The Investigation of Needle Surface Characteristics of *Pinus sylvestris* trees near the Sources of Industrial Pollution

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The objective of the current research was to estimate pollutant effects on the aboveground parts of *Pinus sylvestris* over a 3-year period. NO_2 concentration was estimated as the marker of air quality at the points of Scots pine sampling locations. *Pinus sylvestris* trees were examined in the autumn of 1994-1996 near an oil refinery, a nitrogen fertilizer plant and a cement factory. A comparison of needle surface quality by scanning electron microscopy (SEM), wax amount, gravimetric characteristics and assessment of hydrophobic features of needles in some cases revealed significant differences between the sites. The data obtained show the possibility for a low level of pollutants to induce detectable damage in one-year-old needles.

Key words: nitrogen dioxide, conifers, water loss, contact angles, wettability, wax amount, SEM

Introduction

The second most common acidic pollutant gas is nitrogen dioxide (NO_2). NO_x emissions have increased dramatically over the last 50 years and have not decreased over the last 10 years in the same way as SO_2 emissions (Duyzer, Fowler, 1994; Lorenz et al., 1997). Few measurements of NO_2 concentrations have been made except in city centers. In the United Kingdom, rural NO_2 measurements have shown annual mean values in the range 3-12 $\mu g\ m^{-3}$ (Ashenden & Edge, 1995). Concentrations in the range 4.8-9.7 $nl\ l^{-1}$ are considered typical of rural regions in the FRG (Simonis & Weidner, 1988). In a larger survey of about 20 sites covering 9 European countries the annual mean concentrations in the range 0.5-14.4 $nl\ l^{-1}$ have been recorded for 1983-5 (Schaug et al., 1987).

The average amount of NO_2 in the atmosphere of the main cities and industrial centers of Lithuania (1984-1988) was 0.01-0.09 mg/m^3 (Kairiūkštis et al., 1992) and (1997-1998) 0.02-0.03 mg/m^3 (Environment'98, 1999). In 1995 - 1996 year estimations of NO_2 in Vilnius city (Lithuania) showed the lowest concentrations at the suburban level in the range of 9 - 16 $\mu g\ m^{-3}$ (Perkauskas & Mikelinskienė, 1998). Within 1981-1996 period the mean daily concentration ranged between 1.5-45.0 $\mu g/m^3$ for NO_2 and 0.2-26.0 $\mu g/m^3$ for SO_2. Since 1989 in Lithuania the mean annual background concentration of SO_2 tends to decrease and in very slight form since 1991 this holds for NO_2 (Giedraitis et al., 1999).

Although visual forest tree observations can show the extent of current injury, they give little indication of potential future visible symptoms and there is need to establish objective methods of quantifying the extent of forest damage (Skuodienė & Kairiūkštis, 1996). Plants may differ widely in the rate of production of their total dry weight as well as separate parts. Dry weight/fresh weight ratio difference between current and two-year-old needles was found to show a significant relationship between tree damage in the areas affected by forest decline (Mehlhorn et al., 1988). Gaseous pollutants have been reported to increase the rate of water loss from excised leaves of trees in controlled conditions (Mansfield & Freer-Smith, 1984) and in the field (Huttunen et al., 1981). Cuticular waxes have been shown to be solely responsible for the barrier properties of plant cuticles since an extraction of leaf surface waxes with organic solvents increases cuticular permeability by a factor of about 100 up to 1000. Erosion of waxes of the needle surface was observed as a consequence of gaseous pollutants, simulated acid rain, acid mist or acid fog and basic dust (Turunen & Huttunen, 1990). The use of the epicuticular wax structure as an indicator of air pollution has been mainly based on direct observations of changes in physical form as seen with SEM or on indirect methods, such as determining the wettability of needles by measuring the contact angles of water droplets on needle surfaces (Cape, 1983; Haines et al., 1985). Air pollutants may retard the development of the cuticle and alter the wax synthesis, resulting in a reduced concentration or altered composition of the waxes (Riding & Percy, 1985). Thus, a variety of tests are proposed to evaluate latent injury of trees under air pollution, although, there are many uncertainties left in early diagnosis.

The major objectives in setting up the survey were to determine the concentrations of NO_2 on selected sites situated at different distances from main local pollution sources in Lithuania, as well as to evaluate *Pinus sylvestris* trees under the influence of ambient pollution according to morphophysiological tests proposed to detect latent injury.

Material and methods

Study area. Forest sites near an oil refinery, a cement factory and a nitrogen fertilizer plant - the main local pollution sources in Lithuania - were investigated. During the study period (1994-1996) total emissions from the oil refinery comprised 28.9-31.4 thousand t/year, from the cement factory - 5.8-18.1 thousand t/year and from the nitrogen fertilizer plant - 4.9-5.8 thousand t/year (data provided by Ministry of the Environment of the Republic of Lithuania). Forest sites on podzols (in few cases on gleyic podzols) mainly in north-east direction from each pollution source were selected. Adjacent opened areas in front of woods were places for aerial NO_2 estimations. Near the oil refinery 4 sites (M1-1, M2-2, M4-1, M4-2) with 43-54 years old trees (further named as middle-aged stands of *Oxalido-Pinetum* type) in 5.5 km transecta and 3 sites (M1-2, M2-1, M3) with 15-20-year-old trees (further named as young stands of *Myrtillo-oxalido-Pinetum* or *Vaccinio-myrtillo-Pinetum* type) on a 10.5 km line were selected. Related to the cement factory 4 sites (A1-A4) with 40-67-year-old trees (stands belonging to *Carico-sphagno-Pinetum* or *Vaccinio-myrtillo-Pinetum* type) on a 10 km transecta were examined. Beside the nitrogen fertilizer plant 10 sites (J1-J10) with 15-25-year-old trees (stands of *Oxalido-Pinetum* or *Vaccinio-myrtillo-Pinetum* type) on a 21 km interval were chosen to evaluate pollutant effects on pine stands.

Determination of concentrations of nitrogen dioxide. There were 10 points on sites near the nitrogen fertilizer plant, 8 points in sites near the oil refinery and 4 (AA1-AA9) points on these near the cement factory; those are listed in Table 1. The exposure of tubes was performed during February-March 1994. The diffusion tubes were supplied by Gradko International Limited of Winchester, UK. Two steel mesh discs were soaked in triethanolamine (TEA) and fitted inside one end of the tube. These discs acted as collectors of NO_2 during exposure period. Nitrogen dioxide absorbed by the collectors was determined colorimetrically as NO_2^-. The absorbance of the samples was measured at 520 nm (Ashenden & Bell, 1989; Ashenden & Edge 1995).

Sampling of trees. Scots pine (*Pinus sylvestris* L.), the most common forest tree species in Lithuania, was selected. On each site eight apparently healthy trees were chosen for investigations. The selected trees were assessed visualy according to defoliation, dechromation and needle retention (Nemaniūtė et al., 1997). Sampling was done in September 1994-1996. The material was collected from the middle part of the crown of young trees or from the part of the crown 6-8 m above ground. An extending "squirrel pruner" was used to cut branches. Needles mainly from one-year-old (c+1) shoots were tested and for some tests current-year (c) shoots were used. At each time all type estimations were taken from the same branch. A total of 168 trees on 21 site were sampled each year. The sites were always sampled in the morning.

Epidermal water loss and gravimetric characteristics. Leaf drying rate measurements were made for excised needles. After transportation to the laboratory, twigs were cut from the branch and cut ends placed in water to allow saturation during night. Great care was taken when removing needles from the shoot that the leaves would not be damaged, as cut or broken needles completely ruin the result. Needles were removed using a small scalpel blade, cutting the bark at the needle base. Subsequently the bases of cut needles were coated in petroleum jelly to prevent water loss from the open wounds and then 20 needles from each shoot were placed into labelled open aliuminium plates on a laboratory bench. Each batch of needles was weighed immediately after being excised from shoot and then at intervals over that day and the following days. The temperature and humidity could not be controlled during the period of these measurements, but the needles from different sites were all exposed to the same conditions. In addition, several other measurements of foliar properties likely to have responded to pollutants were made.

The ratios of dry/fresh weight for needles of different age classes have been proposed as indicators of 'decline' (Mehlhorn et al. 1988). Dry weight/fresh weight ratios (D/F) were obtained by weighing out 20 needles gathered from the field at the time of sampling and dry weights were estimated after drying for 3 hours at 105° C. Dry weight/fresh weight ratio difference (R1-R0) was calculated by subtracting the ratio obtained for current-year needles from that for one-year-old needles.

Contact angles. A test of water-droplet contact angles with the leaf surface was carried out using a method described by Cape (1983). Contact angle measurements were made at room temperature, by using distilled water. A total of 15-20 needles of every age class from each of 8 trees per site (totally 120-180 measurements per site) were analysed. A 0.2ml droplet was placed on the center of the abaxial surface of needles by means of a syringe and viewed using a binocular microscope (*50) with protractor graticule. Measurements were carried out using repeating cycle of analysing one branch per each site to avoid artefacts due to surface quality changes per time. The needles were tested within 5 days of collection.

Chloroform - extractable wax. To determine extractable wax, 10 needle pairs were shaken with 40 ml chloroform ($CHCl_3$) for 15 s as it is described by Cape et al. (1988). Chloroform extracts were decanted through a small Buchner funnel under gravity into a sealable bottle. The solution was transferred to a pre-weighed aluminium foil dish, and the remaining solvent was allowed to evaporate in a fume cupboard. The samples were weighed untill a stable weight was reached to ensure the complete evaporation of solvent. The washed needles were placed on aluminium dishes for drying and later used for dry weight determination. Amounts of wax were expressed in terms of percentage of dry weight - d.w.).

Epicuticular wax morphology. The middle part of undamaged needles was secured with the abaxial surface uppermost. The samples were coated with silver using sputter equipment (VUP-4K) and investigated with the JEOL-JSM-IC25S scanning electron microscope (17 kV accelerating voltage). Representative areas of each needle were viewed at magnifications of 100X-10,000X. Areas investigated were the epistomatal chamber, slopes of the epistomatal chamber and the surface round stomata, called 'interstomatal area'. Needle surfaces were rated according to the following classes (Turunen et al., 1991): class I - distribution of crystalloid waxes 100%; class II - distribution of crystalloid waxes 71-99%; class III - distribution of crystalloid waxes 31-70%; class IV - distribution of crystalloid waxes 1-30%; class V - distribution of crystalloid waxes 0%.

Statistical analysis. The ANOVA analysis was used to evaluate the significance of differences between data sets on sites.

Results and discussions

Emissions of nitrogen dioxide. NO_2 as a common pollutant for all 3 regions near the factories was selected despite the fact that it is not of greatest importance according to emmision amounts. The results are shown in the Table. NO_2 values estimated were 2.1-4.1 ppb in the area affected by the nitrogen fertilizer plant, 2.7-4.1 ppb in points near the oil refinery and 1.9-2.9 ppb in the region of the cement factory. The data of the present survey have shown that NO_2 is a pollutant in all points investigated. In our study the concentrations of NO_2 were found to vary across transectas and to some extent were greater at the points near the pollution sources. The levels found in 1994 are comparable with those documented in the carried out in 1993 survey (Kupčinskienė et al. 1996). Our results have shown similarities with the data collected by other researchers in Lithuania (Kairiūkštis et al. 1992). The concentrations determined in our study were low in comparison with the rural parts of other countries: the lowest concentrations of NO_2 throughout Wales had a background level below 4 nl l^{-1}, annual mean concentrations of 12.2 and 11.6 nl l^{-1} were registered on 2 sites in England and 1.7 nl l^{-1} NO_2 on a site in Northern Ireland, while concentrations in the range 4.8-9.7 nl l^{-1} are considered typical of rural regions in Germany (Simonis & Weidner 1988; Ashenden & Bell 1989). Lower NO_2 concentrations of background values and ones near the factories found in our study could be due to the nearly two-

Table. Mean monthly NO_2 concentration (in ppb) near the main local pollution sources of Lithuania (February-March, 1994).

Characteristic of point	Tree site*	Distance (km)**	NO_2 (ppb)
North direction	M1-1	2.0	4.1
North-East direction	M5	2.8	4.0
North-East direction	M4-1	5.4	2.8
North-East direction	M4-2	5.4	2.7
East direction	M1-2	2.4	3.8
East direction	M2-1	3.6	3.0
East direction	M2-2	3.6	2.9
South-East direction	M3	10.4	2.8
West direction	J1	0.5	4.1
North-West direction	J2	0.5	4.0
North-East direction	J3	0.5	4.1
North direction	J4	2.5	3.0
East direction	J6	5.0	2.4
East direction	J5	6.0	2.3
North-East direction	J9	15.0	2.1
North-East direction	J8	19	2.5
North-East direction	J10	22	2.2
East-South direction	A1	0.5	2.9
East-South direction	A2	0.5	2.7
East direction	A3	1.5	2.3
North direction	A4	11.5	1.9

* *Pinus sylvestris* site located in the immediate vicinity of points for exposure of NO_2 tubes
** Point distance from the pollution source

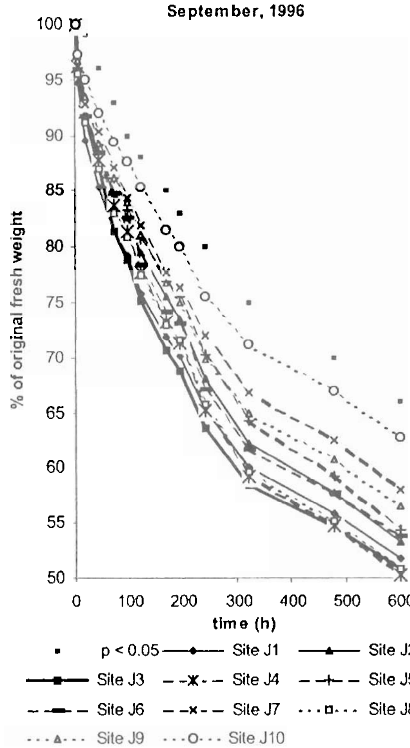

Figure 1. Alternation in one-year-old needles fresh weight over time after excision from trees growing at different distance from the nitrogen fertilizer plant (September, 1996).

fold decrease in productivity of industrial units of Lithuania as compared to the previous decade and the overall lower density of traffic in Lithuania as compared to the countries in West Europe.

Water loss from excised needles. The experiments reported here were designed to investigate the ability of detached one-year-old needles to conserve water after prior exposure to pollution of different level. Generally, quicker water loss was documented during the first 30 h and later drying occured much slower (Fig. 1-4). It could be explained by intensive stomatal closure at the begining of the observation period. In accordance with the data obtained in 1994 significantly quicker water loss was characteristic of J2 (0.5 km from the plant) site needles compared to J4, J7 or J10 sites (after 6, 22 and 30 h period of being excised). In the study conducted in 1995 at the beginning of exposure of needles the most rapid evaporation was common for site J8 ($p<0.05$ for 12h, 24h) and later for site J1, which was close to the factory ($p<0.05$ for 334h, 430h and 598h). In accordance with the data obtained in 1996 during the 43 h-598 h interval site J3 (closely related to the factory) needles dried up at significantly higher speed compared to J10 (Fig. 1). A comparison of the data obtained over 3 year for the sites near the nitrogen fertilizer plant did not reveal any site with a permanent tendency for significantly increased or decreased water loss from excised needles.

A comparison of middle-aged stands (Fig. 2) located at different distances showed only a few transitional effects in the 3-year study period. A different picture was obtained analysing young stands (Fig. 3). In accordance with the data obtained in 1994 in the 5h to 360h period significant differences were found among sites, with the quickest water removal from the needles belonging to site M1-2 (closest to the oil refinery stand). Similar results have been obtained in the assessment made in 1995 where at all time points (6h-598h) needles from site M1-2 dried at the highest speed ($p<0.05$) compared to sites M3 or M2-1. In the last year of observation, no significant differences among young stands were documented, although site 1-2 showed results in the same direction as in previous years.

In most cases the differences among sites near the cement factory were not significant, although showed the same tendency: the highest speed of drying was common for site A1 (1994), sites A1, A2 compared to A3, A4 (1995) and sites A1, A3 compared to A2, A4 (1996; Fig. 4). Significantly quickest drying was documented for A1 needles after 6h and 192h intervals (1994), 6h and 30h intervals (1995). A tendency for quicker water loss from A1 needles in autumn period in 1994-1996 coincides with the results obtained in July-August 1994 by our laboratory (unpublished data).

Dry weight-fresh weight ratios. Dry weight-fresh weight ratios (D/F) are good indicators for needle water

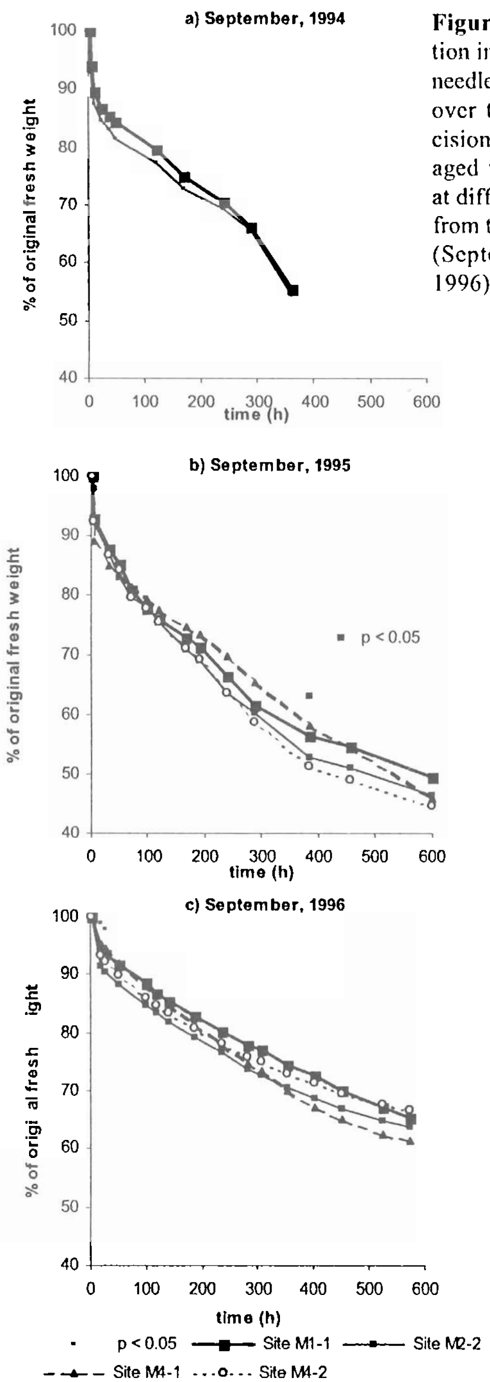

Figure 2. Alternation in one-year-old needles fresh weight over time after excision from middle-aged trees growing at different distance from the oil refinery (September, 1994-1996).

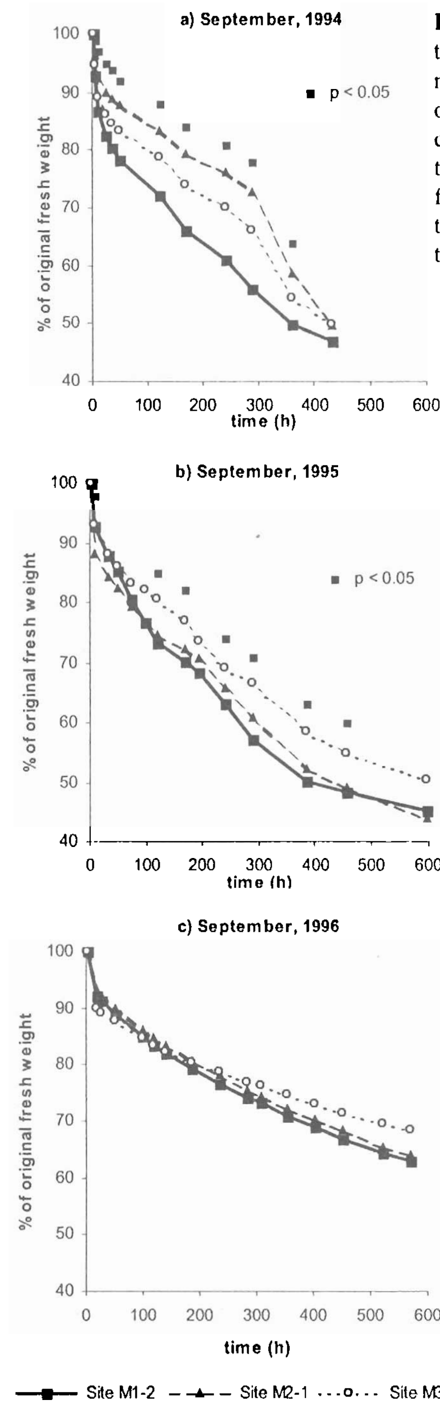

Figure 3. Alternation in one-year-old needles fresh weight over time after excision from young tress growing at different distance from the oil refinery (September, 1994-1996).

balance. Under equal water supply from the soil the differences in this parameter could be related to drying or wetting through the needles and subsequently higher defoliation. In accordance with the literature both effects can be induced by air pollutants (Mehlhorn et al., 1988). On the sites near the nitrogen fertilizer plant D/F ranged between 0.42-0.45 (1994), 0.36-0.39 (1995), 0.39-0.45 (1996) with significant differences ($p<0.05$) among J1-J10 sites during all 3 years. Among middle-aged tree sites (M1-1, M2-2, M4-1, M4-2) near the oil refinery D/F values were the following: 0.46 (1994), 0.37-0.39 (1995), 0.34-0.42 (1996). D/F estimated for older trees were similar to those registered for young (M1-2, M2-1, M3) stands: 0.45-0.47 (1994), 0.37-0.38 (1995) and 0.33-0.40 (1996). Detected differences in water loss among young trees near the oil refinery can not be explained by negligible D/F devia-

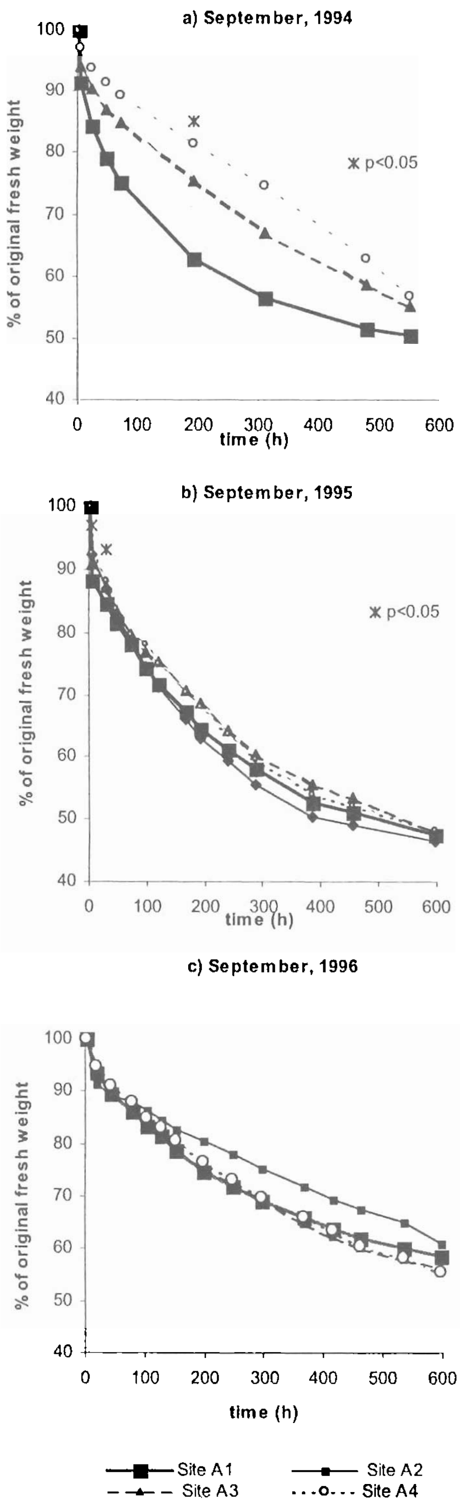

Figure 4. Alternation in one-year-old needles fresh weight over time after excision from trees growing at different distance from the cement factory (September, 1994-1996).

tions and suggest the possibility of surface damage rather than disturbancies in needle supply of water. The scope of D/F (0.46-0.47 in 1994, 0.38-0.40 in 1995, 0.42-0.44 in 1996) for A1-A4 sites resembled the other regions. In all cases gravimetric parameters did not differ significantly among the oil refinery sites as well as between the cement factory sites. D/F obtained from various regions showed similarities in each year, while annual variations were greater: higher values were common in 1994 and 1996 and lower ones in 1995. Generally, dry weight-fresh weight ratios were not related to the speed of water loss from excised needles.

Calculations of dry weight/fresh weight ratio differences between one-year-old and current-year needles (R1-R0) revealed significant variations (0.029-0.055; $p<0.05$) among the J1-J10 sites only in the autumn of 1994 after severe drought, while in subsequent years the deviations were negligible (0.022-0.039 in 1995; 0.029-0.059 in 1996). R1-R0 varied insignificantly among sites of young trees (0.04-0.05 in 1994; 0.02-0.03 in 1995; 0.03-0.04 in 1996) as well as among middle-aged stands (0.04-0.05 in 1994-96) near the oil refinery. Sites near the cement factory (A1-A4) differed according to R1-R0 only in 1994 (0.032-0.047; $p<0.05$), insignificant variation was recorded in subsequent years (0.038-0.044 in 1995; 0.027-0.050 in 1996). Annual and regional variations of R1-R0 were less pronounced as compared to D/F values. Small differences among sites in D/F and R1-R0 indicated at least equal needle supply with water hereby differences obtained examining water loss could be attributed to some type of surface damage including disturbancies in stomatal closure.

Polluted plants may have reduced tolerance to water stress. Various gaseous and aqueous pollutants have been reported to increase the rate of water loss from trees in controlled conditions and in the field (Huttunen et al., 1981; Mansfield & Freer-Smith, 1984; Mengel et al., 1989; Barnes et al., 1990). Several other papers indicate either a reverse effect or no effect of pollution on stomata (Neighbour, Cottam & Mansfield, 1988). There was evidence that the threshold concentration for damage after the period of exposure was at or below 40 nl l^{-1} $SO_2 + NO_2$, while the lowest pollution treatment of 20 nl l^{-1} of both gases hardly affected the transpiration rate of the plants (Neighbour, Cottam & Mansfiel, 1988). Therefore, lack of clearly expressed permanent effects of higher pollutant concentrations on water loss from excised needles in our experiment could be due to quite a low level of pollution.

Needle wettability. The data on the nitrogen fertilizer plant sites are shown in Figure 5. Contact angles on one-year-old needle surfaces significantly varied ($p<0.001$) from 44.7 to 57.4 degrees (1994), from 62.3 to 80.1 degrees (1995) and from 55.5 to 70.7 degrees (1996). In 2 of 3 years the J2 site showed the lowest value and in all 3 years the J6 site had the highest estimate. There

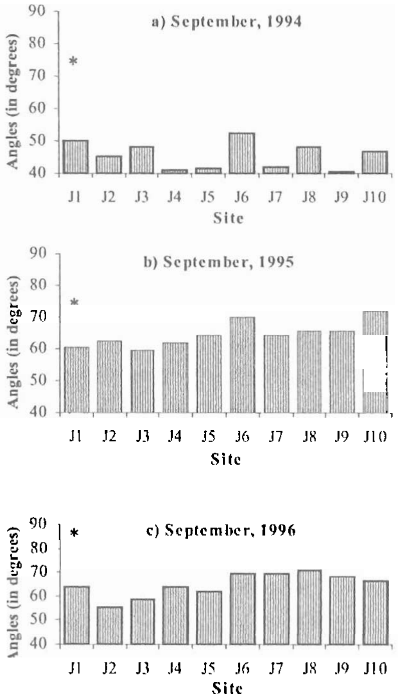

Figure 5. Contact angles (degrees) of water droplets placed on one-year-old needles from trees growing at different distance from the nitrogen fertilizer plant. * - significant difference ($p<0.05$) among sites.

was no close relation between the values of contact angles on the needles and NO_2 concentration on the sites or site distance from the nitrogen fertilizer plant.

In 1994 contact angles ranged from 55.6 to 63.4 degrees ($p<0.001$) for young stands and from 45.1 to 61.5 ($p<0.001$) for middle-aged stands from sites near the oil refinery (Fig. 6). The next year wettability was lower for all sites and varied from 66.0 to 70.5 ($p<0.001$) for young stands and from 65.9 to 68.2 degrees (non-significant differences) for middle-aged stands. In 1996 contact angles on all sites were highest as compared to the results in other year. Estimated degrees ranged from 72.2 to 78.2 ($p<0.001$) for young stands and from 64.8 to 73.7 ($p<0.001$) for middle-aged stands. For young stands wettability varied with each year and did not have a relation to site distance from the pollution source, while among middle-aged stands the lowest values were always documented for M1-1, the site closest to the oil refinery where the highest concentration of NO_2 (table) and the highest defoliation were detected.

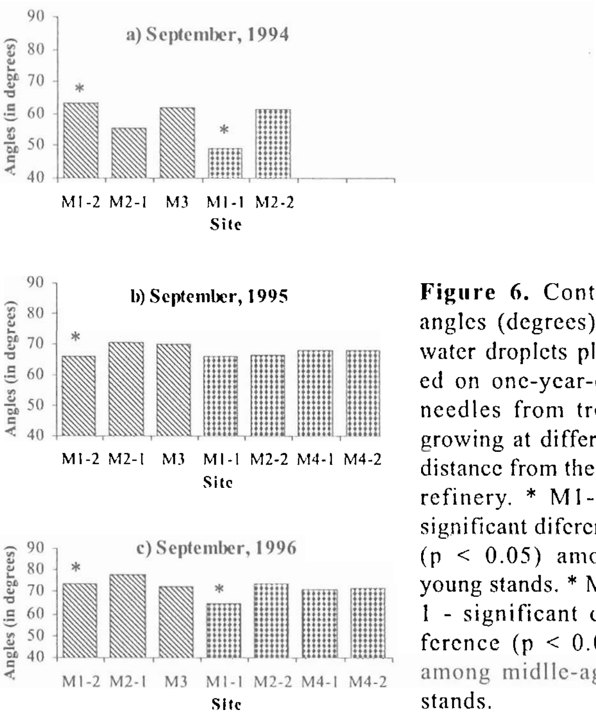

Figure 6. Contact angles (degrees) of water droplets placed on one-year-old needles from trees growing at different distance from the oil refinery. * M1-2 - significant difcrence ($p < 0.05$) among young stands. * M1-1 - significant difference ($p < 0.05$) among midlle-aged stands.

Similar evaluations of contact angles on the needles from the cement factory sites (Fig. 7) revealed significant differences ($p<0.001$) among stands in all 3 years, with variations in the interval of 27.3-42.0 degrees in 1994, 45.6-56.7 degrees in 1995 and 47.3-58.5 degrees in 1996. During the whole observation period site A1 had the lowest values of contact angles of water droplets on the one-year-old needles as compared to the other sites.

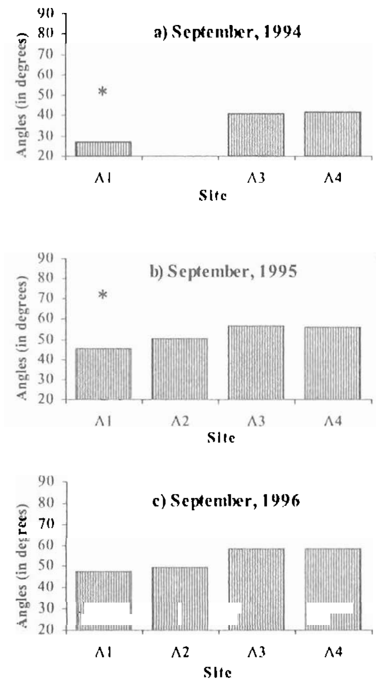

Figure 7. Contact angles (degrees) of water droplets placed on one-year-old needles from trees growing at different distance from the cement factory. * - significant difference ($p<0.05$) among sites.

In different years the data obtained from the nitrogen fertilizer, oil refinery, or the cement factory sites showed the lowest values of contact angles on the needle surface in 1994. The variation in needle wettability may be caused by the interaction between 3 variables: climate fluctuations, pollutants and surface microorganisms (Cape et al., 1995). Variations in wettability may reflect the way in which individual needles and the whole canopy intercept and retain precipitation in a tree (Barnes et al., 1990).

A comparison of data collected in 3 different regions near the pollution sources (see Fig. 5, 6, 7) revealed the lowest values of contact angles for site A1 located near the cement factory. Needle wettability had relationships to the site distance, aerial concentrations of NO_2 and defoliation only for middle-aged stands near the cement factory and oil refinery. In these cases the analysis of variance showed a significant effect of industrial emissions, with smaller contact angles from the trees exposed to the higher pollutants' concentrations.

Microscopical evaluation of the needle surface. In September 1995 the quality of the surface of one-year-old needles was evaluated under scanning electron microscope (SEM; Fig. 8). The mean surface class in stomatal areas of needles from sites near the nitrogen fertilizer plant ranged from 3.5 to 3.9 with the significantly ($p<0.001$) highest value (i.e. the highest structural degradation of wax) for site J2 closely related to the factory. In our previous SEM observations where more precise classification of wax structures was used, J2 showed significantly worst surface conditions compared to the J8 site or relatively 'clean' Kačerginė region (Kupčinskienė 1991; 1992; Kupčinskienė et al. 1996). For young tree sites near the oil refinery average surface class varied from 3.6 to 4.2 ($p<0.005$) while for middle-aged stands from 3.6 to 4.6 ($p<0.001$). Surfaces of one-year-old needles in the immediate vicinity of the oil refinery were more degraded than needles surfaces from adjacent sites. Mean surface class values significantly varied from 3.5 to 3.9 with the biggest area of eroded wax among needles from site A1 ($p<0.005$). Thus, in all regions with the local pollution sources needle surface observations revealed significantly the worst coverage on sites (A1, J2, M1-1, M1-2) closest to the factories. The results about structural degradation of wax on sites near the factories in most cases agreed with the lowest values of contact angles as well as with the greatest defoliation of sampled trees and elevated to a small extent concentrations of aerial NO_2. Among the worst condition sites from various regions the highest structural degradation of wax was detected on site M1-1 adjacent to the oil refinery which is the biggest local polluter in Lithuania. It should be mentioned that site A1, located near the cement factory had the lowest values of needle surface damage according to SEM and the highest values of surface injury according to very low contact angles of water droplets. Such discrepancy could be explained by factors influencing wettability of A1 needles. Presumably both dust par-

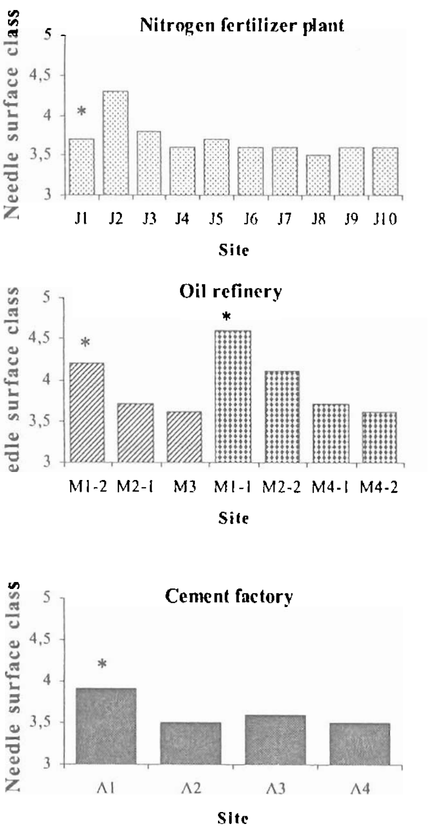

Figure 8. Mean values of surface class of needles from trees growing at different distance from the pollution source (September, 1995). * - significant difference ($p<0.05$) among sites.

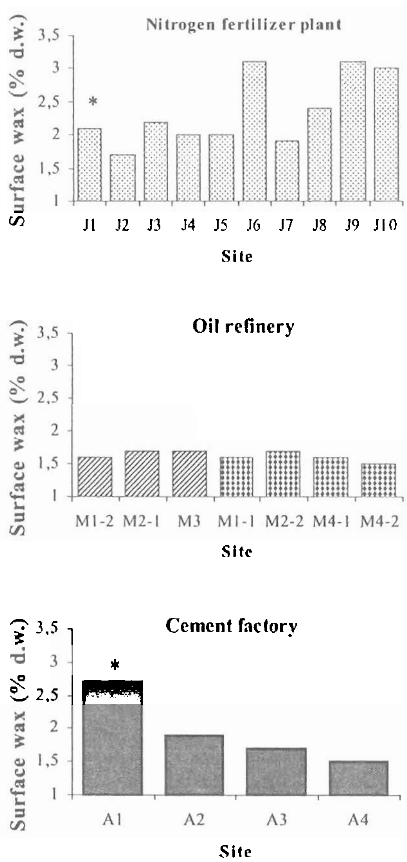

Figure 9. Wax amount (% of dry weight) on the needles from trees growing at different distance from the pollution source (August, 1995).

ticles as well as erosion of wax contributed to the increased moisture on the surfaces.

Chloroform-soluble wax amount. There were significant differences among nitrogen fertilizer sites, although no effects were recorded of site location from the factory on the amount of wax relative to the needle dry weight (Fig. 9). No oil refinery effect could be detected on the total amounts of surface wax. For sites near the cement factory the amount of wax and dust were considerable decreasing (correspondingly $p<0.001$; $p=0.001$, respectively) with increasing site distance from the pollution source. The quantity of wax extracted from the needle surface ranged between 1.7-3.1% dry weight (d.w.) for sites in the nitrogen fertilizer plant region, between 1.5-1.7% d.w. for sites in the oil refinery region and between 1.5-2.7% d.w. for cement factory sites. These results agree with data from literature, where quantity of waxes on pine needles varies from 0.5% to over 2% dry weight. The data concerning changes in wax amount under conditions of simulated or field pollution are very contradicting (Cape et al., 1988; Huttunen, 1994) and our results obtained are in support of it. Pollutant or pH treatment showed a tendency to reduce the concentration of waxes: acid rain-treated Scots pine needles had 50% less epicuticular waxes than in the water controls; the wax quantity of needles of *Picea abies* was considerably reduced by fumigation with 300 mg m^{-3} ozone while UV radiation has been observed to increase epicuticular wax production in plants (Huttunen, 1994). In accordance with our results wax synthesis of the needles may have been changed so that the pines growing in the vicinity of the cement factory protect the needles by producing bigger amount of waxes.

Conclusions

Some gradients of aerial NO_2 concentrations (4.1-2.7; 4.1-2.2 or 2.9-1.9 ppb) were found between the sites located in the immediate vicinity of pollution sources and those situated further from the factories.

In accordance with water loss from excised needles or according to dry weight/fresh weight ratio differences between one-year-old and current-year needles in most cases no relationships were found between the site distance from the factory and water state of examined needles.

Wax amount on the needle surface was significantly increased in trees growing in the neighborhood of the cement factory.

In some cases needle wettability had relationships to the site distance, aerial concentrations of NO_2 and defoliation: indicative differences were detected for middle-aged pine stands near the oil refinery and cement factory.

Needle surface structure investigated by scanning electron microscopy was the most sensitive parameter among all characteristics tested.

In accordance with needle surface parameters investigated, the worst tree condition among sites near the oil refinery was distinguished on site M1-1, while site A1 was poorest among the cement factory stands selected for study.

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МОНИТОРИНГ ПОВЕРХНОСТНЫХ ХАРАКТЕРИСТИК ХВОИ *PINUS SYLVESTRIS*, РАСТУЩЕЙ ВБЛИЗИ ИСТОЧНИКОВ ПРОМЫШЛЕННОГО ЗАГРЯЗНЕНИЯ

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Резюме

Целью данного исследования было изучить влияние загрязнителей на надземную часть *Pinus sylvestris* в течение 3-летнего периода. Концентрация NO_2 измеряли как маркер качества воздуха в точках взятия образцов сосны обыкновенной. Деревья *Pinus sylvestris* изучали осенью 1994-1996 года вблизи нефтяного завода, завода азотных удобрений и цементного фабрика. Сравнение качества поверхности хвои при помощи поверхностной электронной микроскопии, по количеству воска, гравиметрическим характеристикам и по гидрофобными особенностями в некоторых случаях позволило выявить различия между сайтами. Полученные результаты показывают, что у 2-летней хвои в довольно низких концентрациях загрязнители воздуха могут вызвать ощутимые изменения.

Ключевые слова: двуокись азота, хвойные, отдача воды, угол контакта, увлажнение, количество воска, СЭМ.