Fig. 2A.

Tabel 5. Erinevate kasvukohtade okaste g(min) ($\times 10^{-5}$ m s^{-1}) väärtused. Kasvukohtade paiknemine on näidatud joonisel 2A.

Study site	C	C+1	C+2	C+3	C+3/C
3	1.38	2.18	2.73	3.61	2.6
4	1.56	3.45	2.80	4.63	2.9
5	1.54	1.66	1.87	2.85	1.8
6	1.43	2.99	3.42	5.38	3.8
7	1.73	2.84	2.46	3.85	2.2
8	2.31	3.86	4.04	5.05	2.2
9	1.72	2.69	3.60	7.07	4.1
10	1.47	2.22	2.29	3.72	2.5
11	1.81	1.65	2.58	3.68	2.0
12	2.73	3.50	4.00	4.88	1.8
13	1.29	1.87	2.43	2.85	2.2
14	1.49	1.97	1.81	3.24	2.2

Table 6. Differences between g(min) at various study sites. For statistical analysis the values of g(min) for all studied needle age-classes were used. g(min) for the sites presented in rows is significantly higher (a) or lower (b) ($P < 0.05$; Bonferroni T-test) than g(min) for the sites given in columns. Location of the study sites is shown in Fig. 2A.

Tabel 6. Erinevate kasvukohtade g(min) väärtuste erinevused. Statistilisel analüüsil kasutati kõigi nelja uuritud vanuseklassi g(min) väärtusi. g(min) tabeli ridades olevates kasvukohtades on oluliselt kõrgem (a) või madalam (b) kui veergudes olevates kasvukohtades. Kasvukohtade paiknemine on näidatud joonisel 2A.

5	6	8	9	10	11	12	13	14	site
		a	a			a			3
b									4
		a	a	a		a			5
							b	b	6
			a			a			7
				b	b		b	b	8
				b	b		b	b	9
						a			11
							b	b	12

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4. DISCUSSION

4.1. Effect of variability of stomatal antechambers
on water vapour diffusion

The presence of stomatal antechambers causes additional resistance to gas diffusion between the plant leaf and the ambient air. The resistance of the stomatal antechamber (R_{ch}) did not differ significantly in sun and shade needles. However, the resistance calculated per needle area unit (r_{ch}) was different in the shade and sun needles of the same tree. Such a result was caused by higher stomatal density in sun needles. Therefore stomatal density should be taken into account when estimating the role of r_{ch} in transpiration. A significant correlation between light availability and stomatal density was shown by Koppel and Frey (1985). According to this study, however, needles from young trees growing at the open site had low stomatal density similar to that observed in shade needles from an adult tree.

Jeffree *et al.* (1971) showed that the presence of epicuticular wax in antechambers increased their resistance to water vapour diffusion about twice as compared with the respective value measured in the absence of wax. In order to estimate the role of r_{ch} in total water vapour diffusion resistance, different resistance components were calculated (Fig. 11).
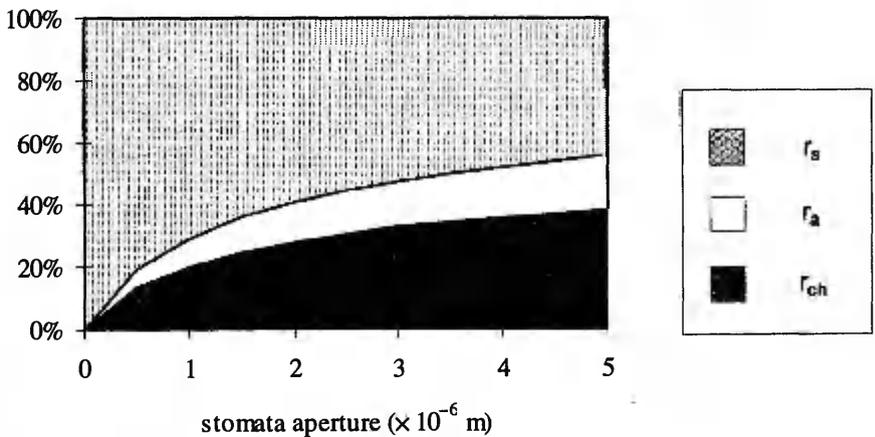
Figure 11. The share of resistance components in total transpiration resistance as a function of stomatal opening. Assumptions: $r_{ch} = 200$ s m^{-1}; $r_a = 90$ s m^{-1}; stomatal pore length — 20×10^{-6} m; stomatal pore depth — 5×10^{-6} m; stomatal density 27.5×10^{6} m^{-2}.

Joonis 11. Takistuse eri komponentide osatähtsus kogu transpiratsioonitakistuses sõltuvalt õhulõhede lahtiolekust. Arvutuse eeldused: $r_{ch} = 200$ s m^{-1}; $r_a = 90$ s m^{-1}; õhupilu pikkus — 20×10^{-6} m; õhupilu sügavus — 5×10^{-6} m; õhulõhede pindtihedus $27,5 \times 10^{6}$ m^{-2}.

Calculations showed that stomatal antechambers played an important role in transpiration in case of widely open stomata. At a stomatal pore width of 4×10^{-6} m, r_{ch} made up about 37% of total transpiration resistance. The role of stomatal antechambers decreased with narrowing of stomatal pores. For example, at stomatal pore width of 0.4×10^{-6} m, r_{ch} made up only 10% of total transpiration resistance.

The effect of r_{ch} on CO_2 uptake is much weaker than on transpiration due to mesophyll resistance to CO_2 diffusion. The presence of antechambers can therefore be considered one of the mechanisms increasing water consumption efficiency (Jeffree et al. 1971). On the other hand, stomatal closure may serve the same function (Larcher 1995). The importance of antechambers in plant water economy is significantly enhanced in case of an incomplete regulation system or damaged stomata.

4.2. Contact angle method as a bioindicator of air pollution

The values of contact angle β on current-year Norway spruce needles, as measured by several scientists, varied between 48 and 105° (reviewed by Cape, Percy 1993). We found wettability of this range only in one tree on Vilsandi Island (site No 15). In all other cases the average contact angle on current-year needles ranged between 110 and 125°. Boyce (1994) demonstrated that in the upper part of the tree crown, β of *Picea rubens* and *Abies balsamea* needles was larger than at the crown bottom. In the current experiment, β of C-needles from various heights did not differ significantly; in the lower part of the tree crown, wettability of needles increased with age much faster compared with the upper crown. However, needles from the lower part of the crown are comparatively well protected from direct solar radiation and the wind. These environmental factors accelerate epicuticular wax degradation (Berlyn et al. 1993). Faster wax degradation and/or change in the roughness of needle surface in shade needles yields no simple explanation.

Opinions differ concerning the direct influence of air pollution on needle wettability. Several authors have shown that O_3 fumigation or acid mist can cause increase in needle wettability (Barnes, Brown. 1990; Franssen 1991 (ref. by Cape and Percy 1993); Percy et al. 1992). However, other authors have not observed such changes (Horntvedt 1988 (ref. by Cape and Percy 1993); Berlyn et al. 1993; Dixon et al. 1997). The use of the droplet contact angle as a tool of biomonitoring is further complicated by the fact that needle wettability is affected by tree age (Boyce 1994; Jagels 1994).

No significant relationship has been found between the contact angle and local air pollution when wettability of spruce needles from different sites of

Estonia were measured. Our results support the opinion of Jagels (1994) that droplet contact angle method can be used for monitoring long-term changes in air pollution by employing fixed model trees. Due to high between-tree variability in case of the contact angle, this method cannot be applied in air pollution monitoring at different sites.

The experiment with needles without the epicuticular wax layer may serve as an example of difficulties arising in the interpretation of collected data. Removal of surface wax reduced needle wettability, which may be caused by increased surface roughness (Jagels 1994). However, SEM photos revealed no significant roughness on the surface of these needles compared with needles with the epicuticular wax layer.

4.3. Variability of epicuticular wax structures

Owing to progress in technical equipment, a large number of studies on leaf surface have been carried out during recent decades. The first SEM photos of epicuticular wax structures were described in the 1970s (Baker, Holloway 1970/71; Davis 1971; Rook *et al.* 1971; Grill 1973). One of the complicated problems in SEM studies is the adequacy of the photo to real structure. There exists serious danger that temperature may rise and wax may fuse during sample coating for SEM observations (Crang, Clomparens 1988). Fusing of wax formations may also result from high accelerating voltage during the use of SEM (Bermandinger-Stabentheiner 1994). Since these artefacts were common in earlier SEM studies, their results should be regarded with caution. In some cases the fused wax layer was even similar to the epidermis and was interpreted as a membrane growing over stomatal alveoli (Günthardt, Wanner 1982). In our pilot studies we obtained photos revealing a fine wax structure by using the same technical equipment as in the present investigation. However, temperature during the coating may have been somewhat too high at least for the samples of adult spruces from various localities of Estonia. In several samples plate-like wax formations were detected. The origin of such plates has been associated with wax erosion in nature (Günthardt-Goerg 1994, Huttunen 1994), or with mechanical injury (Bermandinger-Stabenheiner 1994). Our experiments performed for assessment of the suitability of the method showed that such plates were common on needles which had been kept at temperatures over 60°C.

The majority of wax studies under SEM have been usually carried out with the aim to analyse structural changes in epicuticular wax, caused by air pollutants. Degradation of epicuticular wax structures in the course of ageing has also been widely studied. Much less is known about the natural variability of epicuticular wax structures. Kim (1985) reported that wax degradation on spruce

needles from the northern side of a tree was lower than on needles from its southern side. In samples collected from the upper part of a tree, wax formations were more fused compared with samples taken from the lower part of the tree. Günthardt-Goerg (1994) showed that wax structure on C-needles from the upper part of a tree were less aggregated and flattened than it is on needles from its lower part, and associated this fact with faster degradation of wax structures in shade needles. We found differences in wax structures in case of C-needles: wax structure was more fused in shade needles compared with sun needles. However, since the wax structures of older needles from various heights did not reveal any difference, the opinion of faster wax degradation in shade needles cannot be grounded. It is quite likely that the wax of newly-formed sun needles have a finer structure than the wax of shade needles.

Epicuticular wax structures may vary due to microclimate within the tree crown. Structural changes in the epicuticular wax of the tree can be caused by availability of soil nitrogen (Dixon *et al.* 1997), by the wind (Hoad *et al.* 1994), or by solar radiation. Needle temperature under direct solar radiation and in still air may rise considerably above ambient air temperature (Christersson, Sandstedt 1978). The melting point of several spruce wax components ranges between 45 and 60°C. It is possible that direct sun radiation can heat needles sufficiently to cause melting of some wax components as well as fusing of its structure. Besides the variability caused by environmental factors, there exists genetic variability of the wax structure of individual trees (Bermandinger-Stabentheiner 1994).

Variability of epicuticular wax structure makes its use for air pollution monitoring complicated. The fact that fine tubular wax occurs on the needles of monoclonal trees but not on the needles of adult trees can be associated with differences in methods (preparation, tree age). However, it can also be related to overall decrease in air pollution in Estonia during the time different samples were collected. The study on adult trees was carried out in 1988, the study on monoclonal trees, in 1993. Sulphate emission dropped 40% from 1980 to 1992. The amount of dust and gaseous emissions from stationary sources decreased almost twofold from 1990 to 1993 (Anon. 1993; 1995).

Epicuticular wax structure on needles from several sites showed variability of C- and C+1 needles. However, the results of the study are difficult to explain. For example, needles from monoclonal trees had a finer structure at the site with heavy sulphur load combined with alkaline dust (site No 4 in Kiviõli) compared with sites without significant local air pollution (site No 1 in Putkaste and site No 9 in Tipu). The study of epicuticular wax structure can therefore be regarded as having only a limited monitoring value.

4.4. Minimum epidermal conductance

The causes of continuous decline of conductance (g) during 48 hours of desiccation in controlled conditions are unclear. We analysed the error caused by the assumption that intracellular RH was 100%. For this purpose, needle water potential (ψ) was estimated from the mass of transpired water by using the pressure-volume curve (Hellkvist *et al.* 1974). Further, relative water vapour pressure was calculated from ψ values according to Laisk (1977). The results showed that at room temperature, ψ dropped to –45 bars and relative water vapour pressure in the needles reached 0.97 in 14 hours of desiccation. Consequently, the error resulting from the assumption of water vapour in desiccating needles being saturated is rather small. The real value of g of relative humidity at a given ambient air (RH = 45%) was higher but not more than by 5%. This is not sufficient to account for the measured decline of g. It is likely that there are other mechanisms which caused decrease in g during the experiment. Kerstiens (1996) supposed that decrease in cuticle water content itself affected g(min). In our experiments, there occurred an evident decline of conductance in individual shoots, associated with decrease in shoot water content (Paper VI, Fig. 2), but when the data for all shoots were pooled, this correlation disappeared (Table 4).

Since g(min) has no constant value, method related difficulties will arise concerning the period of shoot weighing as well as comparability of samples collected from different study sites. We assumed that the period of 4...14 hours after detachment was suitable for calculating g(min). However, any other period (for example 8...18 hours) would change the value of calculated g(min). We could not always weigh shoots from several study sites, located far from the laboratory, exactly within 4 to 14 hours after detachment. Although on these occasions branches with sample shoots were preserved in plastic bags to avoid transpiration, but we could not be sure that g(min) was not affected by transport.

Calculated g(min) values for all investigated shoots with C-needles varied in our studies between $0.9...4.5 \times 10^{-5}$ m s^{-1}. These values are in the same range as those reported for *Picea abies* (Cowling and Kedrowski 1980) and for other *Picea* species (Hadley, Smith 1994; Sowell *et al.* 1996). For a winter desiccation model of *Picea rubens,* g(min) of 1.27×10^{-5} m s^{-1} was used (Boyce *et al.* 1992). Minimum epidermal conductance of 10 shoots from the same branch varied in the range of $1.3...4.3 \times 10^{-5}$ m s^{-1}. This range covers almost the whole variability of the measured values of g(min) in different crown positions and at different sites. High variability of g(min) is difficult to explain. Laisk *et al.* (1980) showed the existence of a universal bell-shaped distribution of stomatal apertures. Observation of needles without epicuticular wax under a scanning electron microscope showed that several stomata did not close completely, leaving a 0.2...0.3 μm wide slit between guard cells (Paper VI, Fig. 4). In order

to estimate the possible effect of incompletely closed stomata on g(min), the value of the stomatal conductance of a pore (G_s) was calculated according to Parlange and Waggoner (1970). Calculations showed that only about 6.5% of stomata with a pore width of 0.2 μm accounted for the above described difference in g(min) in case of shade needles (Paper VI, Fig. 5). On sun needles stomatal density was about twice as high as on shade needles (Table 3), and the amount of incompletely closed stomata which caused a similar effect on g(min) was even smaller. The degree of stomatal closure may vary in different shoots, which resulted in the observed lognormal distribution of the collected data.

A number of authors have shown that older needles of several conifer species have higher minimum epidermal conductance compared with younger needles. This increase has been associated with degradation of epicuticular wax (e.g. DeLucia, Berlyn 1986; Hadley, Smith 1994). Throughout two and half years, g(min) for a cohort of needles increased steadily, while we did not note any season or any stage in needle age contributing to the faster decline of g(min). However, g(min) for shade needles without epicuticular wax showed also a significant decline in the course of ageing. This indicates that besides changes in the amount or/and quality of wax, cuticle permeability *per se* or/and the number of dysfunctioning stomata may increase with ageing.

Differences in cuticle thickness and g(min) between wind-exposed and wind-protected shoots have been found in treeline observations (Baig, Tranquillini 1976; Hadley, Smith 1983). Large amount of solar radiation falling on the sun needles of the forest tree may cause faster increase in g(min) in the course of ageing compared with that of shade needles. Although radiation and wind regime are highly different for different crown parts in a tree growing at an open site, there were no differences in g(min) between shoots from different crown positions.

Several pollutants (O_3, acid mist) may affect the water economy of *Picea abies*. After 48 hours of desiccation, RWC in needles treated with acid mist (pH = 3.0) was significantly lower than it was in needles treated with control mist (pH. = 5.0) (Mengel *et al.* 1989). In another experiment, both acid mist and O_3 were found to reduce RWC of treated needles (Barnes *et al.* 1990). However, Schreiber (1994) found that the properties of the cuticular barrier were not different in healthy and damaged needles of *Picea abies* growing in natural conditions. The pilot studies of g(min) carried out at only three sites (No sites 4, 8 and 11) revealed significant differences between the sites. Increase in g(min) in the course of ageing in town (site No 11) was more rapid than in the countryside (site No 8). The most rapid increase in g(min) was observed in an industrial area (site No 4). However, a more recent comprehensive study of g(min) for needles collected from 12 sites of Estonia did not reveal any significant differences in g(min) between different study sites, which could be associated with air pollution. The reliability of the comparison of the absolute values of data collected from different sites is disputable because of

the method related problems discussed above. Therefore, we calculated the ratio of g(min) values for C+3 needles to those for C-needles. The values of the ratio were not related to SO_4^- deposition in snow, or to the presence of local industry at different sites.

The values of minimum epidermal conductance are widely used for modelling wintertime water economy of conifers (e.g. Sowell 1985; Boyce *et al.* 1992). In the present thesis, calculations of wintertime water loss have been made in order to evaluate the significance of the variability of g(min) (Fig. 12).
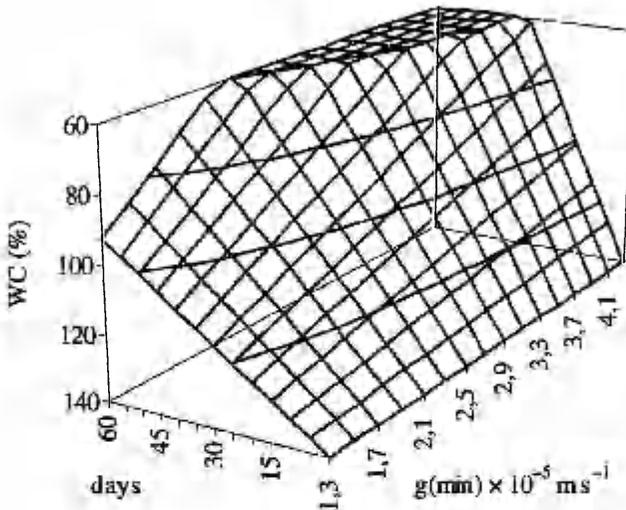
Figure 12. Decrease in needle water content (WC — % of dry weight) as a function of g(min). Assumptions of the model: RH = 80%, air temperature −15°C, initial WC of needles 140%, $S_m = 100\ cm^2\ g^{-1}$.

Joonis 12. Okaste veesisalduse (WC — vee % okaste kuivkaalust) muutus sõltuvalt g(min) väärtusest. Mudeli eeldused: RH = 80%, õhutemperatuur −15°C, okaste algne veesisaldus 140%, $S_m = 100\ cm^2\ g^{-1}$.

The range of g(min) used in this model was equal to that measured in ten individual shoots of a single branch. The model shows that relatively small differences in g(min) may cause appreciable differences in water loss over a long period. Water content drops below 60% of dry weight in needles with g(min) of 3.9×10^{-5} m s^{-1} in 35 days when air temperature is −15°C and RH = 80%. Needles with g(min) of 2.9×10^{-5} m s^{-1} reach the same WC level in 60 days. Water content lower than 60% leads to sublethal damage and irreversible desiccation (Hadley, Smith 1983). The results of this model demonstrate that the average values of g(min) commonly used in calculations of winter desiccation do not describe adequately water loss from different shoots of the tree.

5. CONCLUSIONS

1. Stomatal antechambers of sun needles were significantly larger compared with antechambers of shade needles. The resistance of a single antechamber to water vapour diffusion was similar in all studied needles. The resistance of stomatal antechambers per unit leaf area depended on their number in the needle surface layer. When stomata were fully opened, the percentage of antechamber resistance exceeded that of total transpiration resistance by a third.

2. The droplet contact angle on needle surface was different in various crown positions and changed with needle ageing. However, differences in the contact angle between various sites were not related to air pollution at the site.

3. Epicuticular wax structure on current-year sun needles was less degraded than that on shade needles. Wax structures on older needles from various crown positions were more uniform. Due to the high variability of epicuticular wax structures within a sample no relationship was found between wax degradation and air pollution.

4. Minimum epidermal water vapour diffusion conductance of needles increased with needle ageing, which was not caused merely by epicuticular wax degradation. Minimum epidermal conductance was different in various crown positions of a forest tree. No universal trends were detected in correlation between minimum epidermal water loss and shoot position in the crown of open site trees. At different study sites, minimum epidermal conductance of needles varied in a broad range. However, air pollution was not found to increase minimum water loss from needles.

5. Minimum epidermal conductance measured in shoots from the same branch varied significantly. Scanning electron microscopy revealed incompletely closed stomata on desiccating needles, whose presence may be a cause of variability of minimum water loss.

The results of the investigation showed that the water economy of spruce needles is similar in different localities of Estonia. Both natural variability and differences in local climate have a stronger impact on the share of desiccated needles throughout winter than have differences in the air pollution level in different localities.

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ABSTRACT

This thesis presents a detailed analysis of two components of water vapour diffusion from the needles from *Picea abies* (L.) Karst. — water loss through the stomatal antechambers and minimum water loss through the cuticle.

The dimensions of stomatal antechambers and the number of the stomata per unit leaf area were determined in light and shade needles of 9...100-year-old spruce trees. The results showed that despite the pronounced differences in antechamber dimensions in sun and shade needles, the calculated resistance of a single stomatal antechamber to water vapour diffusion was similar. Antechamber resistance per unit of leaf area depends on stomata density. In the case of fully opened stomata, the proportion of antechamber resistance exceeded a third of the total resistance to water vapour diffusion.

The variability of minimum epidermal water losses has been studied by direct measuring of water loss from the desiccating needles and by two indirect methods which were used to evaluate the changes of the epicuticular wax layer. The indirect methods were the droplet contact angle method showing the wettability of the needle surface and the SEM studies showing the structure of epicuticular wax. In all these cases variability between different needle age-classes and positions in the tree crown were studied. Attention was also paid to the influence of several air pollution situations occurring at various sites of Estonia.

Needle wettability increased in the course of needle ageing. In the lower part of the tree crown the increase of minimum epidermal conductance during needle ageing was faster than in the upper crown. However, the needles from two neighbouring trees showed larger differences in wettability than the trees growing at sites with different air pollution load. Removal of wax decreased wettability. Therefore I conclude that droplet contact angle method is not suitable for monitoring air pollution in Estonia.

Epicuticular wax on current year shade needles was more degraded than in sun needles. In half-year-old needles from adult spruces at 12 sites of Estonia no crystalloid wax was found. Despite the different exposure of the study sites to air pollution, the wax degradation from tubular into flattened structures at the sites was similar. The observations of epicuticular wax structures of young monoclonal spruces five years later showed slower degradation speed. However, great variability in wax structure within a sample and even within the same needle was found. There was no correlation between wax degradation and the overall air pollution level.

In the cause of needle ageing their minimum epidermal conductance increased. The conductance of sun and shade needles of a forest tree was different. At open sites there were no universal trends in correlation of minimum

epidermal conductance and shoot position in the crown. At different study sites the minimum epidermal conductance of needles was different. However, the differences can not be explained by the air pollution. In ten shoots from the same branch the value of this conductance differed threefold, ranging from 1.3 to 4.3×10^{-5} m s^{-1}. No correlation of minimum epidermal conductance and needle water content was found. The variability may be explained by incompletely closed stomata revealed by SEM on desiccating needles. The water loss from these pores and the percentage of them causing the measured difference in conductance were calculated. Since there are suspicions that minimum epidermal conductance may be influenced by air temperature and humidity only sample series examined in the same conditions were compared.

KUTIIKULA JA ÕHULÕHE EESKAMBRITE JUHTIVUS HARILIKU KUUSE (*Picea abies*) OKASTEL

Kokkuvõte

Alates 1970. aastatest on täheldatud Euroopa maades okaspuude massilist suremist. Puude vanemate aastakäikude okkad pudenevad ning okaste keskmine vanus väheneb. Nähtus on levinum saastatud õhuga piirkondades ning seda seostatakse põhiliselt happevihmadega. Üheks kahjustusmehhanismiks on peetud muutusi okaste veemajanduses. Seega osutus vajalikuks uurida veeauru difusiooni okastest ning saada ülevaade difusiooniteedel olevate takistuste suurustest ning varieeruvusest. Põhilist veeauru liiikumisteed läbi õhulõhede on võrdlemisi hästi uuritud. Vähem on uuritud õhulõhede eeskambrite takistust. Samas on teada, et hariliku kuuse (*Picea abies*) saasteainetundlikel kloonidel on okkad väiksemad ja kergemad kui mittetundlikel kloonidel. Järelikult võivad erineda ka nende okaste eeskambrite ehitus ning veeauru juhtivus. Sellealaseid uurimusi pole seni aga tehtud.

Hariliku kuuse okaste välispind allub välismõjudele mitme aasta jooksul. Samaaegselt toimub selle kaudu ka pidev veeauru difusioon. Absoluutväärtuselt on kutikulaarne transpiratsioon küll üle kümne korra väiksem stomataarsest transpiratsioonist (Larcher 1995), kuid ta on pidev ka puule ebasobivate kliimatingimuste korral. Kui kutikulaarne transpiratsioon okastest mingil põhjusel suureneb (näiteks õhu saasteainete toimel) või ebasobiva kliima korral (näiteks pikk talv, mille jooksul puu tüvi on pidevalt külmunud), võib veekadu okastest muutuda letaalseks. Kutikulaarset veekadu on uuritud mäestikus kasvavatel okaspuudel. Vähem tähelepanu on pööratud selle veekao looduslikule varieeruvusele metsapiirkonnas. Samuti puuduvad andmed reaalselt välisõhus eksisteerivate saasteainete mõjust veekao suurusele. Eespool toodut arvestades on käesolevas töös põhitähelepanu koondatud hariliku kuuse kutikulaarse veekao uurimisele, eesmärgiga selgitada välja selle varieeruvus sõltuvalt looduslikest faktoritest ning kasvukoha saastatusest. Samuti on püütud leida vastust küsimusele, kas ja millistel tingimustel võib liigne veekadu okastest põhjustada Eestis kasvavate kuuskede kuivamist.

Vesi aurub taimelehtedest aururõhu erinevuste tõttu rakuvaheruumides ja välisõhus. Veeauru liikumisele avaldatavat takistus jaotatakse sõltuvalt põhjustajast ja regulatsioonimehhanismist mitmeks osaks (jn. 1).

Piirkihi takistus (r_a) on seotud väheliikuva õhukihi tekkega lehepinna lähedal. Tuule kiirusel üle 3...5 m s^{-1} on r_a väärtus enamikul lehtedel väga väike. Madalama tuulekiiruse korral sõltub r_a väärtus lehe morfoloogiast — laiadel, karvastel ning ebatasastel lehtedel on r_a väärtus kõrgem. Okaspuude okkad on kitsad ning seega on nende r_a väike. Hariliku kuuse (*Picea abies*) okastel mõõ-

deti r_a väärtuseks 1 s cm^{-1} juhul, kui tuule kiirus oli 0,25 m s^{-1} ning 0,3 s cm^{-1} tuule kiirusel 1 m s^{-1}. Torkaval kuusel (*Picea pungens*) on mõõdetud r_a 0,46 s cm^{-1} ning Sitka kuusel (*Picea pungens*) 0,12 s cm^{-1} tuule kiirusel 0,35...1,5 m s^{-1}. Nii madalad takistused ei avalda olulist mõju vee aurumisele okastest. Paljud kõrrelised on oma lehti kokku rullides võimelised r_a suurust aktiivselt muutma. Okaspuuokastel sellist võimet pole. Okaste r_a sõltub nende pakkimise tihedusest võrsel. Samas on võrse efektiivne suurus mõjutatud ka teistest kasvutingimustest.

Õhulõhede takistus (r_s) sõltub õhulõhede avatusest, mõõtmetest ja tihedusest lehepinnal, olles ainus osa kogutakistusest, mida taim aktiivselt reguleerib. Ühe lehe kõik õhulõhed pole ühtemoodi avatud. Enamiku õhupilude laius jääb maksimaalsest väiksemaks. Sellest tulenev suurem r_s avaldab rohkem mõju vee aurumisele kui fotosünteesile, sest viimase kogutakistusest moodustab enamuse mesofülli takistus. Veepuuduse korral sulguvad õhulõhed maksimaalselt, kuid vahel pole see täielik. Mõnel juhul on häiritud õhulõhede regulatsioonimehhanism. Ka tolmuosakesed, vetikad ning seenehüüfid võivad mehhaaniliselt takistada õhulõhede täielikku sulgumist.

Paljudel taimedel (sealhulgas enamusel okaspuudest) on õhulõhe sulgrakud sügaval epidermis. Sulgrakkude kohal olevat ruumi nimetatukse õhulõhe eeskambriks. Eeskambrid avaldavad veeauru difusioonile lisatakistust (r_{ch}). Takistuse suurus sõltub eeskambrite suurusest ning õhulõhede tihedusest lehepinnal. Okaspuude õhulõhe eeskambrid on täidetud epikutikulaarse vahakihiga, mille täiendavat mõju r_{ch} väärtusele on raske hinnata. Sitka kuusel arvatakse eeskambri vahakiht suurendavat r_{ch} suurust umbes kahekordseks. Valgus- ja varjuokastel on erisuguse paksusega epiderm ning seega on ka nende õhulõhe eeskambrid eri suurusega.

Kutikulaartakistuse (r_c) põhjustajaks on epidermi välimine kutiniseerunud kiht, mis enamikul taimedest on tõhus kaitse liigse aurumise vastu. Liigiti kutikula paksus ja keemiline koostis erinevad. Igihaljastel taimedel, mis peavad taluma pikaajalist veepuudust, on kutiikula paks ja r_c väärtus kõrge. Kutiikula koosneb orgaanilistes lahustites lahustuvatest lipiididest ja lahustumatust polümeerist kutiinist. Enamikul taimeliikidest on sellessse kihti põimunud polüsahhariididest koosnev võrgustik. Polüsahhariidid on kutiikula all oleva rakuseinaga ilmselt ühenduses ning põhiliseks veeauru difusiooniteeks. Paljudel liikidel (sealhulgas harilikul kuusel) ei ulatu polüsahhariidide võrgustik kutiikula välispinnale. Seetõttu on nende liikide kutikulaartakistus kõrgem kui liikidel, mille polüsahhariidide võrgustik ulatub kutiikula välispinnani (näiteks *Picea sitchensis*).

Harilikul kuusel nagu paljudel teistelgi okaspuudel on okaste pind kaetud epikutikulaarse vahakihiga. Kõige rohkem on vaha õhulõhe eeskambrites ning vahetult nende ümber. Vaha hulk okastel on pärilikult kindlaks määratud. Epikutikulaarse vaha koostisosad on keemiliselt püsivad ning ilmselt ei reageeri otseselt saasteainetega. Vaha kui okaste biosünteesi lõpp-produkt on õhulõhe

sulgrakkude emarakkudel olemas juba enne punga puhkemist ning tema kogus okka vananedes väheneb mõnevõrra. Samuti muutub veidi erinevate vaha-komponentide osatähtsus. Okka vananedes ning mitmete keskkonnafaktorite toimel epikutikulaarse vahakihi välimus muutub. Skaneeriva elektronmikros-koobiga on eristatud mitmesuguseid vahastruktuure. Vastpuhkenud hariliku kuuse okaste eeskambrid on täidetud pikkade, seest tühjade vahatorukestega. Okaste vananedes need torud kleepuvad kokku ja moodustavad võrgutaolise moodustise. Hiljem kämpub vaha veelgi kokku ning moodustab koorikuid. Vahakihi algne ehitus kaob kiiremini saasteainete toimel. Hüdrofoobne vaha-kiht okaste kutiikulal vähendab veekadu läbi okkapinna. Suurema takistusega on noorte okaste kristalloidne ehk "pitsiline" vahakiht, sest ta pikendab oluliselt difusioonitee pikkust. Vahaplaatidel, mille vahele jäävad lõhed, on väiksem mõju vee aurumisele.

Epikutikulaarse vahakihi struktuure uuritakse ka kaudselt kontaktnurga meetodil. Lehepinnal oleva veetilga kontaknurk lehepinnaga näitab lehe märgu-vust ning sõltub lehepinna keemilistest omadustest ja karedusest. Epikutiku-laarse vahakihi karedus väheneb vaha ehituse lihtsustumisel. Mitmed isoleeri-tud vahakomponendid märguvad erinevas ulatuses. Kontaktnurga meetod on lihtne ning biomonitooringus laialt kasutatav. Okaste kontaktnurk varieerub puu võra piires. Vanemad okkad ja saastatud õhus kasvavad okkad märguvad paremini. Okaste töötlemine O_3 ja/või happelise uduga suurendab samuti nende märguvust. Paremini märguvad okkad võivad omakorda rohkem omastada vees lahustuvaid saasteaineid. Kontaktnurga meetodi tulemustesse tuleb siiski suhtuda mõningase ettevaatusega. Kui okkapind vahakihi all on ebatasane, võib vahakihi täielik eemaldamine okaste märgumist hoopiski vähendada. Samuti võivad okkapinnale sattunud osakesed mõjutada okka kontaktnurka.

Kutikulaarset veekadu on kerge mõõta hüpostomataarsetel lehtedel — pärast õhulõhedega lehe külje katmist vettpidava materjaliga saab veeaur liikuda ainult läbi lehe kutiikula. Kutiikula läbilaskvust veeaurule ja teistele gaasidele mõõdetakse ka lehest eraldatud kutiikulal. Lehelt, mille mõlemal küljel on õhulõhesid, on selliste õhulõhedeta kutiikulate saamine keeruline, kitsastelt okastelt aga peaaegu võimatu. Amfistomataarsetel lehtedel hinnatakse tavaliselt kutiikula veeauruläbilaskvust, mõõtes nende massi kadu pärast lehe taimelt eemaldamist. Veeauru difusioonitakistuse väärtuse arvutamiseks on vaja teada ka keskkonna temperatuuri ja suhtelist niiskusesisaldust. Et selle meetodiga on võimatu eraldada veekadu läbi õhulõhede ning kutiikula, soovitatakse termini "kutikulaar-" asemel kasutada "pinna-" või "vähim epidermi-". Viimaste aastate kirjanduses kasutatakse rohkem vähimat epidermijuhtivust g(min) kui -takistust $(g(min)=1/r_c)$, sest juhtivus on võrdeline transpiratsiooni intensiivsusega. Eri autorite mõõdetud g(min) väärtused perekonna *Picea* liikidel on koondatud tabelisse 1. Eri töödes avaldatud g(min) väärtused varieeruvad, sõltudes uurin-guks kasutatud meetodist. Enamik andmeid on arvutatud, kasutades üksiku võrse massi muutust ajas. Eri uuringutes on massimuutuste mõõtmiseks kasuta-

tud erisugust ajavahemikku. Samuti pole võrsete hoiutingimused katse vältel alati samad.

Kutiikula läbilaskvust mõjutavad mitmed kasvutingimused ning saasteained. Kutiikula arengut takistab ebasobiv kliima — tuulepoolsel puupiiril on hariliku kuuse okaste kutiikula paksuseks mõõdetud 30%, võrreldes orus kasvanud okaste kutiikuli paksusega. Suurenenud veekadu okastest võib olla üheks kõrgusliku puupiiri põhjuseks. *Kampfzone* (moondunud puudega ala kõrgusliku puistupiiri ja puupiiri vahel) muld külmub talvel kuni 1 meetri sügavuseni pikaks ajaks ning taimedel ei ole võimalik mulla vett omastada. Sel ajal sõltub taimede ellujäämine kudedes oleva vee hulgast ning aurumise ulatusest. Nendel puudel, mille kutiikula ei ole piisavalt paks ning aurumine on seega küllalt suur, võib kevadeks tekkida veepuudus.

Kahjustunud või kollaseks muutunud okaste veekadu on samuti suur. Hariliku männi okaste veepotentsiaal kevadel oli seda madalam, mida suurem oli okastel oleva väävli hulk. Ka osooniga töötlemine suurendas g(min) väärtust. Happelise udu (pH=3) mõjul okaste veepotentsiaal langes. Eriti suured olid kasvuhoones happelise uduga töödeldud ning töötlemata puude erinevused kevadel päiksepaistel. Kõrgemal temperatuuril veeauru defitsiidi erinevus okaste ja välisõhu vahel suureneb ning transpiratsioon kasvab. Okaste soojenemine varakevadel, kui muld on veel külmunud, põhjustab puude täiendavat kuivamist.

Vähima epidermijuhtivuse väärtusi on kasutatud okaspuude talvise veemajanduse modelleerimiseks. Sowell koos kaasautoritega näitas oma töös, et mudel, mis on tehtud, kasutades laboris saadud g(min) väärtust liigil *Picea engelmannii*, ennustas tegelikkusest tunduvalt suuremat talvist veekadu. Sellist tulemust seostati mudelis kasutatud kliimaandmete ebatäpsusega. Samas oletasid autorid, et g(min) väärtus võib madala temperatuuri ja kuiva õhu tõttu langeda. Vähim epidermijuhtivus võib sõltuda ka kutiikula niiskusesisaldusest. Mudeli tegemiseks on vaja teada veel okastesse juurdetuleva vee hulka. Tüvest on vesi kättesaadav juhul, kui selle temperatuur on üle –4°C. Otsest vee omastamist sulavast lumest, härmatisest ja udust läbi okka või võrse telje pinna on peetud tavaliselt võimatuks. Siiski demonstreeris Katz koos kaasautoritega, et hariliku kuuse võrse telg imab 200 min jooksul pealepritsitud veest 80 mm^3 1 g kuivmassi kohta. Imamine toimus läbi telje koore mööda parenhüümirakke ksüleemini.

Käesolevas töös on pööratud tähelepanu põhiliselt õhulõhe eeskambritele ning kutiikulale kui veeauru difusiooni takistajatele. Kummagi takistuse suurust taim aktiivselt ei reguleeri, kuid mõlemad varieeruvad sõltuvalt kasvutingimustest. Samuti avaldavad mõlemad olulist mõju taime veemajandusele äärmuslikus olukorras — õhulõhede eeskambrid täiesti avatud õhulõhe korral, kutiikula veedefitsiidi ja/või õhu saastatuse korral. Käesolevas töös on veeauru difusiooni maksimaalselt suletud õhulõhede korral püütud hinnata otseste ja kaudsete meetoditega alates 1984. aastast. Eriakirjanduses on selle aja jooksul

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jõutud paljude väga oluliste tulemusteni, muutunud on seisukohad nii mitmeski metoodikat puudutavas küsimuses. Selleks on kaasa aidanud uuringuteks vajaliku aparatuuri pidev täiustumine. Käesolevate teeside kirjutamise ajaks olemasolevaid teadmisi on kasutatud ka varasemate tööde analüüsil ning seega ei ühti teesides esitatavad tulemused vahel artiklites leiduvatega.

Teesides uuritakse järgmisi probleeme:

1. Õhulõhe eeskambrite muutlikkus puuvõra eri osades, eeskambrite suuruse mõju nende poolt avaldatavale veeauru difusiooni takistusele.
2. Kontaktnurga meetodi sobivus Eesti saastatuse hindamiseks.
3. Epikutikulaarse vahakihi struktuuride muutlikkus võra eri osades, õhu saastatuse mõju nendele struktuuridele.
4. Vähima epidermijuhtivuse muutlikkus okaste vananedes, eri võraosades ning erisuguse õhusaastatuse korral.
5. Vähima epidermijuhtivuse muutlikuse põhjused.

Töös on kasutatud hariliku kuuse erivanuselisi võrseid Eesti eri paigust (tabel 2; jn. 2A). Enamik uuringuid on tehtud looduses kasvavate täiskasvanud puude võrsetega. Monoklonaalsetel noortel puudel uuriti epikutikulaarse vahakihi struktuure. Õhulõhe eeskambrite mõõtmed leiti okka piki- ning ristlõikudelt tehtud mikrofotodelt. Saadud tulemustest arvutati ühe eeskambri takistus veeauru difusioonile (R_{ch}). Õhulõhede pindtihedus loeti mikroskoobi all ning kasutati õhulõhe eeskambrite poolt okka pinnaühikule tekitatava veeauru difusioonitakistuse (r_{ch}) leidmiseks. Kontaktnurk määrati suurendusaparaadis horisontaalselt oleva okka ning sellele asetatud veetilga suurendatud kujutiselt. Kutiikula märgumise mõõtmiseks kasutati okkaid, millelt epikutikulaarne vahakiht oli maha pestud.

Skaneeriva elekronmikroskoobiga uuriti epikutikulaarse vahakihi struktuure ning õhulõhede avatust. Eri kasvukohtadest pärit täiskasvanud puude okkad kaeti kullaga otse katoodi all. Vahemaa proovi ja katoodi vahel oli 20 mm. Et selline katmismeetod tõi endaga kaasa temperatuuritõusu, mis võis epikutikulaarset vaha sulatada, siis paigutati teised proovid katoodist kaugemale ning nende pind pöörati katoodi suhtes nurga alla.

Eri kasvukohtadest pärit okaste epikutikulaarse vaha struktuure hinnati viiepallise skaalaga (fotod artiklis II, jn. 2). Õhulõhede lahtiolekut uuriti mitmetel erivanuselistel okastel. Enne kullaga katmist eemaldati eetriga nendel okastel olev vahakiht. Harilikult uuriti vähimat epidermijuhtivust, mõõtes kaalukadu okastest 4...14 tunni jooksul pärast katse algust. Samal ajal mõõdeti ruumi temperatuur ning RH. Saadud tulemustest arvutati g(min). Juhtivuse muutlikkus ühe oksa piires arvutati massikaokõveratest, mis saadi võrse kaalumisel iga 10 minuti tagant. Kogu selle katse jooksul olid need võrsed kliimakambris (t = 22°C, RH = 45%). Ühe aastakäigu okaste vähimat epidermijuhtivust uuriti kahe ja poole aasta jooksul. Teisi okkaid mõõdeti sügiseti. Ainult g(min) mõõtmine täiskasvanud metsapuul toimus kevadel enne pungade puhke-

mist. Okaste pindala mõõdeti otseselt, kasutades 10 okast võrselt, või klaaskuulikeste meetodil.

Eeskambrite mõõtmed ühe oksa piires ei varieerunud oluliselt. Ka olid üksteisega sarnased samas kasvukohas kasvanud noorte puude eeskambrid. Erinevusi leiti ühe puu valgus- ja varjuokaste eeskambrite mõõtmetes (tabel 3). Samuti erinesid erivanuseliste puude eeskambrid. Kõige rohkem varieerus eeskambrite sügavus, sõltudes epidermi paksusest. Ühe õhulõhe takistus veeauru difusioonile oli uuritud puudel sarnane, kuna pikemad ja laiemad kambrid olid ühtlasi ka sügavamad (jn. 3). Õhulõhede pindtihedus põhjustas r_{ch} väärtuste rohkem kui kahekordse erinevuse.

Okaste märgumine suurenes nende vananedes võra alaosas kiiremini kui ülaosas (jn. 4). Eri kasvukohtades varieerus β suurtes piirides (jn. 5). Kõige väiksem kontaktnurk oli ühel Vilsandi kuusel. Sama kasvukoha teise kuuse β oli üks kõrgemaid mõõdetutest. Kõrvuti kasvavate puude märgumine varieerus mõnel juhul rohkem kui eri kasvukohtade oma. Olulisi erinevusi uuritud kasvukohtade okaste märgumises ei leitud. Epikutikulaarse vahakihi eemaldamine okastelt vähendas nende märgumist (jn. 6).

Noore okka eeskambrid sisaldavad amorfsete vahatorukeste struktuuri, mida kirjanduses nimetatakse kristalloidseks. Metsapuu viimase aasta valgusokastel oli vahakiht kristalloidsem ehk "pitsilisem" kui varjuokastel (jn. 7A; B). Okaste vananedes kleepus nende vahakiht kokku ning vanematel valgus- ja varjuokastel vaha ehituse erinevusi enam ei täheldatud. Käesolevas töös loobuti vahakihi välimuse hindamisel statistika kasutamisest. Kõrvuti olevate eeskambrite vahakiht võis olla väga erinev (artikkel III, jn. 3). Kõige rohkem varieerus vaha välimus viimase aastakäigu okastel. Vanemate okaste eeskambrites olev vahakiht oli ühtlasema välispinnaga. Mõnel juhul oli võrsesisene vaha välimuse muutlikkus sama suur kui erinevatel puudel (artikkel III, tabel 3). Seetõttu kasutati erinevate kasvukohtade okaste vahakihi kirjeldamiseks ainult eri eeskambrite vahale antud hindepallide keskmisi väärtusi. Üheltki looduslikult kasvavalt täiskasvanud puult ei leitud täiesti kristalloidset vaha. Noortel monoklonaalsetel puudel oli kahe viimase aastakäigu vaha paremas olukorras kui samavanuselistel täiskasvanud puu okastel. Mõlemal juhul erinesid eri kasvukohtades peamiselt kahe viimase aastakäigu okkad. Vanemate okaste vahakiht oli kõikides kasvukohtades ühesugune. Eri kasvukohtade vahakihi ehituses ning kadumise kiiruses ei leitud olulisi erinevusi.

Ilma epikutikulaarse vahakihita õhulõhe uuringud SEM-i abil näitasid, et pärast kuivamist oli enamik okkal olevaid õhulõhesid suletud. Siiski jäi mõnel juhul õhulõhe osaliselt avatuks ning õhulõhe sulgrakkude vahel võis näha 0,2...0,3 µm laiust pragu. Lahti jäänud õhulõhede arvu okka pinnaühikul oli võimatu määrata, sest enamik õhulõhe eeskambreid polnud mikroskoobi vaateväljas otse.

Esimestel kuivamise tundidel oli veekadu okastest kiire. Umbes kolme tunni pärast muutus kaalukadu aeglasemaks õhulõhede maksimaalse sulgumise tõttu (jn. 8). Ometi jätkus kogu 48-tunnise katse käigus okaste juhtivuse (g) langus. Selline pidev g langus tekitab küsimuse, milline ajavahemik üldse sobib vähima epidermijuhtivuse g(min) mõõtmiseks. Eelnevates katsetes olime selleks kasutanud tavaliselt ajavahemikku 4...14 tunnini pärast võrse lõikamist. Et esialgne kiire kaalukadu on selleks ajaks lõppenud ning okaste veesisaldus on samas veel küllalt kõrge, tundub see olevat parim ajavahemik g(min) mõõtmiseks. Vähima epidermijuhtivuse väärtused olid lognormaalse jaotusega (artikkel, V jn. 3). Seega kasutati statistilisel analüüsil andmete logaritmilist teisendust. Töös on esitatud iga katseseeria kohta erinevate proovide g(min) väärtuste geomeetriline keskmine. Ühe oksa eri võrsete g varieerus suures ulatuses (artikkel VI, jn. 3). Vähim epidermijuhtivus jäi vahemikku 1,3 ja 4,3 × 10^{-5} m s^{-1} geomeetrilise keskmisega 2,26 × 10^{-5} m s^{-1}. Võrse g(min) väärtuse ning võrset iseloomustavate arvuliste tunnuste lineaarset seost ei leitud (tabel 4). Okaste vananedes nende vähim epidermijuhtivus kasvab (artikkel V, jn. 2). Ühe aastakäigu okaste uurimine mitme aasta jooksul näitas, et g(min) pideva tõusu käigus sellel ajavahemikul oli ka lühikesi perioode, mille vältel g(min) langes (artikkel V, jn. 4). Nimetatud g(min) väärtuse kõikumisi ei olnud võimalik seletada labori õhutemperatuuri ega RH muutustega. Metsas kasvava puu erivanuselistel valgusokastel oli g(min) kõrgem kui sama aastakäigu varjuokastel. Okaste vananedes kasvas g(min) mõlemat tüüpi okastel võrdse kiirusega. Kõikidel uuritud prooviokstel suurenes vähim epidermijuhtivus pärast epikutikulaarse vahakihi eemaldamist. Statistiliselt oluline oli see muutus noorematel (C ja C+1) okastel. Ka ilma epikutikulaarse vahata kasvas g(min) okaste vananedes, kuigi statistiliselt usaldusväärne oli see muutus ainult varjuokastel. Avamaastikul kasvavate kuuskede okaste g(min) väärtus ei sõltunud okaste kasvukohast puu võras (artikkel V, jn. 1). Kasvukoha ilmakaar avaldas küll mõlemal uuritud puul mõju viimase aasta okaste g(min) väärtusele, kuid erinevatel puudel oli see mõju vastupidine. Samuti ei leitud sõltuvust vähima epidermijuhtivuse ja õhulõhede pindtiheduse vahel. Sellest võib järeldada, et õhulõhe sulgrakkude välispinna kaudu ei toimu oluliselt suuremat veekadu kui läbi kutiikula.

Eri kasvukohtades mõõdetud g(min) väärtused on esitatud tabelis 4. Viimase aasta okastel oli g(min) kõige kõrgem Alskas (prooviala nr. 12), C+3 okastel Tipus (prooviala nr. 9). Vähima epidermijuhtivuse muutumise kiirus okaste vananedes (C+3/C) oli kõige kõrgem Tipus (prooviala nr. 9) ning kõige madalam Vigalas ja Alskas (proovialad nr. 5 ja 12). Kõik nimetatud kasvukohad asuvad suurematest õhusaasteallikatest kaugel ning nende lähedal ei ole nimetamisväärset tööstust. Lumega sadenes kõikides nendes piirkondades 0,10 kuni 0,15 g sulfaatioone ruutmeetrile (jn. 2B). Piirkondades, mille õhusaastatus eeldati olevat kõrgem — proovialal nr. 7 (Rakkes, 3 km kaugusel lubjatehasest) ning proovialal nr. 4 (Kiviõlis, 2 km kaugusel põlevkivikeemiakombinaadist) — polnud okaste g(min) väärtus oluliselt kõrgem kui teistes kasvukohtades.

Õhulõhe sulgrakkude kohal olevad eeskambrid avaldavad lehe gaasivahetusele täiendavat takistust. Valgus- ja varjuokastel ühe eeskambri avaldatav R_{ch} ei erinenud. Olulised r_{ch} väärtuste erinevused olid seotud õhulõhede pindtihedusega. Järelikult tuleb r_{ch} mõju hindamisel kogutakistusele pöörata põhitähelepanu õhulõhede pindtiheduse väärtusele. Õhulõhede pindtihedus sõltub tavaliselt okaste valgustingimustest. Samas on õhulõhede pindtihedus noorte puude okastel sama madal kui täiskasvanud puu varjuokastel.

Eeskambrites olev epikutikulaarne vaha suurendab nende takistust veeaurule umbes kaks korda. Uurimaks r_{ch} osatähtsust kogu veeauru difusioonitakistuses, arvutati transpiratsiooni intensiivsus takistuse eri komponentide olemasolu puhul (jn. 11). Arvutused näitasid, et hästi avatud õhulõhede korral on õhu-eeskambrite takistus transpiratsioonile oluline. Kui õhulõhe oli lahti 4×10^{-6} m, siis r_{ch} moodustas umbes poole kogu transpiratsioonitakistusest. Õhulõhe sulgudes eeskambrite osatähtsus vähenes. Kui õhupilu laius oli $0,25 \times 10^{-6}$ m, moodustas r_{ch} ainult umbes 13% kogu transpiratsioonitakistusest

CO_2 omastamisele mõjub eeskambrite takistus vähem kui transpiratsioonile, sest CO_2 difusiooni mõjutab oluliselt mesofülli takistus. Seega võib eeskambreid vaadelda kui mehhanismi, mis tõstab taime veekasutuse kasutegurit. Sama ülesannet täidab ka õhulõhede mittetäielik avanemine. Õhulõhe eeskambrite osatähtsus taime veemajanduses muutub väga oluliseks juhul, kui õhulõhed saavad viga või kui nende regulatsioonimehhanism kahjustub.

Hariliku kuuse viimase aastakäigu okaste kontaktnurk β on eri autorite andmetel vahemikus 48...105°. Nii vähe märgusid käesolevas töös ainult ühe Vilsandis kasvava puu (prooviala nr. 15) kuuseokkad. Kõikidel teistel puudel oli β väärtus 110...125°. Kirjanduses on andmeid, et okaspuude võra ülaosas on okaste märguvust väiksem kui alaosas. Käesolevas töös viimase aastakäigu okaste märguvus puu piires oluliselt ei varieerunud. Võra alaosas suurenes okaste märguvus nende vananedes kiiremini kui puu ülaosas. Selliseid tulemusi on raske seletada, sest võra ülaosas on otsese päikesekiirguse ning tuule mõju okastele suurem. Mõlemad nimetatud tegurid kiirendavad epikutikulaarse vahakihi struktuuride hävimist. Tegureid, mis põhjustavad võra alaosa okaste suuremat märguvust, on raske oletada.

Arvamused õhu saastatuse mõjust okaste kontaktnurgale on vastandlikud. Mitmes töös väidetakse, et O_3 ja/või happeline udu suurendab kliimakambris kasvavate okaste märguvust. Samal ajal ei leia teised autorid sõltuvust õhu saastatuse ning okaste kontaktnurga vahel. Peale O_3 ja sademete happelisuse võivad looduslikes tingimustes kasvavate okaste märguvust mõjutada okastele sadenevad tahked osakesed (kaasa arvatud tolm). Okaste β sõltub ka puu vanusest. Eesti eri piirkondades kasvavate okaste märgumise mõõtmine ei näidanud β ja kasvukoha õhu saastatuse seost. Kontaknurga mõõtmise tulemused viivad samale arvamusele, mida on esitanud dr. Jagels: okaste kontaktnurga mõõtmine on kasutatav pikaajaliste muutuste jälgimiseks ühes piirkonnas samadel puudel.

Eri puude ning kasvukohtade märguvuse erinevusi on raske interpreteerida, sest mitmesugused looduslikud tegurid võivad varjutada õhu saastatuse võimalikku mõju. Kogutud andmete seletamisel tekkivate raskuste näiteks on tulemused, mis saadi uurides β suurust okastel, mille epikutikulaaarne vahakiht oli eemaldatud. Pärast vahakihi eemaldamist okaste märguvus vähenes. Sellise tulemuse põhjustajaks on kirjanduse andmetel okkapinna suurem karedus võrreldes epikutikulaarse vahakihiga. Ometi ei näita SEM-i abil saadud fotod vahata okastel mingeid olulisi ebatasasusi, võrreldes epikutikulaarse vahakihi tekitatutega.

Tänu tehnika arengule on viimastel aastakümnetel lehtede välispinda palju uuritud. Esimesed sellealased tööd tehti 1970. aastatel. Kasutatava tehnika tase mõjutab saadavaid tulemusi oluliselt. Preparaadi ülekuumutamine kullaga katmisel võib sulatada lehtedel olevat vahakihti. Samasugust mõju võib avaldada ka liiga kõrge kiirenduspinge mikroskoobis. Varajasemates uuringutes olid sellised artefaktid tavalised ning seega tuleb nende tulemustesse suhtuda kriitiliselt. Mõnel juhul oli kokkusulanud vahakiht nii sarnane epidermiga, et seda peeti õhulõhet katvaks membraaniks. Sama aparatuuriga, mida kasutati käesoleva töö tegemiseks, on saadud ka täiesti kristalloidse vaha kujutisi. Sellegipoolest jääb püsima kahtlus, et vähemalt eri kasvukohtadest pärit täiskasvanud puude epikutikulaarne vahakiht on proovide tegemise käigus liiga kuumenenud. Eri tööde käigus on leitud epikutikulaarse vaha plaatjaid moodustisi, mida peetakse sageli vaha loodusliku hävinemise tulemuseks. Samasugused plaadid tekkivad ka vahakihi mehhaanilisel vigastamisel. Oma töös leidsime selliseid "plaate" proovideit, mille temperatuur kullaga katmise käigus tõusis üle 60°C.

SEM-iga uuritakse tavaliselt saasteainete põhjustatud muutusi epikutikulaarse vaha ehituses. Esialgse vahastruktuuri hävimine okaste vananedes on tõestatud paljudes töödes. Hoopis vähem on teada vahastruktuuride looduslikust muutlikkusest. V. S. Kimi (1985) andmetel on võra lõunapoolsetel okastel rohkem kokkusulanud vahamoodustisi kui põhjapoolsetel. Sama uurimuse kohaselt oli vahastruktuur võra ülaosas rohkem kahjustunud kui alaosas. Günthardt-Goerg näitas oma töös, et varjuokaste vaha kokkukämpumine on umbes kuu aega varajasem kui valgusokaste oma. Meie töö tulemused näitasid viimase aastakäigu okaste vahakihi ehituse erinevusi: varjuokastel oli näha vähem "vahapitse" kui valgusokastel. Vanemate okaste vahakihis erinevusi ei leitud. Tulemuste lahknemine võib olla põhjustatud võra mikrokliima erinevustest. Vahakihi välimuse muutus võib olla tingitud lämmastiku olemasolust mullas, tuulest ja päikesekiirgusest. Otsese päikesekiirguse käes olevate okaste temperatuur võib tõusta oluliselt üle õhutemperatuuri. Vaha eri komponentide sulamistemperatuur jääb vahemikku 45...60°C. Seega on täiesti võimalik, et otsene päikesekiirgus soojendab okkaid sedavõrd, et mõned vahakomponendid sulavad ning vahastruktuurid kleepuvad kokku. Samas pole epikutikulaarse vahakihi ehitus ka eri puude okastel alati samasugune.

Vahakihi välimuse suured erinevused tekitavad raskusi õhu saastatuse mõju hindamisel. Suurem "pitsilise" vahakihi olemasolu monoklonaalsetel puudel

erinevalt täiskasvanutest võib olla põhjustatud puude erinevast vanusest või muutustest metoodikas. Samal ajal võib see olla tingitud õhu saastatuse vähenemisest Eestis uuringutevahelisel perioodil. Täiskasvanud puid uuriti 1988. aastal, monoklonaalseid puid 1993. aastal. Ajavahemikul 1980...1992 vähenes Eestis õhku paisatud sulfaatioonide kogus 40%. Erinevate kasvukohtade vahastruktuurid varieerusid kahe viimase aastakäigu okastel. Siiski on saadud tulemusi raske seletada. Näiteks oli monoklonaalsete kuuskede okastel, mis kasvasid kõrge väävlisaastega piirkonnas (prooviala nr. 4) epikutikulaarses vahakihis rohkem kristalloidset struktuuri kui piirkondades, kus oluline õhusaaste puudus (prooviala nr. 1 ja 9). Saadud tulemustest võib järeldada, et epikutikulaarse vahakihi kasutamine õhu saastatuse hindamiseks pole Eesti oludes põhjendatud.

Juhtivuse pidevat langust 48 tunni jooksul alates katse algusest on raske seletada. Pikaajaline g langus toob kaasa probleemi laborist kaugemal asuvate okaste uurimisel, sest kui määrata okaste veekadu hilisemal ajavahemikul, on g(min) väärtus ilmselt väiksem kui 4...14 tunni jooksul määratust. Juhtivuse arvutamisel on võetud eelduseks, et okaste RWC on 100%. Arvutused näitasid, et tegeliku RWC väärtuse arvestamine g(min) arvutamisel suurendab 14 tunni möödudes g(min) väärtust kuni 5%. Seega ei saa RWC väärtuse muutumist pidada juhtivuse langemise põhjuseks. Kerstiens (1996) oletas, et g(min) väärtusele mõjub kutiikula veesisaldus. Käesolevas töös tehtud katsed ei näidanud erinevate okaste g(min) ja veesisalduse seost.

Kõikide uuritud C-okaste g(min) varieerus vahemikus $0{,}9...4{,}5 \times 10^{-5}$ m s^{-1}, jäädes samasse vahemikku teiste autorite tulemustega. Kümnel ühe oksa võrsel mõõdeti g(min) vahemikus $1{,}3...4{,}3 \times 10^{-5}$ m s^{-1}, mis näitas, et ühe oksa piires on g(min) muutlikkus peaaegu sama suur kui kõikide uuritud puude ning kasvukohtade puhul. Sellist muutlikkust ühe oksa piires on raske seletada. A. Laisa (Laisk *et al.*, 1980) töö tulemustest võib järeldada, et kõik õhulõhed ei sulgu üheaegselt. Ilma epikutikulaarse vahakihita okaste uurimine SEM-iga näitas, et osa õhulõhesid ei sulgu täielikult ka pärast ööpäevast kuivamist. Osaliselt lahtijääva õhulõhe mõju hindamiseks okaste veemajandusele arvutati tema juhtivus (G$_s$). Hiljem arvutati osaliselt lahtijäänud õhulõhede vajalik arv okkapinnal, mis tagaks kümne võrse uurimisel saadud g(min) erinevuse (artikkel VI, jn. 5). Selgus, et umbes 6,5% varjuokka õhulõhedest peab jääma avatuks 0,2 μm, et põhjustada g(min) muutlikkus, mis leiti ühe oksa võrsete uurimisel. Kuna valgusokastel õhulõhede pindtihedus on suurem, on seal vajalike lahtiste õhulõhede suhtarv väiksem. Erinev mittetäielikult sulguvate õhulõhede osatähtsus võrsetel võib olla kogutud andmete lognormaalse jaotuvuse põhjuseks.

Okaste vananedes nende g(min) tõuseb. Tavaliselt on seda seostatud epikutikulaarse vahakihi vananemisega. Mitmeaastane katse ühe aastakäigu okastega ei lubanud oletada ühtegi ajavahemikku (ega seega ka kliimafaktorit), mis oleks

g(min) suurust mõjutanud rohkem kui teised. Ilma epikutikulaarse vahakihita okaste g(min) tõusis samuti okaste vananedes. Seega tuleb oletada, et peale epikutikulaarse vaha muutuste on veel mingi mehhanism, mis suurendab vanemate okaste g(min) väärtust. Selleks võivad olla okaste kutiikula enda muutused. Samuti võib okaste vananedes tõusta osaliselt lahtijäävate õhulõhede arv.

Kirjandusest on teada, et tuulele avatud ning tuule eest kaitstud okaste g(min) erineb. Metsapuu valgusokaste kõrgem g(min), võrreldes sama puu varjuokastega on ilmselt põhjustatud valgusokastele langevast suuremast päikesekiirgusest. Lagedal kasvava kuuse eri kõrgustele ning võra külgedele mõjuv tuul ning päikesekiirgus erinevad samuti. Ometi ei erinenud oluliselt lagedal kasvava puu eri piirkondadest pärit okaste g(min). Järelikult ei mõjuta mikrokliima erinevused nende puude võrsete võimalikku veekadu kuivamise käigus.

Mitmed autorid on näidanud, et happeline udu ja O_3 mõjutavad hariliku kuuse okaste veemajandust. Samas väidab Schreiber (1994), et tervete ja kahjustunud okaste kutiikula läbilaskevõime gaasidele ei erine. Esialgsed uuringud kolmes Eesti piirkonnas näitasid, et g(min) väärtus linnas (prooviala nr. 11) kasvas kiiremini kui maal (prooviala nr. 8). Kõige kiirem oli g(min) tõus tööstuspiirkonnas (prooviala nr. 4). Vähima epidermijuhtivuse määramine 12 proovialal üle Eesti ei näidanud kasvukohtade g(min) väärtustes mingeid erinevusi, mida saaks seostada õhu saastatusega. Kasvukohtade g(min) absoluutväärtuste võrdlemine on mõnevõrra küsitav seoses eespool käsitletud raskustega metoodikas. Samal ajal ei mõjutanud eri kasvukohtade õhu saastatus ka g(min) muutuse kiirust võrse vananedes (arvutatud kui C+3 ja C okaste g(min) väärtuste suhe).

Vähima epidermijuhtivuse väärtusi kasutatakse sageli okaspuude talvise veemajanduse modelleerimisel. Käesolevas töös arvutati võimalik veekadu okastest mitmesuguste g(min) väärtuste puhul (jn. 12). Uuritav vähima epidermijuhtivuse vahemik oli sama, mis saadi kümne sama oksa g(min) mõõtmisel. Mudelist selgub, et suhteliselt väike g(min) erinevus põhjustab suuri muutusi okaste pikaajalises veekaos. Seega ei kirjelda tavaliselt mudelites kasutatav mitme võrse keskmine g(min) kuigi hästi eri võrsete veesisaldust.

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ИЗМЕНЧИВОСТЬ И ЭКОФИЗИОЛОГИЧЕСКАЯ РОЛЬ ПРЕДВАРИТЕЛЬНЫХ ДВОРИКОВ УСТЬИЦ ХВОИ ЕЛИ ЕВРОПЕЙСКОЙ

Излагаются результаты изучения предварительных двориков устьиц на хвое разновозрастных елей европейских, а также на хвое различной морфоструктуры. Приведены расчетные величины их сопротивления диффузии водяного пара. Рассматривается значимость предварительных двориков устьиц в регуляции интенсивности транспирации.

У листьев растений с ксероморфной структурой устьица часто погружены в эпидерму, так что над ними образуется углубление, так называемый предварительный дворик устьиц [4]. Устьица с предварительными двориками свойственны и хвое ели европейской [12]. Известно, что предварительные дворики устьиц составляют определенное сопротивление диффузии углекислого газа из атмосферы в мезофилл листа и водяного пара в обратном направлении. Таким образом, их можно рассматривать как один из факторов, регулирующих фотосинтез и транспирацию. Среди малочисленных литературных источников, посвященных изучению предварительных двориков устьиц, имеется несколько работ, в которых сделана попытка оценить величину их диффузионного сопротивления и таким образом определить значимость в экологической физиологии растений [5, 11]. Однако отсутствуют данные об изменчивости размеров предварительных двориков и о размахе экоморфологически обусловленной регуляции физиологических процессов.

В настоящей работе рассматривается изменчивость размеров предварительных двориков устьиц ели европейской на хвое различной морфоструктуры (теневой и световой) и на хвое деревьев разного возраста. Также приводятся результаты расчета диффузионных сопротивлений предварительных двориков и анализируется их влияние на транспирацию.

Материал и методика

Объектом исследования служила световая и теневая хвоя соответственно с вершины и из нижней части кроны 100-летнего модельного дерева из района Вооремааской экологической станции (Эстонская ССР), а также хвоя из крон шести отдельно растущих деревьев 9—66 лет, находящихся вблизи полевой базы той же станции.

От каждой пробной ветки брали по одному побегу, а с каждого побега пять хвоинок, на которых под измерительным микроскопом МИ-1 с увеличением 40 пересчитывали число устьиц. Счет устьиц вели на каждой грани хвоинок на трех отрезках длиной 1 мм. На срезах тех же хвоинок измеряли ширину каждой грани. Для измерения размеров предварительных двориков изготовляли временные препараты поперечных срезов хвоинок и эпидермиса. Размеры определяли на увеличенных микрофотографиях. На препаратах эпидермиса для получения ясных контуров предварительных двориков эпикутикулярный воск растворяли этиловым эфиром. Средние размеры получены, как правило, не менее чем из 30 замеров.

Исходя из анатомических параметров, сопротивление устьиц и предварительных двориков к испарению проанализировал Ванге [5], который наблюдал при слегка загубленных устьицах Zebrina pendula

Schnizl. восемь последовательных компонентов сопротивления. При этом сопротивление предварительных двориков составляло только 2—5% от суммарного сопротивления. Ли и Гейтс [11] разделили устьице и предварительный дворик хвои сосны веймутовой в поперечном разрезе на 14 отрезков различной геометрической формы. Учитывая изменчивость устьиц и особенно их предварительных двориков даже на одном листе растения, такая детальность нам кажется неоправданной.

По нашему представлению, геометрическое строение предварительного дворика ели европейской целесообразно рассматривать как состоящее из двух геометрических форм (рис. 1), а возникающие при диффузии газов сопротивления — из четырех компонентов: сопротивления внешней эллипсообразной поры дворика — r_{e1}, сужающейся верхней части — r_{e2}, поры узкой нижней части — r_{e3} и сопротивления нижнего углубления — r_{e4}.

Расчетные формулы для определения диффузионного сопротивления круглых пор и мелких трубочек разработаны в начале нашего века [6]:
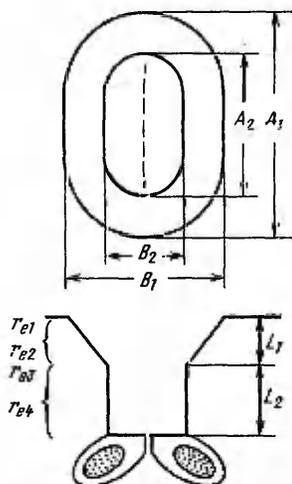
Рис. 1. Схематическое изображение предварительного дворика устьица ели европейской. Указана локализация различных компонентов сопротивления диффузии и замеры, использованные в расчетах диффузионного сопротивления. Условные обозначения см. в тексте и таблице

$$\text{Для поры } r_p = {}^1\!/_2 Dd, \qquad (1)$$

$$\text{Для трубки } r_t = 4l/D\pi d^2, \qquad (2)$$

где D — коэффициент диффузии газа (для водяного пара при 14° $D = 0,24$ см²·с⁻¹), d — диаметр поры и l — длина трубочки. При определении диффузии газов через поры с эллипсообразным отверстием в формулу (1) следует включить эффективный диаметр эллипса (т. е. диаметр круга, периметр которого соответствует периметру данного эллипса), а в формулу (2) — площадь эллипса. В случае, когда длина полуосей эллипса более или менее равна, вместо d в формулу (1) можно включить $(A+B)/4$, где A и B — длина полуосей эллипса. Общее сопротивление предварительного дворика получается суммированием этих компонентов. Таким способом можно рассчитать сопротивление диффузии через одиночный предварительный дворик. Обычно сопротивление выражают на единицу площади листа. Для получения этой величины сопротивление одиночного предварительного дворика надо разделить на поверхностную плотность устьиц.

Результаты и обсуждение

Анатомическое строение предварительных двориков и поверхностная плотность устьиц. Данные об анатомических замерах предварительных двориков приведены в таблице. Оказывается, больше всего различается по размерам глубина предварительных двориков. При этом у световой хвои старых деревьев они на 50—60% глубже, чем у их теневой хвои и у световой хвои молодых деревьев. Остальные размеры среди различных проб варьируют намного меньше.

Как показало наше исследование, в кроне взрослой ели существует тесная корреляция между поверхностной плотностью устьиц и световыми условиями произрастания хвои [3]. Но у молодых деревьев, растущих на открытой местности, поверхностная плотность устьиц напоминает больше теневую, чем световую хвою взрослых деревьев в лесу. Следовательно, можно полагать, что у ели существует онтогенетический фак-

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Размеры предварительных двориков устьиц (10^{-6} м) и поверхностная плотность устьиц, шт·мм$^{-2}$

Хвоя	Длина		Ширина		Глубина		Поверхностная плотность
	верхней части A_1	нижней части A_2	верхней части B_1	нижней части B_2	верхней части L_1	нижней части L_2	
Дерево 1							
Световая, с высоты 34 м (n=31)	62,0±4,7а	33,0±3,5	41,3±6,3	16,3±2,3а	12,2±2,5а	15,8±1,9а	47,5
Теневая, с высоты 12 м (n=27)	56,6±4,4	28,4±3,4	34,8±5,0	15,4±1,5	8,6±1,9	12,6±1,8	26,9
Дерево 2							
С высоты 2 м (n=38)	56,8	27,5	39,2	12,46	11,1а	11,2	27,0
Дерево 3							
С высоты 2 м (n=30)	55,8	31,6	34,7	13,9	7,1	10,3	37,7

Примечание. Дерево 1 — растет в ельнике сложном, возраст 100 лет; дерево 2 — свободно растущее, возраст 66 лет; дерево 3 — свободно растущее, возраст 10 лет. Для дерева 1 приведены стандартные отклонения средних величин. Величины, обозначенные различными буквами, статистически различны на уровне вероятности 0,95. n — количество изученных предварительных двориков в проᶦе.

тор регуляции количества устьиц на единицу площади листа, существование которой у травянистых растений показано в работе Н. Тиха [14].

Анатомическое строение устьиц пяти соседних и почти одновозрастных (9—12 лет) деревьев практически не различается. Поэтому в таблице приведены данные лишь для одного из них. Однако индивидуальные различия поверхностной плотности устьиц на побегах этих деревьев (32,2—45,5 устьиц на 1 мм²) могут быть почти соизмеримы с изменением этой величины у побегов одного дерева, растущих в совершенно различных световых условиях.

Диффузионное сопротивление предварительных двориков. Результаты расчета сопротивления диффузии водяному пару через предварительные дворики представлены на рис. 2. Несмотря на довольно значительные различия в размерах предварительных двориков отдельных побегов (например, между световой и теневой хвоей модельного дерева 1), их сопротивление различается очень мало. Это вызвано тем, что более глубокие предварительные дворики одновременно и шире, и длиннее. Увеличение ширины и длины компенсирует увеличение сопротивления за счет увеличения глубины. Максимальное различие сопротивления предварительных двориков у изученных деревьев составляет около 30% (2675—3754 с·см⁻³).

В экологическом смысле более важным, чем сопротивление отдельных устьиц, является среднее сопротивление листа, т. е. сопротивление, выраженное на площадь поверхности. В формировании этого параметра отчетливо выражается влияние варьирования поверхностной плотности устьиц (рис. 2). За счет ее различия величина $r_е$ может измениться двукратно: от 0,64 до 1,36 с·см⁻¹.

В приведенных расчетах предварительные дворики рассматриваются как свободные для диффузии газов пространства (рис. 3, *а, б*). Однако известно, что у хвойных они заполнены отложением эпикутикулярного воска [7, 9] (рис. 3, *в, г*). Эти восковые структуры увеличивают сопротивление, но насколько — определить весьма трудно. Сделаны лишь отдельные попытки оценить влияние этих структур на диффузию газов. Так, Джеффри с соавт. [9] на основе микрофотографий, сделанных сканирующим электронным микроскопом, предположил, что восковые отложения в предварительных двориках ели ситхинской увеличивают сопротивление примерно в 2 раза. Однако эта цифра весьма приближенная. Как показано в дальнейших работах, микроструктура эпикутикулярного воска очень изменчива. Она зависит от возраста хвои, влияния

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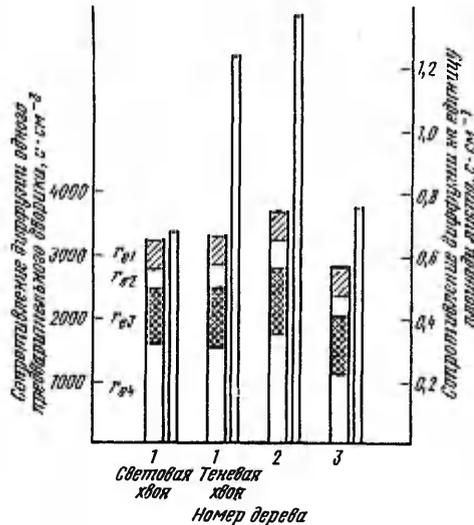
Рис. 2. Сопротивление диффузии водяного пара через
предварительные дворики.
Левые столбики — сопротивление одного предварительного дворика (указаны компоненты сопротивления), правые столбики — сопротивление, выраженное на поверхность площади хвои

метеорологических факторов и уровня загрязнения воздуха [8]. Те «кристаллические» микротрубочки воска, какими оии представлялись Джеффри с соавт., с возрастом хвои подвергаются слиянию, образуя «аморфный» воск (рис. 3, *г*). В условиях атмосферного загрязнения восковые пробки в предварительных двориках подвергаются сильной эрозии. Все эти явления обязательно влияют на сопротивление предварительных двориков и усложняют анализ их влияния на экологические функции хвои.

Экологическая значимость предварительных двориков. Предварительные дворики устьиц составляют дополнительное сопротивление диффузии углекислого газа и водяного пара, влияя таким образом на интенсивность фотосинтеза и транспирации. Для количественной оценки их влияния на эти процессы приходится учитывать и другие компоненты сопротивления диффузии.

Сопротивление устьичных щелей можно рассчитать согласно формуле Брауна и Эскомба, модифицированной Парлангом и Ваггонером [13], учитывая глубину, длину и ширину устьичных щелей. На рис. 4 приведены результаты расчетов транспирации на примере световой хвои модельного дерева 1, а также кроме сопротивления устьичных щелей общее сопротивление устьиц и предварительных двориков. При этом аналогично рассуждению Джеффри с соавт. [9] допускается, что восковые структуры увеличивают сопротивление пустых двориков вдвое. При составлении рис. 4 учтено и сопротивление пограничного слоя побега, установленное ими по скорости испарения воды с покрытых гипсом побегов [2]. Предварительные дворики могут оказывать существенное антитранспирационное влияние лишь в случае открытых устьиц. Так, при ширине устьичных щелей 4 мкм доля предварительных двориков больше половины общего сопротивления. По мере закрытия устьиц общее сопротивление увеличивается и доля предварительных двориков уменьшается. Так, если ширина устьичных щелей 0,25 мкм, то устьичное сопротивление составляет 87, а сопротивление предварительных двориков — 13% от общего сопротивления.

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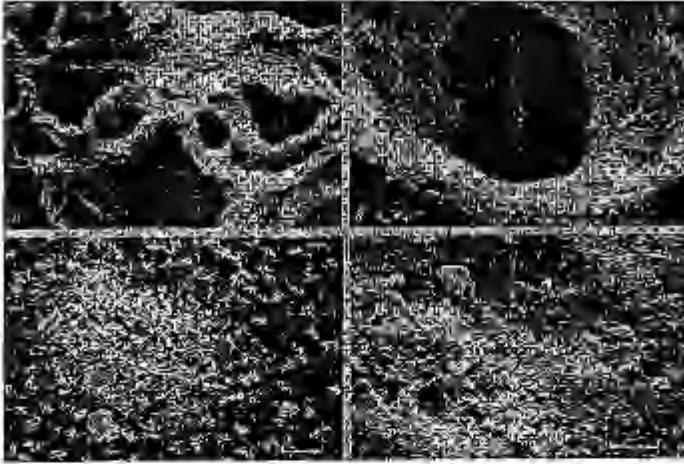
Рис. 3. Устьица ели европейской под сканирующим электронным микроскопом: *а* — разрез устьичного аппарата. Эпикутикулярный воск размазан по предварительному дворику при разрезании хвоинки. Пунктирной линией показано очертание предварительного дворика. *б* — вид предварительного дворика сверху. Воск удален эфиром. *в* — структура «кристаллического» воска на только что распустившейся хвое. *г* — структура «аморфного» воска в предварительном дворике на 4-летней хвое. Длина белых столбиков соответствует 10 мкм.
Фотографии сделаны на СЭМ «Тесла *BS*-300» в лаборатории электронной микроскопии Таллинского политехнического института У. Каллавус

Вопросы экологической значимости предварительных двориков рассматривались в работах Х. Вальтера [1], В. Лархера [10] и Джеффри с соавт. [9]. Они пришли к выводу, что предварительные дворики транспирацию уменьшают в большей мере, чем фотосинтез, поскольку на фотосинтез ограничивающее влияние оказывает и внутрилистовое

Рис. 4. Регуляция интенсивности транспирации светового побега ели в зависимости от степени открытости устьиц: *1* — предполагаемая транспирация, если бы устьица находились на поверхности хвои; *2* — предполагаемая транспирация в случае отсутствия устьиц, т. е. если предварительные дворики открывались прямо в мезофилл; *3—5* — транспирацию ограничивают все компоненты сопротивления (скорость ветра 1,0 (*3*); 0,2 м·с⁻¹ (*4*); 0, т. е. абсолютный штиль (*5*)). Условия расчета: температура воздуха и хвои 20°, относительная влажность 60%, сопротивление предварительного дворика вместе с восковым наполнением 2 с·см⁻¹
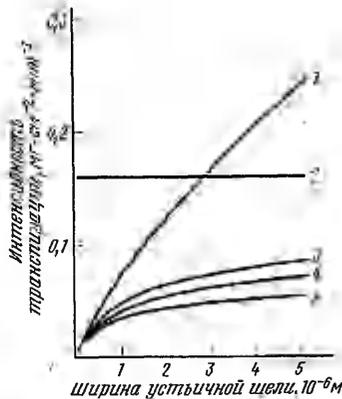
сопротивление (сопротивление мезофилла). Таким образом, предварительные дворики уменьшают транспирационное соотношение, т. е. количество потерянной растением воды на единицу образовавшегося органического вещества.

Нам кажется, что эти гипотезы не объясняют полностью роль предварительных двориков. В отношении транспирации, фотосинтеза и их соотношения не имеет значения, изменяется ли сопротивление устьичной щели или предварительного дворика. Предварительные дворики можно скорее всего рассматривать как своего рода предохранительную

систему, как некоторое дополнительное сопротивление, подключенное последовательно к устьицам и защищающее растение от чрезмерного засыхания в случае «аварий» или неадекватной регуляции устьичных щелей.

Выводы. 1. Предварительные дворики устьиц ели европейской оказывают на диффузию водяного пара значительное влияние, особенно при открытых устьицах, ограничивая тем самым интенсивность транспирации.

2. Хотя размеры предварительных двориков устьиц могут изменяться в довольно широких пределах, их общее расчетное сопротивление довольно константно. Можно предполагать, что в действительности из-за различий структуры эпикутикулярных восковых отложений реальное сопротивление предварительных двориков намного выше расчетных величин.

3. Больше, чем изменчивость размеров предварительных двориков устьиц, на сопротивление диффузии газов между листом и атмосферой влияет изменчивость поверхностной плотности устьиц.

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VARIABILITY AND ECOLOGICAL SIGNIFICANCE OF STOMATAL ANTECHAMBERS IN NORWAY SPRUCE

Variability of stomatal antechamber sizes and of the number of stomata per unit area in Picea abies Karst. needles were determined. Light and shade needles of 9—100-year-old spruce trees (the Estonian SSR) were analysed. Despite the pronounced fluctuations of antechamber sizes in light and shade needles, the calculated resistance of them to water vapour diffusion was similar. Resistance of stomatal antechambers depends on their number in the needle surface layer. The degree of stomatal opening affects both the antechamber resistance and transpiration intensity. When stomata being fully opened, the percentage of antechamber resistance may exceed a half of the total transpiration one.

II

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VARIABILITY IN CUTICULAR RESISTANCE OF
PICEA ABIES (L.) KARST. AND ITS SIGNIFICANCE
IN WINTER DESICCATION

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Abstract. The variability of cuticular transpiration resistance (r_c) was investigated in *Picea abies*. Needles of different crown positions, age, and air pollution load were measured. Cuticular resistance declined gradually with the needle age, so that in four-year-old needles it constituted about half of that in the current-year needles. In an individual tree, the values of r_c were much higher in the shade needles than in the sun needles. The experiments in which wax was removed with ether revealed that the role of epicuticular wax in the total r_c decreased with needle age. In the current-year needles, the resistance attributable to wax exceeded 40% of the total r_c; in C+3 needles it was as small as 18%. At the sites with polluted air the decrease in r_c during needle ageing was faster than at the control site. Calculations with wintertime air temperature and humidity used in a model showed that damage resulting from decreased cuticular resistance and consequent needle desiccation may appear after 22 days of continuously frozen soil in the four-year-old needles from the polluted site. Sun and shade needles from the control site can tolerate periods of permanent frost for 37 and 60 days, respectively.

INTRODUCTION

The rate of cuticular transpiration is considerably lower than the rate of stomatal transpiration, especially in xerophytes and conifers (Larcher, 1975). However, under extreme conditions small variations in wintertime water economy may have crucial effects on the survival of conifers, thus playing the main role in determining both the altitudinal treeline in the mountains and the polar timberline (Tranquillini, 1979, 1982). A similar term 'urban timberline' has been proposed (Laine et al., 1984) for localities where human impact is the most important stress factor determining the growth of trees.

Cuticular resistance to water vapour diffusion (r_c) decreases with needle age (e.g., Fowler et al., 1980; Cape & Fowler, 1981). This phenomenon has been attributed to the gradual degradation of the epicuticular wax (Grill, 1973; Sauter & Voß, 1986). Air pollution and acid rain increase the rate of wax erosion (Percy & Riding, 1978; Huttunen & Laine, 1981; Crossley & Fowler, 1986; Rinallo et al., 1986; Schmitt et al., 1987).

Thus it seems logical to assume that the rate of decrease in r_c during needle ageing could serve as an indicator of pollutant concentrations.

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However, as Huttunen (1978) has stated, there may be great variability in cuticular resistance among individual trees. No investigations are known to us in which cuticular resistance of sun and shade needles has been studied on the same tree.

The aim of the present study was to investigate the variability of resistance to cuticular transpiration in different parts of an individual tree, the variability between trees of the same age, and the differences in r_c between trees growing under different air pollution loads.

We have analysed the role of epicuticular wax and cuticle in total cuticular resistance, and we have estimated the possible influence of the age- and pollutant-induced decrease in r_c on winter desiccation of needles.

MATERIAL AND METHODS

Sample trees. The data were gathered from the following four sources in Estonia:

(1) a 100-year-old tree of 38 m in height growing in a closed canopy stand in Roela 1 sample area, Vooremaa Ecological Station, 43 km north of Tartu. The shoots for measurements were taken from the upper (30 m) and lower (12 m) parts of the crown;

(2) five 12- to 15-year-old self-sown trees growing in a close group in the open near the main laboratory of the Vooremaa Ecological Station, 2 km from site 1. The shoots were taken from the western side of the crown, 1 m above the ground. These trees were used to estimate the variation in r_c amongst trees;

(3) an 80-year-old tree growing 2 km northwest of the Kiviõli Oil-Shale Chemical Plant, a region in which alkaline oil-shale dust is deposited. The pH of snow water can reach 11.3 in this region (Laur & Mandre, 1987);

(4) a 35-year-old tree from a hedge in the northern part of Tartu, a site with slight, complex air pollution (Frey et al., 1988).

Measurement procedure. Measurements were made on shoots of each of the four age classes: current year (C), and three previous years (C+1, C+2, and C+3). The measurements were made in the middle of May, two weeks before budbreak. Thus the C needles were almost one year old, C+1 needles two years old, etc.

For each sample, r_c was estimated on ten separate shoots. The twigs were cut off from the tree, placed in a plastic bag, and taken to the laboratory. The shoots were detached from the twig and hung in the open air in the laboratory. The cut surfaces of the shoots were covered with latex glue to prevent evaporation. Air humidity and temperature in the room were measured with a ventilated psychrometer and the water vapour pressure deficit was maintained at about 1.0 kPa. As the boundary layer resistance at the air circulation rate in the laboratory (0.2—0.4 m·s^{-1}) was negligible as compared to cuticular resistance (Jarvis et al., 1976), the cuticular resistance to water vapour diffusion was calculated as

$$r_c = (\varrho_{sat, Tn} - \varrho_{sat, Ta} \times RH) \times t \times A/\Delta m,$$

where ϱ_{sat} is the saturating water vapour concentration at needle (Tn) and air (Ta) temperature, RH—relative air humidity, Δm—water loss, t—time of the exposure, and A—the total needle surface area. Needle temperature was considered to be equal to air temperature. It was assumed that the stomata were closed completely and the cuticular phase of transpiration was reached after 4 hours of desiccation. The water loss was estimated by weighing the shoots on a torsion balance 4 hours after their detachment and again 10 hours later. The accuracy of weighing was

0.1 mg. Baig & Tranquillini (1980) have suggested that cuticular transpiration should be measured after a 24-hour initial desiccation period. We could not do this as by that time the shoots had started to shed needles, especially the older shoots from the polluted sites.

Total needle surface area was estimated by the bead method (Thompson & Leyton, 1971).

To determine the influence of epicuticular wax on r_c, the wax was removed by rinsing the shoots in ethyl ether for 1 minute.

The number of stomata was counted under the microscope at 1 mm intervals at the base, middle, and top of the four sides of the needles; five needles constituted a sample. Stomatal density was expressed on the total needle area basis.

RESULTS

Effect of needle age and position. In all the investigated trees the cuticular resistance decreased with needle age (Fig. 1). In general, the values of r_c in the four-year-old needles were half those in the current-year needles. There were pronounced differences in r_c among shoots from different crown positions in the forest-grown tree (Fig. 1). The shade needles from the crown base had much higher ($\times 1.6$ to 2.2) resistance in all age classes than the sun needles from the top of the crown.
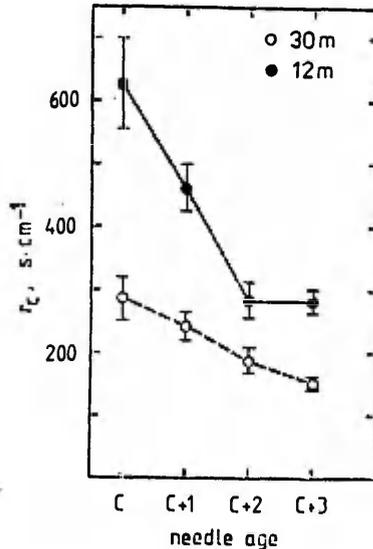
Fig. 1. Cuticular transpiration resistance of the shoots from the upper and the lower part of the crown of the 100-year-old tree. Error bars: $\pm$SE.

Variability between the trees. There were large differences in the r_c of C-needles of the five open-grown trees, the values of r_c varying from 650 ± 50, 620 ± 60, 440 ± 50, 430 ± 20 to 420 ± 30 s·cm^{-1}. On the average, however, r_c of the needles from young open-grown trees was closer to that of the shade needles rather than to the sun needles of the forest-grown adult tree (Fig. 2). There was no correlation between stomatal density and cuticular resistance.
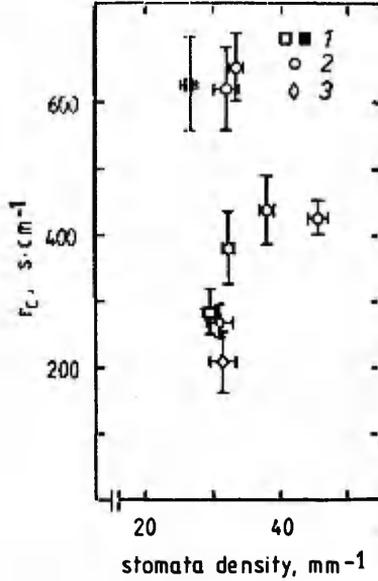
Fig. 2. The relationship between r_c and stomatal density in the current year sun (open symbols) and shade needles (closed symbols) of the 100-year-old control tree (*1*), 12—15-year-old trees (*2*), and a 35-year-old tree in a polluted area (*3*). Error bars: ±SE.

Effect of epicuticular wax. In all the samples measured, removal of epicuticular wax resulted in a decrease of the cuticular resistance (Fig. 3). However, the differences in r_c of the treated and untreated shoots decreased with needle age. The contribution of the wax layer to the total r_c in the C-needles was 44%, in the C+1 needles 33%, and in the C+3 needles only 18%.
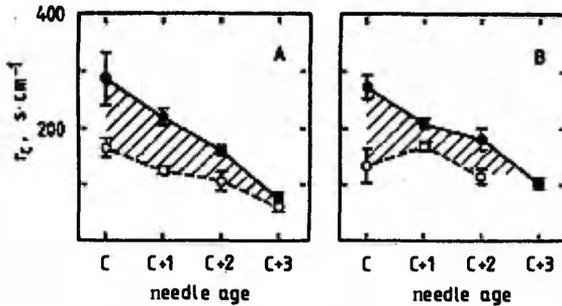
Fig. 3. Cuticular transpiration resistance of the shoots from Tartu (*A*) and from the oil-shale region (*B*). Dots denote untreated shoots, open circles mark epicuticular wax removed. The hatched area corresponds to the resistance of wax layer. Error bars: ±SE.

Effect of air pollution. Unfortunately, at present we lack exact quantitative data on the deposition of air pollutants at the investigated sites. Pilot studies have revealed a rather heavy alkaline dust deposition at the Kiviõli site and a slight alkaline deposition at the Tartu site.

At both sites, the r_c values of C-needles (about 280—290 s·cm^{-1}) were almost the same as the values of the sun needles of the control tree from the Tartu site (Fig. 3). The decrease of r_c with needle age, however, was much more rapid in both polluted sites. In the control plot, r_c of C+3 needles decreased 1.9-fold as compared to the C-needles. At the Kiviõli site, the ratio was 2.7, and at the Tartu site, 3.6.

DISCUSSION

The general trend found in the present investigation—the decrease of r_c with the advancing of needle age—is consistent with earlier findings (e. g. Fowler et al., 1980). This result may be explained by gradual degradation of the epicuticular wax, as revealed in several other coniferous species (e. g. in Jeffree et al., 1971; Grill, 1973). In *Picea abies*, the epicuticular wax undergoes with ageing both chemical changes and reduction in quantity (Günthardt-Goerg, 1986).

The differences in r_c of sun and shade needles are not easy to explain. There may exist several processes which result in a more rapid degradation of the epicuticular wax and/or the cuticle in more exposed leaves. In the timberline ecotone the abrasion of cuticular wax by windborne ice crystals resulted in a decrease of the cuticular resistance of the wind-exposed needles of *Picea engelmanni* (Hadley & Smith, 1986). Baker & Hunt (1986) have demonstrated that the mechanical impact of rain droplets can cause the erosion of the crystalline wax structures of some species with glaucous leaves. These effects may lead to bigger differences in r_c of older needles from different crown positions. In our case, the situation was quite the opposite: the relative differences in r_c of the sun and shade needles were greater in younger needles (Fig. 1). One possible reason for the higher resistance of shade needles could be their somewhat lower stomata number per unit of the needle area. The stomata may close not tightly enough and there may occur water vapour diffusion through the stomatal apparatus during the desiccation experiment, which could be higher than that of the non-stomatal epidermis. The lack of correlation between stomatal density and r_c (Fig. 2) shows that this mechanism did not occur. We have no information on the differences in the diffusion resistance of stomatal and non-stomatal parts of epidermis in conifers. It has been shown that in *Prunus laurocerosus*, a species with hypostomatous leaves, the cuticular conductance of the non-stomatal adaxial epidermis is higher rather than lower than that of the stomatal-bearing abaxial epidermis (Meidner, 1986). This indicates that the stomata close very tightly.

The decrease of cuticular resistance with age in the trees growing in polluted areas was somewhat faster than in the control tree (Figs. 1, 3). In order to compare the rate of the decrease in r_c, a ratio was used that may be called the ageing factor of r_c (AF):

$$AF = r_{c \ (C-needles)}/r_{c \ (C+3 \ needles)}.$$

The values of AF for the sun and shade needles of the control tree were 1.9 and 2.2, respectively; for the city-grown tree, 3.6; and for the tree from the alkaline-polluted site, 2.7.

It has been demonstrated on several coniferous species that air pollution can cause an accelerated degradation of epicuticular wax structures

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(Fowler et al., 1980; Huttunen et al., 1981; Huttunen & Laine, 1981, 1983; Crossley & Fowler, 1986; Sauter & Voß, 1986), thus increasing their permeability to water vapour (Cape & Fowler, 1981). Alterations in the wax structure have been observed after spraying the needles of *Abies alba* and *Picea abies* with weak solutions of inorganic acids (Rinallo et al., 1986; Schmitt et al., 1987).

Our experiments with wax removal demonstrated that it was not only epicuticular wax degradation that was responsible for the impairment of cuticular transpiration resistance. The permeability of the cuticle *per se* increased with needle age as well. However, the wax degradation was much faster. Thus the share of the wax resistance ($r_{wax} = r_c - r_{c, \text{wax removed}}$) in the total cuticular resistance decreased significantly with needle age. In C-needles, r_{wax} contributed 44% of the total r_c. Its share decreased to 41, 33, and 17% in C+1, C+2, and C+3 needles, respectively.

Model calculations of the wintertime water losses from the needles were made in order to evaluate the physiological significance of the decrease in r_c on the whole-tree level. For the driving force of transpiration the daily values of absolute air humidity and air temperature, averaged for January and February, 1984, were used (Метеорологический ..., 1985). The initial conditions for the calculations were the following: the initial needle water content—117.5%, needle specific area— 50 mm$^2 \cdot$ mg^{-1} (d. w.). Needle temperature was taken equal to air temperature. The criteria for needle water content, leading to its sublethal damage and irreversible desiccation, were defined as the average values for *Picea abies* during the winter months according to Pisek & Larcher (1954).

Under these conditions the water content of the shade needles for the control tree ($r_c = 500$ s $\cdot$ cm^{-1}) would approach the limit of drought resistance after 47 days of permanent frost, and after 60 days the needles would desiccate to the critical damage level (Fig. 4). For the sun shoots of the control tree and shade C needles of the polluted trees ($r_c = 300$ s $\cdot$ cm^{-1}) these levels would be exceeded in 27 and 37 days, respectively. For C+3 needles of the polluted tree ($r_c = 180$ s $\cdot$ cm^{-1}) the initial damage would occur after 17 days and the critical limit of desiccation would be reached after 22 frosty days.
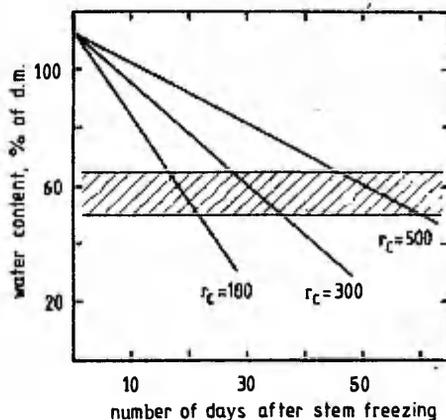
Fig. 4. Desiccation of needles with different r_c during a period of continuously frozen soil. The hatched area denotes the region of sublethal damage as defined by Pisek & Larcher (1954).

The above calculations are extremely simplified. In sunny and calm days the temperature of sunlit needles may rise about 7 to 8 °C above the ambient air temperature (Christersson & Sandstedt, 1978), thus increasing drastically the driving force of transpiration. Hence, our estimation of the decrease of the needle water content may be considerably underestimated, especially for the exposed parts of the crown. On the other hand, the snow and hoarfrost, covering the shoots for extensive periods, may serve as protection against desiccation.

The decreased cuticular resistance of sun needles from the upper part of the tree combined with the high evaporation demand may be one of the mechanisms that lead to the well-known top-drying of conifers.

Huttunen (1978) has found great differences in air pollution effects of different provenances and even individual trees of *Pinus sylvestris* and *Picea abies*. We have noticed the high individual variability of trees with respect to their r_c, which may be one of the factors causing selective desiccation damage. This variability makes difficult to use r_c of randomly sampled self-sown trees as an indicator of the air pollution level. Further experiments with clone material are needed to evaluate this possibility.

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III

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СМАЧИВАЕМОСТЬ ХВОИ КАК ИНДИКАЦИОННЫЙ ПРИЗНАК ЗАГРЯЗНЕННОСТИ ВОЗДУХА

Загрязнение, хвоя, эпикутикулярный воск, ель европейская, сосна обыкновенная, смачиваемость.

Поверхность листьев большинства растений покрыта слоем эпикутикулярного воска, что служит барьером между кутикулой и окружающей средой. Этот слой толще у ксероморфных растений, и его основной функцией считают уменьшение испарения воды из листьев [5, 6]. У хвойных, обычно у молодой хвои, восковой слой относительно толстый и имеет кристаллическую структуру. Основные компоненты воска — алкоголи, сложные эфиры, альдегиды и жирные кислоты [10, 11]. Со старением хвои восковой слой теряет свою первоначальную структуру, некоторые компоненты уносятся или испаряются. Такой деградированный восковой слой не в состоянии полностью выполнять свою функцию, и потери воды из хвои увеличиваются. Скорость деградации воска зависит от многих климатических факторов. Особенно быстро деградирует воск в условиях загрязненности воздуха [5, 24].

Учитывая вышеизложенное, некоторые зарубежные ученые используют изменение структуры эпикутикулярного воскового слоя у хвойных как индикационный признак загрязненности воздуха. В литературе известны разные методы изучения состояния эпикутикулярного воска на хвое. Некоторые авторы изучают его под электронным микроскопом [16, 19, 21—23], другие используют косвенные методы: определение химической структуры воска [11, 12], измерение потери воды из хвои при закрытых устьицах [21], а также изучение смачиваемости поверхности хвои. Последний метод представляет собой измерение угла между поверхностью хвои и находящейся на ней каплей воды [4, 18]. Этот метод был избран в настоящей работе, так как он отвечает многим требованиям, предъявляемым к индикации среды. Он прост, не требует сложной аппаратуры, работу можно проводить с большим количеством проб в полевых условиях.

Методику для измерения смачиваемости поверхности листьев разработал Т. Е. Фогг [8]. Смачиваемость поверхности зависит от шероховатости и химических свойств листа [9, 14], т. е. от морфологической структуры эпикутикулярного воска и его химического состава. Чем рыхлее структура, тем больше остается воздуха между воском и жидкостью, тем меньше смачиваемость хвои. Как правило, у гидрофобных растений смывание слоя эпикутикулярного воска с поверхности листа увеличивает смачиваемость, а у гидрофильных — уменьшает ее [9, 13].

Повышение смачиваемости поверхности увеличивает возможный контакт растворенных в воде загрязнителей с листом и тем самым повышает риск их воздействия на лист, а также позволяет им быстрее попасть в ткани листьев [2, 7]. Высокая смачиваемость поверхности хвои или листьев может стать дополнительной причиной заболеваний деревьев в промышленных районах. Так как
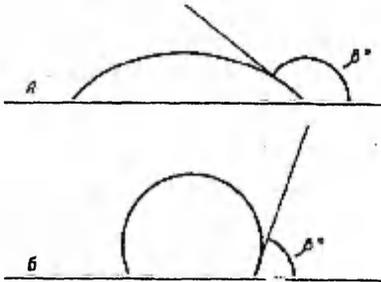
Рис. 1. Определение смачиваемости у гидрофильных (*А*) и гидрофобных растений (*Б*)
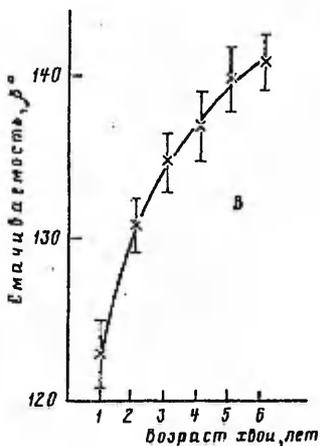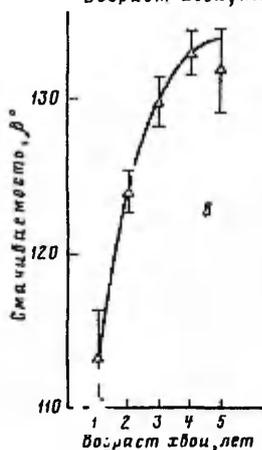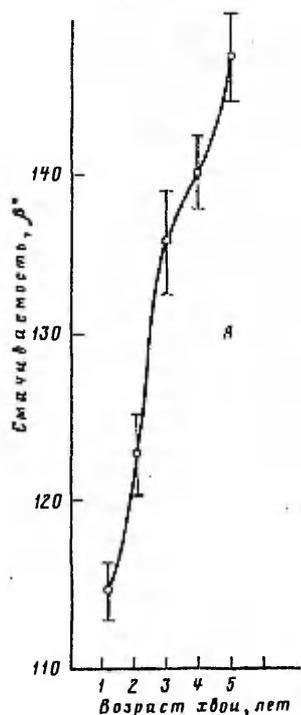
Рис. 2. Смачиваемость хвоинок ели на высоте 2 м (А), 10 м (Б) и 18 м (В). Пробы взяты с ели, растущей вблизи Воремааской экостанции в хороших световых условиях. На графике показаны средние ошибки проб

в Эстонии ель европейская повреждена в большей мере, чем другие хвойные, то основное внимание в настоящей работе уделено этой породе. Было изучено природное варьирование смачиваемости хвои и различие в ее смачиваемости при разных уровнях загрязнения. К сожалению, в литературе мы не нашли материалов об изучении смачиваемости хвои ели европейской в других районах Европы, поэтому сравнение своих данных с результатами других авторов проведено по сосне обыкновенной.
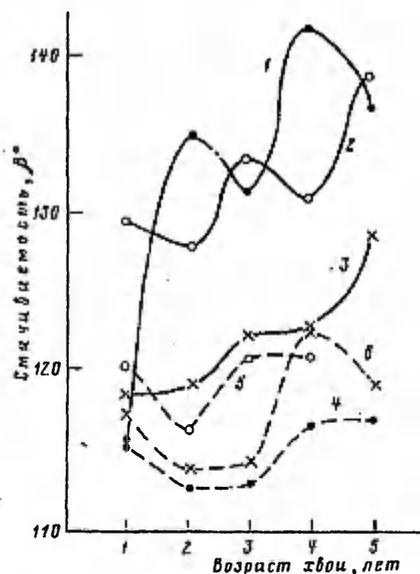
Рис. 3. Влияние эпикутикулярного воска на смачиваемость хвоинок трех ветвей одной ели в Паганамааском ландшафтном заказнике: 1—3 — с воском; 4—6 — воск удален

Материал и методика

Пробы хвои ели европейской и сосны обыкновенной взяты с деревьев, растущих вблизи Вооремааской экологической станции Тартуского университета, а также в промышленном районе Кивиыли (г. Тарту) в Паганамааском ландшафтном заказнике и Вилсандиском госзаповеднике. Все пробы хвои взяты с двухметровой высоты у 20-летних и более старых деревьев, растущих в хороших световых условиях. Для установления варьирования смачиваемости в кроновом пространстве у одного дерева пробы взяты с разных высотных слоев. Все пробы собраны в сухую погоду.

Хорошие обзоры о методике исследования смачиваемости приведены Н. К. Адамом [3], а также Дж. Т. Мартином и В. Е. Джунипером [20]. Если раньше смачиваемость измеряли у вертикально расположенных листьев [8, 18], то сейчас в большинстве случаев измеряют угол между горизонтальным листом и находящейся на нем каплей жидкости.

В данной работе хвоинки закрепили горизонтально и на них наносили шприцем капли дистиллированной воды объемом 1—3 мкл. Для измерения угла изображение хвои спроецировали диапроектором на экран. Угол между каплей воды и поверхностью хвои измеряли угломером (рис. 1). Для того чтобы смачиваемость была соразмерна с измеряемым углом, определяли угол β. Использованный многими исследователями так называемый контактный угол α в этом случае вычисляется так: $\alpha = 180° - \beta$.

В некоторых опытах эпикутикулярный воск удаляли с поверхности хвои этиловым эфиром в течение 10 мин. Во избежание случайной ошибки в каждой пробе вычисляли средний угол для десяти хвоинок.

Сопротивление кутикулярной транспирации измеряли на 4-м и 14-м часу после отрезания побега от дерева, учитывая потерю массы на единицу площади хвои. В то же время учитывали температуру и влажность воздуха в лаборатории.

Результаты

В связи с тем, что сведения о смачиваемости хвои ели европейской в литературе отсутствуют, провели ряд опытов для уточнения методики. Вначале изучали смачиваемость верхней и нижней грани хвои. Она оказалась следующей:

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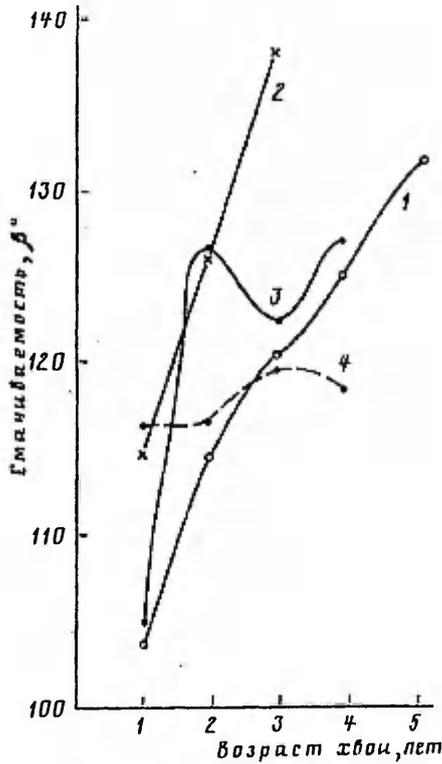
Рис. 4. Смачиваемость хвоинок сосны: *1, 2* — вблизи Вооремааской экостанции; *3* — в г. Тарту (*1—3* — с воском); *4* — воск удален (г. Тарту)

Возраст годичного прироста, лет	Верхняя грань, $\beta°$	Нижняя грань, $\beta°$
1	126	127
2	129	129
3	134	132
4	144	139
5	144	140

Разницы в смачиваемости верхней и нижней грани хвои не обнаружено, поэтому необходимость измерения смачиваемости обеих граней отпала [13, 25]. Позднее изучали только анатомически верхние грани хвои ели.

Несмотря на то что смачиваемость хвои с разной высоты в кроне различается, не обнаружено прямой зависимости этой величины от высоты прикрепления хвои. Со старением хвои ее смачиваемость увеличивается на всех высотах (рис. 2). У хвои с удаленным воском такое возрастное изменение значительно уменьшается. При этом почти во всех случаях удаление воска приводит к уменьшению, а не к увеличению смачиваемости хвои. Сильнее меняется при удалении воска гидрофобность старой хвои (рис. 3). Такой же результат получен при исследовании хвои сосны (рис. 4).

В природе смачиваемость — весьма вариабельный параметр, у побегов соседних деревьев и даже разных приростов одного дерева она может различаться довольно значительно. Для установления причины этого явления кроме смачиваемости измеряли кутикулярную транспирацию хвои:
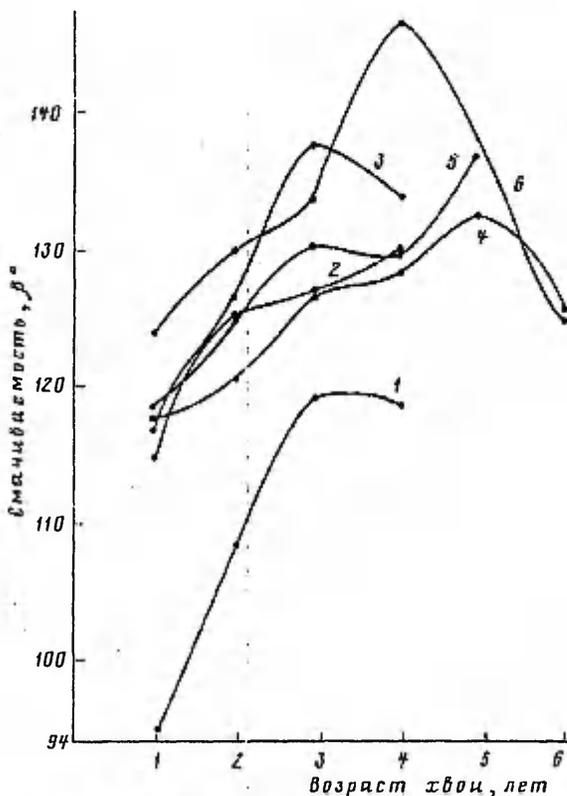
Рис. 5. Смачиваемость хвоинок ели из разных районов Эстонии: *1* — Вилсандиский заповедник; *2* — Паганамааский ландшафтный заказник; *3* — Вооремаа; *4—6* — сланцехимический комбинат в Кивиыли; *5* — г. Тарту

Возраст годичного прироста, лет	Смачиваемость, $\beta°$	Кутикулярное сопротивление R, $с·см^{-1}$
2	119	379
2	132	347
2	127	306
3	120	104
3	128	107
3	138	211

Как видно, корреляция между кутикулярным сопротивлением и смачиваемостью скорее позитивная, чем негативная.

Несмотря на неодинаковое загрязнение воздуха, существенных различий в смачиваемости хвои в разных районах Эстонии не обнаружено (рис. 5). Значительно отличается по смачиваемости только хвоя с о. Вилсанди.

Обсуждение

Из проведенной работы следует, что по смачиваемости хвоинок одного побега нельзя судить о смачиваемости других хвоинок дерева. Различие в смачиваемости хвоинок в пределах одного побега незначительно и поэтому причиной гетерогенности между ветвями в пределах одного

дерева может быть их различная экспозиция по отношению к солнечной радиации, ветру, депозиции пыли и другим внешним воздействиям. Все указанные причины вызывают изменение структуры и химического состава воска [7, 15, 17]. Со старением хвои эпикутикулярный воск выветривается, его химический состав и морфоструктура изменяются. Очевидно, эти изменения приводят к тому, что старые хвоинки смачиваются лучше. Различие в гидрофобности хвои одновозрастных, рядом растущих деревьев может быть вызвано генетической вариабельностью деревьев. Для устранения генетически обусловленной вариабельности целесообразно проводить опыты на клоновом материале.

Следует обратить внимание на факт, что хуже смачиваются те хвоинки, у которых воск смыт или кутикулярное сопротивление не очень высокое. Если взять за основу, что кутикулярное сопротивление хвоинок зависит непосредственно от толщины и спелости покрывающего воскового слоя, то можно предполагать, что эпикутикулярный воск смачивается — как у ели европейской, так и у сосны обыкновенной — лучше, чем кутикула. Правильность такой теории приводит к сомнению в возможности употребления смачиваемости хвои в качестве индикатора загрязнения воздуха. На это указывают и данные, полученные из мест с разным уровнем загрязненности в Эстонии.

Наши результаты отличаются от результатов Дж. Н. Кейпа [4]. Так как методика в обеих работах одинаковая, то значительно лучшая смачиваемость хвои сосны обыкновенной в Эстонии по сравнению с деревьями Центральной Великобритании может быть вызвана или генетическими различиями, или, если все-таки использовать теорию о пригодности смачиваемости в качестве индикатора загрязнения, существенно большей загрязненностью воздуха в Эстонии. В свете такой теории можно объяснить и низкую смачиваемость хвои ели на о. Вилсанди. Этот остров — самое удаленное место от суши в Эстонии, и чистоту его атмосферы отмечали многие ученые [1].

Нельзя, однако, полностью исключить того, что на молодую хвою действует загрязнение, нарушающее структуру воска на хвое, и последующие возрастные или вызванные дополнительным загрязнением изменения почти не проявляются. Для проверки данной гипотезы необходимы специальные эксперименты. Но результатам данной работы можно заключить, что смачиваемость природного материала хвои ели европейской и сосны обыкновенной в условиях Эстонии нельзя применять при индикации уровня загрязненности.

Выводы. 1. Смачиваемость хвои как у ели европейской, так и у сосны обыкновенной улучшается со старением хвои. Хорошая смачиваемость становится дополнительным фактором заболевания в загрязненной атмосфере.

2. Сравнение наших данных с данными из зарубежной литературы показывает, что хвоинки сосны в Эстонии смачиваются гораздо лучше, чем в Великобритании. Причиной этого могут быть либо генетические различия, либо большее загрязнение атмосферы в Эстонии.

3. Хуже смачиваются хвоинки, у которых эпикутикулярный воск смыт. Кутикула старой хвои более гидрофобна, чем воск.

4. Смачиваемость хвоинок в районах с разным загрязнением в Эстонии существенно не различается, поэтому смачиваемость нельзя использовать в качестве индикатора загрязнения воздуха.

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WETTABILITY OF NEEDLES AS AN INDICATOR OF AIR POLLUTION

Wettability of needles in Picea abies L. and Pinus sylvestris L., growing in regions of various pollution in Estonia, is considered. The contact angle between the surface of a needle and a water drop is mostly influenced by structure and chemical composition of epicuticular wax. With aging the wax is destroyed, the needles become more hydrophylic. The needles, in which wax is removed off with ethyl ether, are wetted poorer. Wettability of needles is a variable parameter, therefore it cann't be used in indicating the pollution level in Estonia. Possible reasons of a gap between the data obtained and given in literature are discussed.

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ТЕРМОДИНАМИЧЕСКИЕ ПОКАЗАТЕЛИ ВЛАГООБЕСПЕЧЕННОСТИ ДЕРЕВА

Сосна обыкновенная, термодинамический подход, водные потенциалы и потоки в дереве, запасы воды в почве.

Применение термодинамического подхода при изучении динамики водного обмена дает возможность раскрыть многие важные особенности этого процесса. Одним из вопросов, требующих детального рассмотрения, является вопрос об оценке влагообеспеченности растений по термодинамическим показателям. Обычно обеспеченность растений почвенной влагой оценивают по величине влажности почвы. Р. Слейчер [8], а в дальнейшем и другие авторы [15, 16] показали, что эта оценка может быть более точной, если в качестве показателя использовать «ответ» самого растения.

Исходя из термодинамических представлений, движение воды в системе почва—растение—атмосфера (ПРА) происходит по градиенту водного потенциала (Ψ); при этом, чем больше Ψ на

IV

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

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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).

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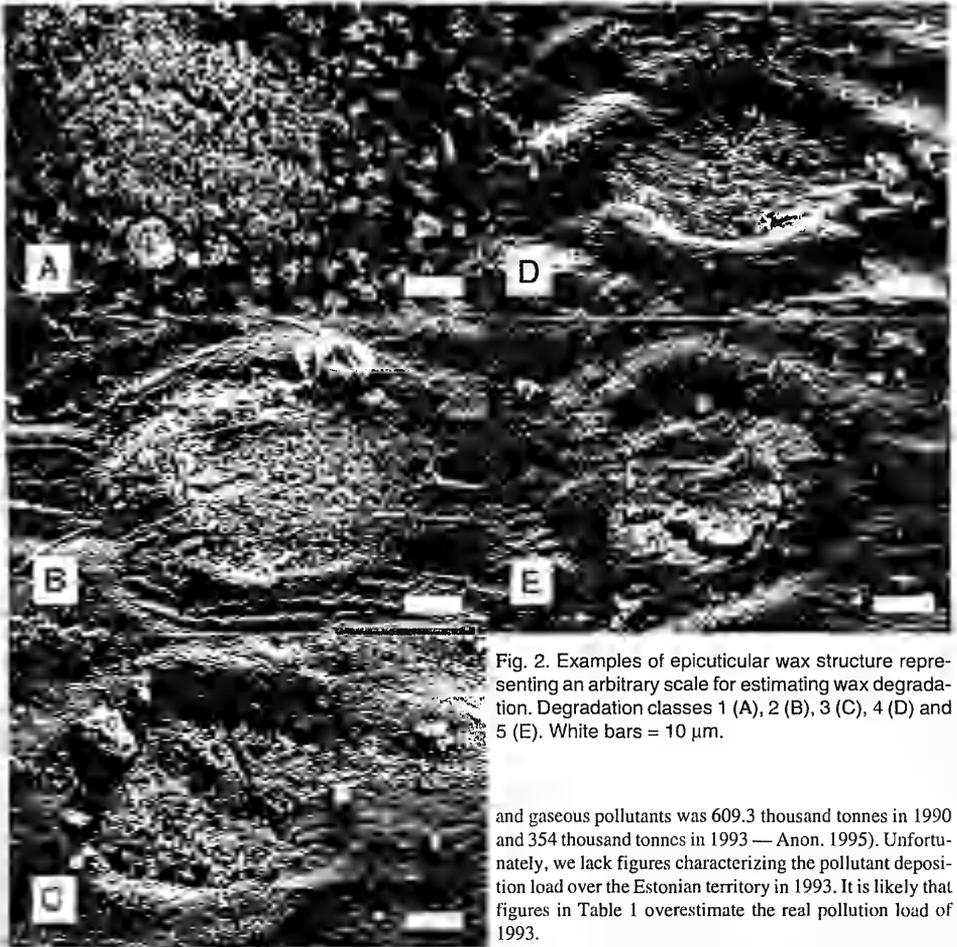
Fig. 2. Examples of epicuticular wax structure representing an arbitrary scale for estimating wax degradation. Degradation classes 1 (A), 2 (B), 3 (C), 4 (D) and 5 (E). White bars = 10 µm.

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.

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

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.

Site	C	C+1	C+2	C+3	C+4
		Needle age class			
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 30 estimates.

Site	C	C+1	C+2	C+3
		Needle age class		
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)
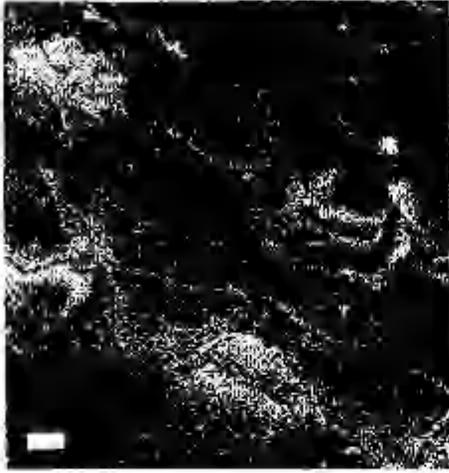
Fig. 3. Variability of wax structure in neighbouring stomatal antechambers. The degradation classes of wax cover on the stomatal antechambers: upper left — 3, upper right — 5, lower left — 4, lower right — 2. White bar = 10 μm.

Fig. 4. Epicuticular wax in the stomatal antechamber of a current-year needle of spruce from Northern Sweden. 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.

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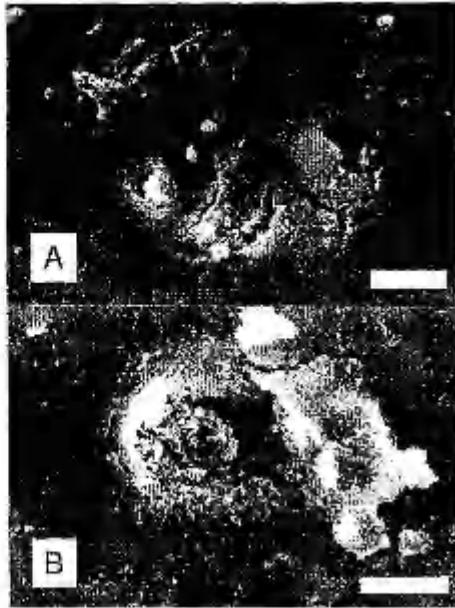
Fig. 5. Under the amorphous wax plate (A) fine tubular wax structures can be found (B). White bars = 10 µm.

tion (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).

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.

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V

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Minimum epidermal conductance of Norway spruce (*Picea abies* (L.)
Karst.) needles: influence of age and shoot position in the tree crown.
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Minimum epidermal conductance of Norway spruce (*Picea abies*) needles: influence of age and shoot position in the crown

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Minimum epidermal conductance (g_{min}) of *Picea abies* (L.) H. Karst. needles was estimated for various positions and age classes. The values of g_{min} showed a lognormal distribution. There were no universal trends in correlation between g_{min} and shoot position in the crown. The values of g_{min} increased significantly in the course of needle ageing. All sample series displayed high variability, which may be caused by differences in stomatal closure and/or in cuticular conductance *sensu stricto*.

Keywords: cuticle, epicuticular wax, minimum epidermal conductance, needle ageing, *Picea abies*

INTRODUCTION

Cuticular transpiration constitutes only a small part of water losses from conifer needles. However, in extreme conditions cuticular water loss may play an important role in the water economy of conifers. It has been shown that transpiration through the needle surface can determine the survival of conifers at the alpine timberline (Baig & Tranquillini 1974) or in polluted areas (Barnes & Davison 1988). Due to many methods-related difficulties in measuring the cuticular conductance of amphistomatal leaves or needles "minimum epidermal conductance" (g_{min}) is a more correct term to use when values are calculated from leaf weight-loss curves in desiccation experiments (van Gardingen *et al.* 1991). The value of g_{min} changes in time and is influenced by temperature and cuticle water content (Kerstiens 1994).

The values of g_{min} for conifer needles are closely related to the quantity and structure of epicuticular wax. Koppel and Heinsoo (1994) showed that removal of epicuticular wax from Norway spruce (*Picea abies* (L.) H. Karst.) needles can increase g_{min} by up to 44%. The quantity and structure of epicuticular wax of conifer needles has been widely investigated. Epicuticular wax on newly formed needles has a microcrystalline fine structure. With needle ageing wax degrades and forms fused tubes and platelets (e.g., Huttunen & Laine 1983, Ber-

madinger *et al.* 1988). At the same time g_{min} of older needles increases (Hadley & Smith 1994, Schreiber 1994).

Air pollution accelerates the rate of wax "ageing" (e.g., Crossley & Fowler 1986, Sauter & Voß 1986). Changes in epicuticular wax structure may be caused also by the micromechanical action of precipitation, and wind and dust particles (Günthardt-Goerg 1994). It has been shown that wax structure may vary on different needle sides of the same needle (Günthardt-Goerg 1994), or on the needles of the same age class, collected from different crown positions (Kim 1985). Unfavourable climatic conditions may also harm cuticle development and the layer of epicuticular wax of newly formed leaves. In tobacco (*Nicotiana tabacum* L.) the biosynthesis of epicuticular wax is affected by UV-radiation (Barnes *et al.* 1994). The influence of wind on cuticle development and g_{min} has been reported for several deciduous trees growing in upland hills (Hoad *et al.* 1994) and for conifers growing at timberline (Baig & Tranquillini 1976, van Gardingen *et al.* 1991).

In conifer species the values of measured g_{min} vary on a large scale even in experiments with one species (reviewed by Kerstiens 1996). At the same time the mean values of g_{min} for one species may show very high standard errors in one experiment(e.g., Hadley & Smith 1990, Koppel & Heinsoo 1994). This variability may cause problems when the values of g_{min} are used for modelling wintertime water loss from needles. The aim of this study was to analyse the spatial variability of minimum epidermal conductance within the crown of Norway spruce and to estimate changes in g_{min} during needle ageing.

MATERIAL AND METHODS

Sample trees

Shoots for investigation were collected from three approximately 70-year-old spruces growing in an open site close to the Vooremaa Ecological Station, Central Estonia. Two trees (Nr. 1 and 2) were used for studying the variability of g_{min} within the crown. The study was carried out on current-year shoots(C) and on two-year-old (C + 2) shoots in autumn 1995. The shoots were collected from the top (30 m),

middle (14 m) and bottom (2 m) of the crown, both from the northern and southern sides of the tree. Six shoots were sampled from distal and proximal branch parts for each height/year class. The branches were severed and taken to the laboratory in plastic bags.

One tree (Nr. 3) was used to study changes in g_{min} during needle ageing. One cohort of shoots (sprouting at the end of May 1985) was monitored through two and half years (from June 1985 until October 1987). In the same tree g_{min} of shoots was estimated for four previous year classes in spring before budbreak(marked as C + 1, C + 2, C + 3 and C + 4). For this, shoots were collected from the height levels of 6 m and 18 m (eastern side) as well as 2 m (eastern and western sides). Sample size was ten shoots per age class/position.

Measurement of minimum epidermal conductance (g_{min})

The shoots were detached from the branch in the laboratory and the cut surface of the twig was covered with latex glue to prevent evaporation. The shoots were hung up in a closed room at dim light. Air humidity and temperature were measured with a ventilated Assmann psychrometer. Needle temperature was considered to be equal to air temperature. It was assumed that stomata were closed after 4 hours of desiccation. The values of g_{min} were calculated over the period 4—14 hours from the beginning of the experiment. Total needle surface area was estimated by the glass bead method (Thompson & Leyton 1971).

Minimum epidermal conductance was calculated as:

$$g_{min} = \Delta m / ((\varsigma_{sat\,Tn} - \varsigma_{sat\,Ta} \times RH) \times t \times S),$$

where Δm is water loss in the period, ς is the saturating water vapour concentration at a given needle (Tn) and air (Ta) temperature, RH is relative air humidity, t is the length of the period, and S is the total needle surface area. The surface area of needles was many times larger than that of the twig. Therefore the water loss through the twig bark was assumed to be negligible. For statistical analysis SAS System GLM procedure for classes was used.

RESULTS

Data distribution

Analysis of g_{min} demonstrated a lognormal data distribution. Therefore the geometric mean of non-transformed data was used instead of the arithmetic mean. For statistical analysis lognormal transformation of the data was used.

Spatial variability of g_{min} in the crown

Statistical analysis showed that there were no universal trends in correlation between shoot position in the crown and g_{min} (Table 1). In tree Nr. 1 shoots taken from the northern side of the tree had larger values of g_{min} compared to those taken from the southern side. In tree Nr. 2, the results were the opposite. The role of the quadrant was significant only for C-needles. Analysis of the joint effect of several factors did not reveal any general trends either.

The mean values of g_{min} for tree Nr. 1 are used to describe the behaviour of minimum conductivity in the tree crown (Fig. 1). The mean values of g_{min} for C-needles within the crown of this tree varied in the range $1.7–4.3 \times 10^{-5}$ m s^{-1}. The values of g_{min} in C + 2 needles were always about twice as large.

Needle age and g_{min}

Minimum conductance of needles increased steadily during needle ageing (Fig. 2). According to statistical analysis, the largest differences in g_{min} occurred during the third growing period of needles. Differences between g_{min} of C + 1 and C + 2 as well as between C + 3 and C + 4 needles were not statistically significant. The distribution histograms of g_{min} showed a significantly higher variability in older age classes. The frequency class with the largest number of measurements was almost the same for all age groups. However, for older shoots the distribution curves extended towards higher g_{min} values (Fig. 3).

The study of g_{min} needles over two years demonstrated that g_{min} declined rapidly in the first weeks after budbreak. Afterwards g_{min} increased steadily with needle ageing. At the same time large fluctuations of g_{min} occurred against the background of this increase. For example, in April 1986, g_{min} was about twice as high as in March 1986. In May 1986, g_{min} dropped to the level of March (Fig. 4). The variability of g_{min} did not correlate with water vapour deficit calculated from air climate parameters of the laboratory.
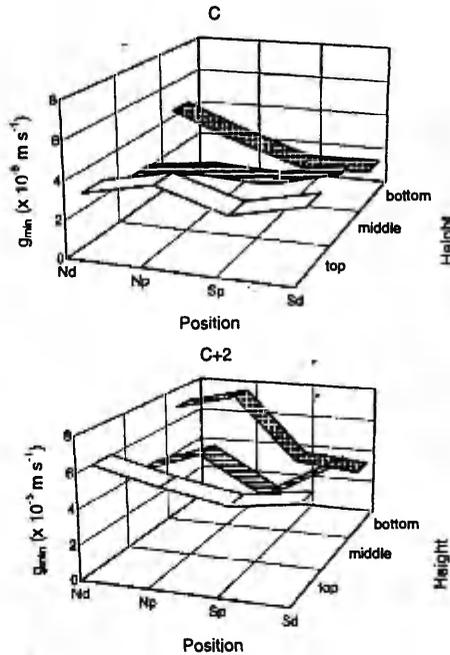
Fig. 1. Pattern of minimum transpiration conductance of C and C + 2 shoots in the crown of tree Nr. 1. Height: 2 m (bottom), 14 m (middle) and 30 m (top); position: northern distal (Nd), northern proximal (Np), southern proximal (Sp) and southern distal (Sd).

Table 1. Influence of various factors on g_{min} of trees Nr.1 and 2. C: current-year needles; C + 2: two-year-old needles. Quadrant: northern vs. southern side of the tree; distance: distal vs. proximal part of the branch; age: C *versus* C + 2 needles. *** = $p < 0.005$; ** = $p < 0.01$; n.s. = not significant.

	Tree Nr. 1		Tree Nr. 2	
	c + 2	c	c + 2	c
Factors:				
Height	n.s.	n.s.	n.s.	***
Quadrant	***	***	n.s.	***
Position	n.s.	n.s.	**	***
Age	***		***	
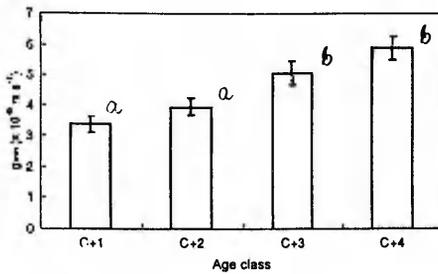
Fig. 2. Changes in g_{min} during needle ageing in tree Nr. 3. Bars: ± S.E. (n = 40). Columns with different marks are statistically different (p < 0.05; Bonferroni T-test).

DISCUSSION

Baur (1997) showed that water permeability and organic solute mobility in nonstomatal plant cuticles are characterised by lognormal distribution. Normal distribution was found in cuticles when cuticular waxes were extracted. Thus lognormal distribution can be attributed to the presence of the wax layer. Epicuticular wax consists of a mix of different components with different water permeability. Considerable variation in its composition might cause the described permeability distribution. Our results demonstrated that the values of g_{min} in the desiccating amphistomatal needles of *Picea abies* are also characterised by lognormal distribution. However, the reasons of this distribution pattern are unclear. It may be caused partly by the above-described phenomenon, but an alternative/additional explanation can be suggested as well. It has been shown that evaporation from stomatal leaf surfaces includes a stomatal component (Kerstiens 1996). If the degree of stomatal closure differs for the shoots, the lognormal distribution of g_{min} is expected.

The values of g_{min} are supposed to depend on the quantity and structure of epicuticular wax (Schulze 1989, Turunen & Huttunen 1990). Epicuticular wax lost its original structure most rapidly in the top of the tree crown and on the adaxial surface of needles (Kim 1985, Günthardt-Goerg 1994). It is possible that such differences might cause changes in the minimum cuticular conductivity of needles occupying different positions in
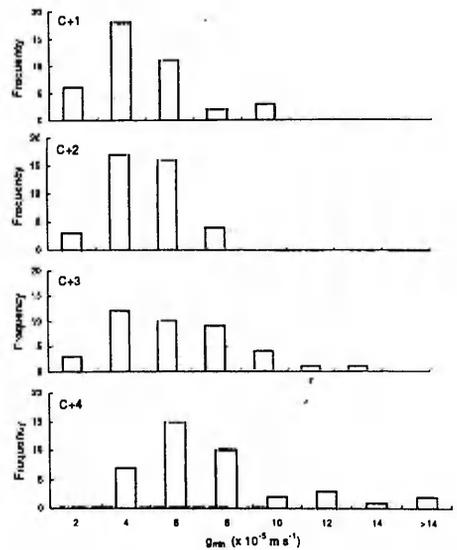
Fig. 3. Frequency distribution of g_{min} of various age classes (n = 40).

the crown. In treeline experiments, differences in cuticle thickness and g_{min} between wind-exposed and wind-protected shoots have been found (Baig & Tranquillini 1976, Hadley & Smith 1983). The amount of solar radiation and wind climate differ essentially for various heights and positions in the crown. However, we did not find any significant differences in g_{min} between shoots occupying different crown positions. Therefore, we conclude that microclimate differences within the crown do not influence g_{min}.

Permeability of the current year cuticles of *Picea abies* with respect to pentachlorophenol differs statistically from that of older cuticles (Schreiber 1994). In *P. engelmannii* Engelm. needles, g_{min} increased after the first wintering of needles in the Central Rocky Mountains, USA (Hadley & Smith 1994). At the same time there were no significant differences in g_{min} of older year classes. We found that g_{min} increased gradually with needle age until the fifth year at least. The largest differences in g_{min} were revealed between C + 2 and C + 3 shoots(Fig. 2). Different results might be explained by the more stable wax structure of *P. abies* or/and by milder climatic conditions.

The distribution of g_{min} values for different age class needles (Fig. 3) shows that only a relatively small proportion of older needles have lost their original capacity to minimize uncontrollable water loss during ageing. It has been claimed that one of the main causes of increase in g_{min} of older needles is the degradation of initial epicuticular wax structure during needle ageing (Huttunen 1994). On the other hand, g_{min} of older needles was significantly higher even if their wax layer was removed (Koppel & Heinsoo 1994). This indicates that besides changes in the amount and quality of wax, cuticle permeability or/and the number of dysfunctioning stomata may increase with needle ageing.

The influence of climate on g_{min} during needle ageing is yet unclear. Our investigation of the needles of one year class throughout two years showed a fast decline of g_{min} during the first weeks of growth (Fig. 4). This is most likely due to the presence of immature cuticle as well as additional evaporation through the twigs of a newly sprouted shoot. Conductance drops in the course of shoot maturation. In mature needles, g_{min} increases steadily for years with no significant differences between the seasons (Fig. 4). High variability of the calculated mean of g_{min} over many years is probably caused by different climatic conditions and/ or most likely, by differences in stomatal closure. As has been found, permeability of the plant cuticle and hence g_{min} depends on temperature and humidity (Kerstiens 1994, Sowell *et al.* 1996). At the same time no correlation was found either between g_{min} on the one hand and room humidity or temperature on the other.

The minimum conductance of *Picea abies* needles, as calculated by a number of authors, varies in the range of 0.3–8.7×10^{-5} m s^{-1} (Kerstiens 1996). There are several reports about large variations of g_{min} in one experiment. Hadley and Smith (1990) calculated the variation coefficient of g_{min} in *Abies lasiocarpa* (Hook.) Nutt. needles as 16%. The mean variation coefficient in our experiment over two years was 14.8%. At the same time the variation coefficient of the mean values of g_{min} measured throughout one year (January–December 1986) was 39.6%. Such high variability of g_{min} should be kept in mind when modelling wintertime water economy in conifers.
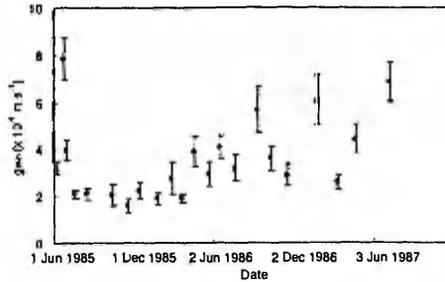
Fig. 4. Changes in g_{min} during two and half years after sprouting of the studied shoots. Bars: ± S.E.

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VI

Heinsoo K, Koppel A. 199X.
Minimum epidermal conductance in Norway spruce varies within the branch.
(manuscript).

MINIMUM EPIDERMAL CONDUCTANCE IN NORWAY SPRUCE VARIES WITHIN THE BRANCH

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Abstract

Gravimetrically measured minimum epidermal conductance (g_{min}) in Norway spruce (*Picea abies* (L.) Karst.) shoots from the same branch varied in the range $1.3–4.3 \times 10^{-5}$ m s^{-1} in a desiccation experiment. Scanning electron microscopy revealed incompletely closed stomata on needles after the experiment. Calculations based on stomatal aperture length and width as well stomatal density showed that the variability of g_{min} can be explained by incomplete closure of a small fraction of stomata during shoot desiccation.

Key words: Cuticular conductance, *Picea abies*, scanning electron microscope, stomata, variability.

Introduction

Extensive evidence has been gathered, demonstrating the crucial effect of conifer needles' ability to control water loss on their wintertime water economy along the altitudinal gradient in mountains (1; 5; 9; 11; 22; 24). The mechanism of enhanced cuticular conductance has been explained earlier by immature or thinner cuticle membrane (1; 4; 5; 24), by mechanical damage caused to the cuticle by snow or dust particles at high windspeed (8; 26), or by the effect of air pollutants on cuticle and epicuticular wax. Fumigation experiments have shown that O_3 and/or acid mist may increase water loss from conifer shoots significantly (2;19). Studies on conifers growing at different sites at different pollution loads have not yielded such unique results. Several authors have shown quite high local variability of cuticular conductance within shoots of the same age class and position (see figures in 8; 15). High variability of measured values complicates the comparison of different sample series and evaluation of the effect of air pollution on the water economy of shoots in stress conditions.

Measurement of g_{min} in conifer needles is complicated since in conifers it is impossible to separate cuticular and stomatal components of water loss during desiccation experiments (13). That is the reason why the terms "minimum transpiration" and "minimum conductance" (g_{min}) have been proposed instead of "cuticular transpiration" and "cuticular conductance" (7; 26). Grace (7) suggested that increased g_{min} in *Pinus sylvestris* at higher altitudes was caused by incomplete closure of dysfynctioning stomata rather than by the thinner or less developed cuticle. Kerstiens (14) showed that a very

small fraction of incompletely closed stomata may result in considerable increase in g_{min}. On the other hand, g_{min} may be influenced by water content in the cuticle (14).

The aim of the present study is to analyse variability of g_{min} in individual shoots within the same branch of Norway spruce (*Picea abies* (L).Karst.) and to assess the contribution of incompletely closed stomata to g_{min}.

Materials and Methods

Ten current-year shoots were cut at the western side of a 60-year-old Norway spruce growing in a suburb of Tartu, Central Estonia, in October 1996. Samples of lateral shoots were taken, one shoot per day, from a single branch about 2 m from the ground. After excision, the cut end of the shoot was covered with latex glue to prevent evaporation, and the shoot was hung on a special stand, placed on a plate of the balance (Mettler Toledo PB 303 Delta Range) which in its turn was kept in a climatic chamber (t = 22°C, RH = 45%). The shoot was weighed automatically at a 10-minute interval, and data were logged into the computer. The air in the chamber was stirred with a ventilator (wind-speed approximately 1 m s^{-1}) to reduce boundary layer resistance. Conductance (g) was calculated as:

$$g = \frac{\Delta m}{(\varsigma_{sat,Tn} - \varsigma_{sat,Ta} \times RH) \times \Delta t \times A},$$

where Δm is water loss during time interval Δt, ς_{sat} — saturating water vapour concentration at needle (Tn) and air (Ta) temperature, RH — relative air humidity, and A — total needle surface area. Needle temperature was considered to be equal to air temperature. At the beginning of the experiment shoot water content was 136...147% of shoot dry mass.

One shoot was desiccated for more than 48 hours, the others, for 22...24 hours. The minimum transpiration conductance of needles (g_{min}) was calculated over a 10-hour period (4 to 14 hours from the beginning of desiccation). For the estimation of needle surface area, 10 randomly selected needles were sampled after the end of desiccation and two diagonals (a and b) were measured with micrometer in the middle of the needle. Average needle length (l) was measured using a ruler after aligning the 10-needle sample in a row. Needle cross section was approximated to the rhomboid.

Needle area (A) was calculated as:

$$A = 2l \times \sqrt{a^2 + b^2}.$$

Shoot needle area was calculated by using the proportion of the dry mass of all shoot needles and 10 sampled needles (oven-dried at 80°C for 24 h). The relative water content (RWC) of the shoot was calculated as :

$$RWC = \frac{m_t - m_{dry}}{m_0 - m_{dry}} \times 100,$$

where m_t is shoot mass at time t, m_{dry} — shoot dry mass, and m_0 — shoot mass at the beginning of desiccation.

2

Shoots without any visible evidence of damage were collected from the same branch and kept in the same conditions as desiccated shoots. After desiccation, needles were detached and rinsed in ethyl ether to remove the epicuticular wax layer. The samples were covered with gold by using the JEOL Fine Coat Ion Sputter JFC-1000 and examined in the scanning electron microscope JEOL JSM 840 A at accelerating voltage 7 kV and current 0.3×10^{-12} A at the Centre for Materials Research of Tallinn Technical University.

Results

During the first hours of desiccation both *RWC* and conductance declined rapidly, after 3 hours the decline attained an almost linear character, indicating the onset of the classical "cuticular phase" of transpiration, i.e. closure of the major part of stomata (Fig 1). Needle *RWC* dropped from 81.3 to 72.2% within a 10-hour period during which g_{min} was calculated. After 48 hours *RWC* declined to 46.2%.

The error caused by the assumption that water vapour in the needles was saturated was estimated. For this, needle water potential (ψ) was calculated from the mass of transpired water by using the pressure-volume curve (10). Further, relative water vapour pressure was calculated from ψ values according to Laisk (17). The results showed that ψ dropped to −45 bars and relative humidity in the needle to 0.97 after 14 hours of desiccation. Consequently, the error, resulting from the assumption about water vapour in desiccating needles being saturated is quite small. The values of g at a given ambient air relative humidity (0.45) were overestimated not more than 5%.

Shoot water content was different at the fourth hour of desiccation, which may have been caused by the different initial water content of individual shoots collected within a two-week period (Fig. 2).

Individual shoots displayed a considerable variation of g (Fig. 3). The values of g_{min} differed threefold, ranging from 1.3 to 4.3×10^{-5} m s^{-1}. The mean of 10 shoots was 2.26×10^{-5} m s^{-1} (SE = 0.35×10^{-5}). In most shoots g declined during desiccation. Decline rate by linear approximation was 0.0059 and 0.028×10^{-5} m s^{-1} per hour for two shoots which displayed the highest and the lowest g values, respectively. One shoot behaved erratically showing slight increase in g over time. No significant correlation of g_{min} either with shoot area, or with needle or twig dry mass was found. Also, initial needle water content m_0/m_{dry}, which characterises needle water status at the beginning of desiccation, did not correlate with g_{min}, either.

Observation of the stomata with a scanning electron microscope after desiccation showed that most of the stomata were completely closed. However, several stomata did not close completely, leaving a 0.2..0.3 µm wide slit between guard cells (Fig 4). The proportion of incompletely closed stomata is hard to estimate, since a number of stomata are inclined in relation to the viewing direction.

Discussion

It has been demonstrated in a number of studies that g_{min} in conifer shoots increases along the altitudinal gradient (1; 5; 7; 9; 11; 22; 24). The values of g_{min}, estimated on the basis of detached shoots in the laboratory have been used to estimate possible water loss in natural conditions and to predict needle survival in case water transport into needles is hindered by the frozen soil or stem (4; 15; 23).

We found in our experiment that leaf conductance to water vapour varied largely between neighbouring shoots originating from the same branch. Values of g_{min} ranged from 1.3 to 4.3×10^{-5} m s^{-1}. This range covers almost the whole variability of g_{min} in conifers (reviewed in 14). In the majority of shoots, g declined during desiccation. This decline in time can be partly attributed to the decrease in water content (14). The dependence of calculated g on leaf water status has been shown both in conifers (3; 9) and in deciduous trees (12). In individual shoots the decline of g, associated with decrease in shoot water content, was evident, but when data for all shoots were pooled, this correlation disappeared. Instead, there was slight opposite correlation: g for shoots with higher water content tended to be lower (Fig 2). This phenomenon is plausible if there exists another conductance component besides humidity-dependent cuticular conductance. Some shoots behaved erratically, displaying slight increase in g over time (Fig 3). When reviewing earlier published data (e.g. Fig 1 in 22), the same phenomenon becomes evident. This effect can be caused by slight re-opening of stomata in the course of needle desiccation. Parker (20) showed that during dehydration the needles of *Pinus strobus* and *Pinus nigra* decreased in size, while the epidermis and the hypodermis bent inward. It is likely that some stomata may increase their aperture even during small needle shape change.

It has been assumed in earlier publications on cuticular conductance in conifers that stomata close tightly during needle dehydration, and diffusion can be attributed only to the cuticle. The authors of more recent papers have suggested that not all stomata close completely, and both the cuticular and stomatal components of transpiration are involved in desiccation experiments (7; 14; 26). Kerstiens (14) demonstrated that a very small fraction of slightly opened stomata may contribute significantly to the values of g_{min}. There may exist multiple external causes for incomplete stomatal closure or dysfunction. For example, simulated acid rain increased the number of abnormally developed stomatal complexes in *Pinus sylvestris* (25), and stomatal closure was mechanically disturbed by pollutant particles in *Pinus nigra* (6). Examination of stomata in the scanning electron microscope after the end of the desiccation experiment confirms that incompletely closed stomata do occur in Norway spruce needles in the absence of external stress factors (Fig. 4).

Laisk, Oja and Kull (18) showed the existence of a universal bell-shaped stomatal aperture distribution in two species with a different stomatal apparatus: barley (*Hordeum vulgare*) and horse bean (*Vicia faba*). This means that during stomata closure, decrement of stomatal aperture is uniform for all stomata regardless of aperture size. Approximately 1% of stomata belonged to the maximum pore width class of about 1 μm. There is no reason to suggest that stomata in conifers behave differently.

In order to estimate the possible effect of incompletely closed stomata in measured g_{min}, we calculated stomatal conductance (g_s) to water vapour according to Parlange and Waggoner (21):

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$$g_s = \frac{\pi abnD}{\ln(4a/b)b+l} \, ,$$

where l, a and b are the depth, semiwidth and semilength of stomatal pore, respectively, D — water vapour diffusion coefficient , n — stomata density. For narrow stomatal pores, stomatal conductance is much smaller than the conductance of stomatal antechambers (which is about $0.5 \times$ cm s^{-1}), and the latter may be omitted from calculations in case of very narrowly opened stomata (16). In calculations we used the following mean parameter values obtained for shade shoots of Norway spruce in Estonia: $a = 25$ µm, $b = 5$ µm and $n = 2750$ stomata per cm^2 (15). From these data it is possible to find the conductance of a single stomatal pore as a function of stomatal openness as well as the proportion of stomata which must remain open in order to cause decline in g_{min} (Fig 5). For example, if only as few as 6.5% of stomata have a pore width of 0.2 µm (see Fig 4), stomatal transpiration alone causes increase in g_{min} by 3×10^{-5} m s^{-1}, which explains the difference between the minimum and maximum values of g_{min} in the measured set of shoots. In our earlier studies we have found stomata with a pore width of 1.8 µm on the needles taken from the top of the dominant spruce and treated as described above. Only about 1% of stomata opened to this degree can account for the above-mentioned increase in g_{min}. Stomata density in sun shoots can be almost twice as high as it is in shade shoots (16). Consequently, the proportion of incompletely closed stomata may be smaller to bring about the same effect.

Grace (7) suggested that stomata density *per se* might influence the variation of g_{min}. However, we did not find any relationship between stomatal density and g_{min} in Norway spruce shoots of different morphological structure and age in our earlier study (14).

Our results indicate that there occurs incomplete closure of stomata during needle desiccation, which can account for a major part of variation of the minimum conductance of Norway spruce shoots. High natural variability complicates the comparison of g_{min} of shoots growing in different areas at different loads of air pollution.

Acknowledgements

Figure legends

Fig 1. Changes in *RWC* and *g* of a typical shoot during desiccation. The arrows mark the time for which g_{min} is calculated.

Fig 2. Relationship between shoot water content $(m_t - m_{dry})/m_{dry}$ and *g* in ten individual shoots. Linear regression lines (solid) are given for two shoots with the highest and for two shoots with the lowest *g* values. Broken line marks the linear regression for the shoot, which displayed slight increase of *g* in time.

Fig 3. Epidermal conductance *g* of individual shoots against time. Linear regression lines are given for the same shoots, as in Figure 2.

Fig 4. Scanning electron microphotos of Norway spruce stomata after 24 h of desiccation. Epicuticular wax was removed with ethyl ether. Most stomata are completely closed (A), but some stomata remain incompletely closed. The width of the stomatal pore in (B) is approximately 0.2 μm.

Fig 5. Calculated proportion of incompletely closed stomata, resulting in additional *g* of 3.0×10^{-5} m × s^{-1}, which corresponds to the difference between the maximum and minimum values of g_{min} of the measured shade shoots (solid line), and the conductance of a single stoma as a function of stomatal aperture (broken line).

6

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Figure 1.

Figure 2.

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Figure 3.

Figure 5.

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Figure 4 a, b

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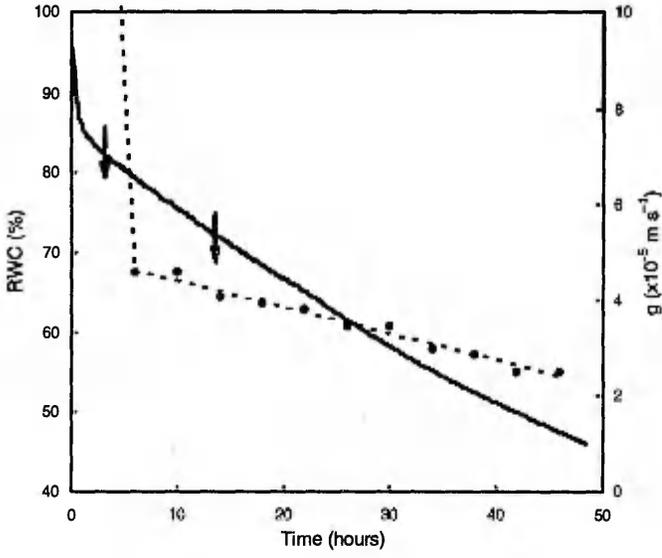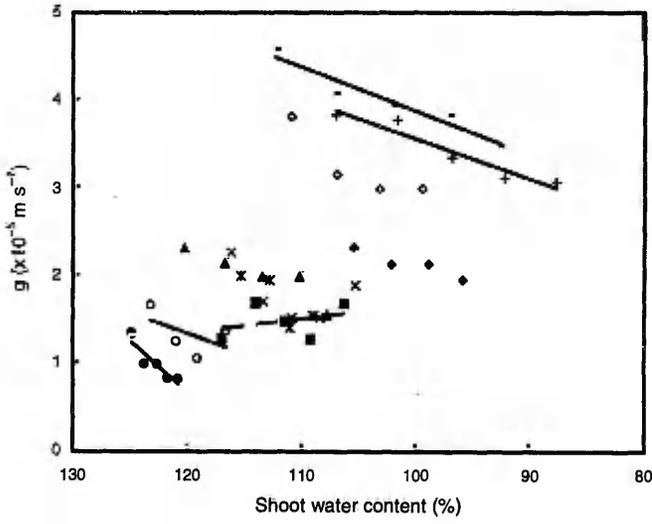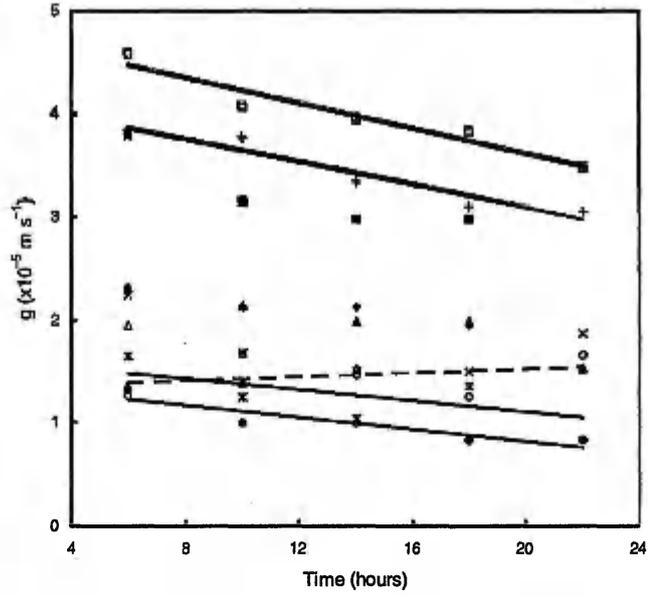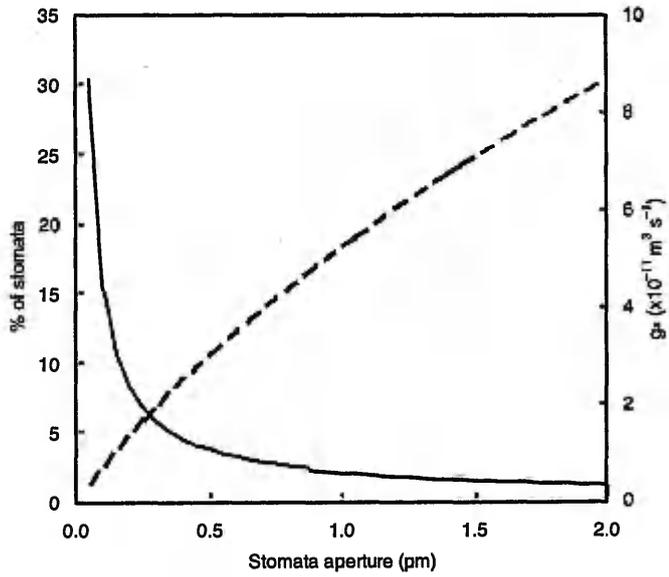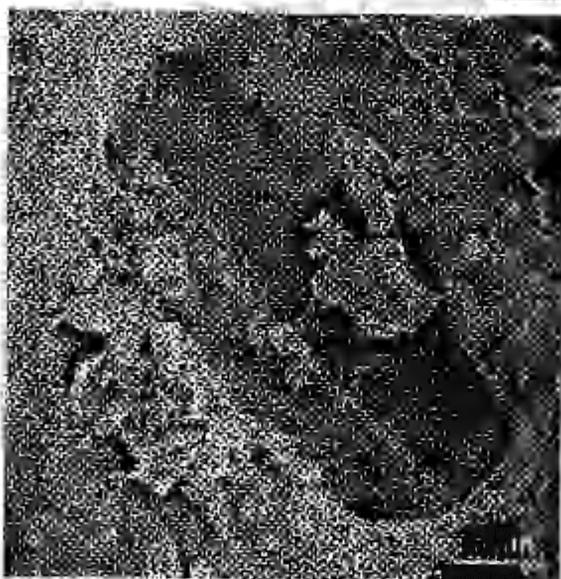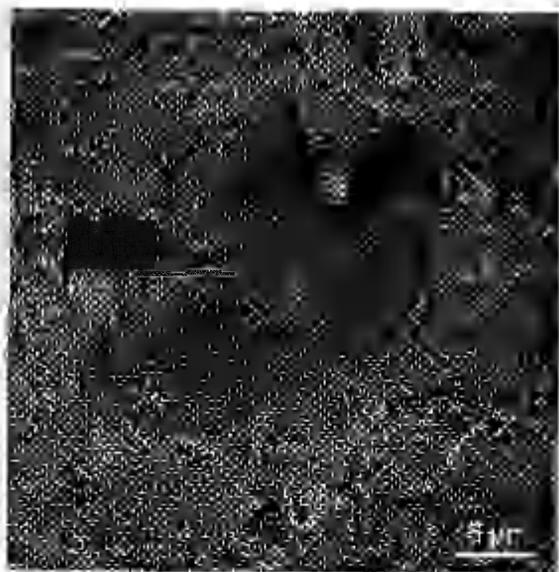
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