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EFFECT OF AIR POLLUTION ON THE VOLATILE OIL IN NEEDLES OF SCOTS PINE *(PINUS SYLVESTRIS L.)*

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ILMAN EPÄPUHTAUKSIEN VAIKUTUS MÄNNYN NEULASTEN HAIHTUVIEN ÖLJYJEN MÄÄRÄÄN

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The amount of volatile oil and monoterpene composition in pine needles were studied in trees growing near two factories and in the city of Kuopio. The amount of volatile oil increased with increasing injury class in trees growing near the fertilizer factory. The amounts of volatile oil in trees near the pulp mill differed in the various injury classes. More oil was found in younger needles. The greatest differences in monoterpene composition were in the amounts of camphene, β-pinene, myrcene and tricyclene.

INTRODUCTION

Air pollutants effect the amount and composition of volatile oil in the needles of conifers. SOLBERG and ADAMS (STEWART et al. 1973) described microscopic changes caused by fluoride in *Pinus ponderosa* needles. Epithelial cells of the resin canal hypertrophied and underwent plasmolysis. In many specimens the epithelial cells within the resin canal enlarged until they completely occluded the openings (STEWART et al. 1973). DÄSSLER (1964) observed that needles of 6- to 8-year-old *Picea exelsa* contained less oil after the trees had been exposed to SO_2. The total yield of oil was found to be significantly less in injured *Pinus ponderosa,* although total amount of monoterpenoids remained the same (COBB et al. 1972). The significance of volatile oil and so-called secondary metabolic compounds has been studied in an attempt to evaluate the mechanisms of plant resistance (HANOVER 1975, SMELYANETS 1977) and allelopathy (PUTNAM and DUKE 1978). Changes in oil composition, as well as the effect of tree age, needle age, season and other environmental factors, have been studied (JUVONEN 1966, ZAVARIN 1971, DEMENT et al. 1975, HILTUNEN 1976, GERSHENZON et al. 1978).

The aim of this study was to determine the effects of air pollutants on the amount of volatile oil and the monoterpere composition in 1- to 3-year-old needles of 80-year-old Scots pine *(Pinus sylvestris L.).* The study areas were located: 1) in the city of Kuopio, 2) near a fertilizer factory in Siilinjärvi, and 3) near a pulp mill in Sorsasalo. Trees at these locations are exposed principally to sulphur dioxide and fluoride emissions.

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MATERIALS AND METHODS

The chemical and fertilizer factory of Kemira Ltd., situated near the Siilinjärvi study area (25 km north of Kuopio), emits 3.0 10^6 kg SO_2, 0.4 10^6 kg NO_2, 0.25 10^6 kg NH_3, and 1.0 10^3 kg F^- yearly (T. Karjalainen, M. Eng., Kemira Ltd., personal communication 1977).

The main pollutant from the pulp mill of Savon Sellu Ltd. situated near the Sorsasalo study area (7 km north of Kuopio), is sulphur dioxide, ca. 7 10^6 kg yearly (P. Joutsenoja, M. Sc., Savon Sellu Ltd., personal communication 1977).

Emissions of SO_2 in the city of Kuopio are ca. 10 10^6 kg yearly, based on an emission inventory (Meteorological Institute of Finland 1977) and fuel oil consumption in Kuopio (Öljyalan keskusliitto ry. 1978).

The forest area around Lake Kapeinen near Heinävesi was used as the control area. The nearest noteworthy source of air pollution is the city of Varkaus, 25 km away.

The trees in the study areas were classified in 1976 according to visible injuries. Four damage zones were defined on the basis of these results (LEHTIÖ et al. 1980). Samples were taken at the same time (June and July) from mature trees in the damage zones for analysis of their sulphur content and the study of volatile oils. Samples consisted of 3–4 branches taken from different sides of each tree at a height of 5–10 m. These were collected from 1–6 mature trees, located at least 30 m apart, representing the average degree of injury at the sampling site. The branches were gathered in plastic bags and placed in a refrigerator the same day. 1- to 3-year-old needles were taken from the branches after a few days. One lot of needles was used for dry-weight determinations (dried at 105°C over ninght), and the rest were deep frozen in plastic bags at – 18°C. 50 g of fresh needles was homogenised in distilled water and the oil then extracted by steam distillation (HILTUNEN 1976). The composition of the volatile oil was studied by means of gas chromatography (Carlo Erba Stumentazione 230). A glass capillary column (30 m, ϕ 0.35 mm) with a FFAP stationary phase was used. The carrier gas was nitrogen, the inlet pressure being 0.6 kp/cm^2. The temperature was raised from 40°C to 220°C at a rate of 4°/min. The sample was injected at 175°C. Values for the peaks were obtained using an integrator (Infotronics 304). The monoterpenes were identified by their mass spectra and retention times. A Jeol JMS D-300 with helium as the carrier gas was used. The separation conditions were the same as in the gas chromatography analysis.

RESULTS

The quantity of volatile oil decreased with increasing age of the needles in trees growing in Kuopio and in Sorsasalo. A similar trend was also found in Siilinjärvi and Kapeinen (Table 1). At Siilinjärvi the amount of volatile oil (% of fresh- and dry weights of needles) increased, especially in the high-carene group (where the amount of 3-carene exceeds 10 %, HILTUNEN 1976) with increasing injury class (Table 2). At Sorsasalo the oil content of various injury classes differed only slightly (Table 2).

There was slight positive correlation between the total amount of monoterpenes and injury class in low-carene trees at Sorsasalo. In these calculations, monoterpenes are expressed as percentages of the total oil recorded on the gas chromatogram (Table 2).

Amounts of individual monoterpenes found in this study were approximately the same as those reported previously (Fig. 1, HILTUNEN 1976). The correlation between the amount of monoterpenes and injury class are presented in Table 3. In repeated analyses of a single oil sample, n-hexanal (Fig. 3), α-terpinene, 2-hexenal (Fig. 2), cis- and trans-β-ocimene, and β-carene produced a coefficient of variation greater than 10. The amount of n-hexanal increases with increasing injury class in both the low- and high-carene groups (in the low-carene group the amount of 3-carene is below 10 %). The

Table 1. Amount of Scots pine needle oil (ml oil/100 g dry, 1-, 2- and 3-year-old needles) in trees in the various study areas in 1976 and 1977 (all damage zones combined).

Taulukko 1. Männyn neulasten öljymäärä (ml öljyä/100 g 1-,2- and 3-vuotiaita kuivia neulasia) tutkimusalueittain vuosina 1976 ja 1977 (kaikki vaurioluokat yhdistetty).

Study area *Tutkimusalue*		Age of needles (y) – *Neulasten ikä (v)* 1	2	3	Test variable – *Testisuure* N	F	r_s
Kapeinen	1976	1.11	0.97	0.87	27	1.74	
	1977	1.28	1.21	1.08	58	2.15	– 0.172
Kuopio	1976	1.19	1.05	0.98	66	6.30**	
	1977	1.16	1.00	0.86	33	5.41**	– 0.371*
Sorsasalo	1976	1.21	1.08	0.91	24	3.50*	
	1977	1.21	1.10	0.97	33	3.85*	– 0.397*
Siilinjärvi	1976	1.07	0.99	0.85	33	1.65	
	1977	1.10	1.07	0.92	42	1.57	– 0.198

N = number of trees. – *puiden lukumäärä*

F = the F-value of variance analysis (for needles of various ages). – *varianssianalyysin varianssisuhde (eri-ikäisille neulasille).*

r_s = the Spearman rank correlation coefficient (for needles of various ages). – *Spearman'n järjestyskorrelaatiokerroin (neulasten iän suhteen).*

°p<o.1, *p<0.05, **p<0.01, ***p<0.001

Table 2. Correlation between the amount of pine needle oil (ml/g fresh and dry needles) and injury class, correlation between the total amount of monoterpenes and injury class and F-value for the amount of oil in various classes (two-year-old needles in 1978).

Taulukko 2. Männyn naulasöljyn (ml/g tuoreita ja kuivia neulasia) ja vaurioitumisasteen suhde, monoterpeenien kokonaismäärän ja vaurioluokan suhde ja varianssisuhde eri vaurioluokkia edustaville puille (2-vuotiaat neulaset, kerätty 1978).

Study area *Tutkimusalue*	Correlation between amount of oil (ml/g) and injury class *Öljymäärän (ml/g) suhde vaurioluokkaan* Fresh, *Tuoreet* r_s[1])	N[2])	Dry, *Kuivat* r_s	Correlation between monoterpenes and injury class *Monoterpeenien suhde vaurioluokkaan* r_s	N	Test variable *Testisuure* F[3])	N
Sorsasalo high-carene *korkea-kareeninen*	– 0.065	39	– 0.128	0.016	44	2.32°	71
low-carene *matala-kareeninen*	– 0.008	23	– 0.102	0.401°	19		
Siilinjärvi high-carene *korkea-kareeninen*	+ 0.321*4	37	+ 0.370*	0.091	41	1.39	70
low-carene *matala-kareeninen*	+ 0.120	31	+ 0.202	– 0.216	27		

[1]), [2]), [3]) and [4] see Table 1. – *vertaa taulukko 1.*

Table 3. Correlations between the amounts of monoterpenes and the injury class and F-value of variance analysis for the same material studied in 1978.

Taulukko 3. Monoterpeenien ja vaurioluokan välinen korrelaatiokerroin (r_S) ja varianssisuhde (F) vuonna 1978.

Characteristic *Tunnus*	Sorsasalo Low-carene *Matalakareeninen* N[3] = 22 r_s[1])	F[2])	High-carene *Korkeakareeninen* N = 42 r_s	F	Siilinjärvi Low-carene *Matalakareeninen* N = 32 r_s	F	High-carene *Korkeakareeninen* N = 38 r_s	F
1. Tricyclene, *Trisykleeni*	–0.011	0.27	–0.187	2.54	0.314°	1.29	0.032	1.25
2. α-Pinene, *α-Pineeni*	–0.104	0.69	–0.063	0.34	–0.197	3.20	0.039	0.07
3. Camphene, *Kamfeeni*	–0.075	0.36	–0.193	2.90*	0.321°	1.57	–0.034	3.03°
4. n-Hexanal, n-Heksanaali	0.380°4	2.25	0.429**	1.19	0.302°	0.74	0.465**	4.97*
5. β-Pinene, *β-Pineeni*	–0.182	0.25	–0.083	1.40	0.429*	1.82	0.199	0.59
6. Sabinene, *Sabineeni*	0.072	2.15	0.119	1.16	0.118	1.07	0.117	0.40
7. 3-Carene, *3-kareeni*	0.404°	1.45	0.154	0.73	–0.155	2.25	0.025	0.23
8. Myrcene, *Myrseeni*	–0.203	0.85	–0.019	0.57	0.362*	2.74	0.078	1.23
9. α-Teprinene, *α-Terpineeni*								
10. Limonene, *Limoneeni*	0.044	0.12	0.037	1.01	0.047	0.20	0.109	0.72
11. β-Phellandrene *β-Fellandreeni*	0.184	0.01	0.271°	0.58	0.153	0.11	0.061	0.89
12. 2-Hexenal, *2-heksenaali*	0.354	1.54	0.142	0.73	0.061	1.05	0.333*	2.41
13. Trans-β-ocimene *Trans-β-osimeeni*	–0.089	0.61	0.165	0.91	0.467**	2.85	0.289°	3.45*
14. Cis-β-ocimene *Cis-β-osimeeni*								
15. p-Cymene, *p-symeeni*		1.52	0.350*	10.06***		0.58	0.021	1.26
16. β-Carene, *β-kareeni*			0.193	0.78			0.103	0.04
17. Terpinolene, *Terpinoleeni*	0.157	1.03	0.030	0.89	–0.171	0.89	–0.080	0.92

[1]), [2]), [3]) and [4] see Table 1. – *Vertaa taulukko 1.*

injury classes of trees growing at Sorsasalo differed significantly in the amount of camphene in the high-carene group and in the amount of 3-carene in the low-carene group. The amount of myrcene, β-pinene, camphene, and tricyclene increased in trees of the low-carene group at Siilinjärvi, and the amount of camphene differed in various classes of the high-carene group. Similar results were obtained in 1977 (LEHTIÖ, unpublished results) for camphene in the high-carene group at Sorsasalo and Siilinjärvi and for β-pinene in the low-carene group at Siilinjärvi.

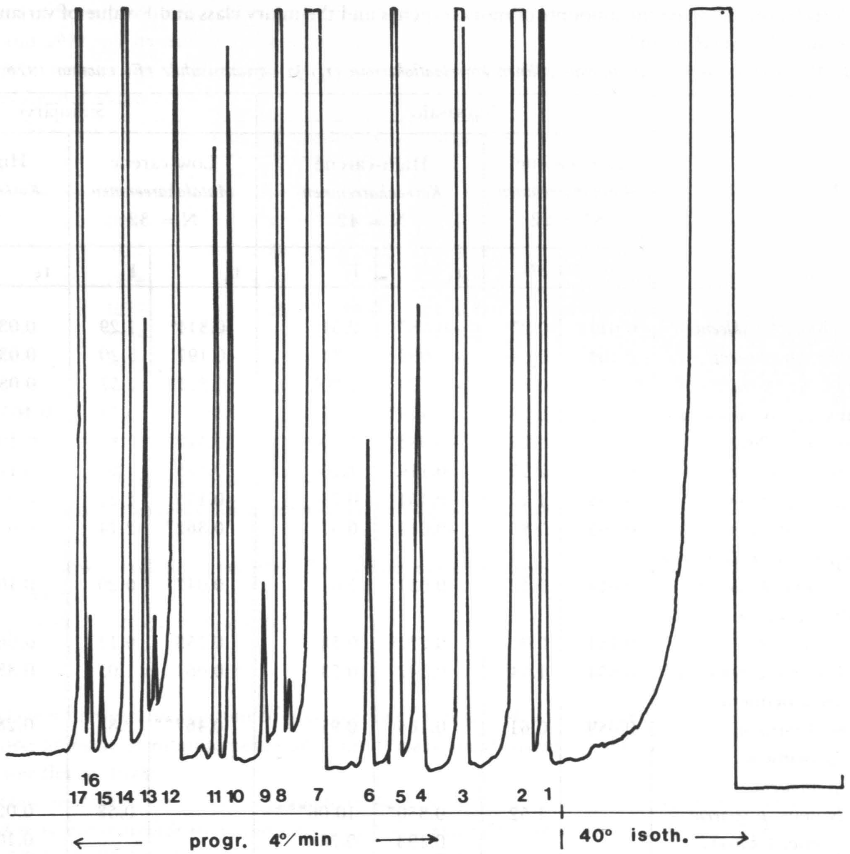

Fig. 1. Glas chromatogram of monoterpene hydrocarbons from Scots pine. The peak numbers refer to the numbers of the monoterpenes shown in Table 3.
Kuva 1. Männyn neulasöljyn monoterpeeniosan kaasukromatogrammi. Piikkien numerot vastaavat taulukon 3 numeroita.

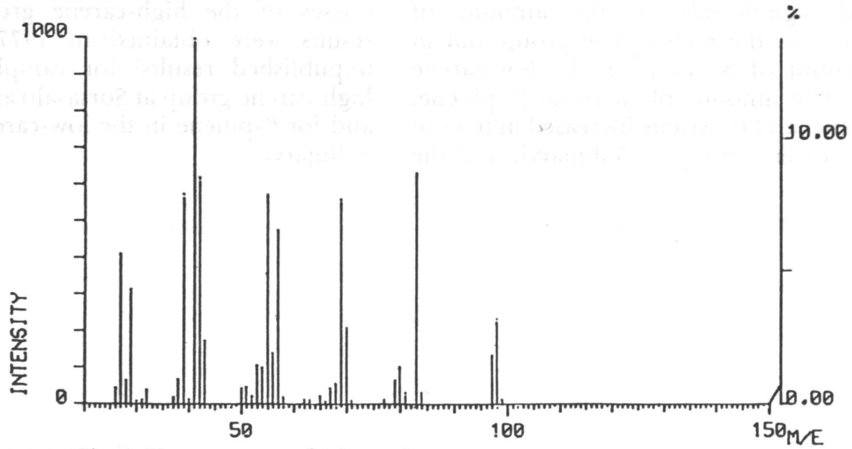

Fig. 2. Mass spectrum of 2-hexenal
Kuva 2. 2-Heksenaalin massaspektri

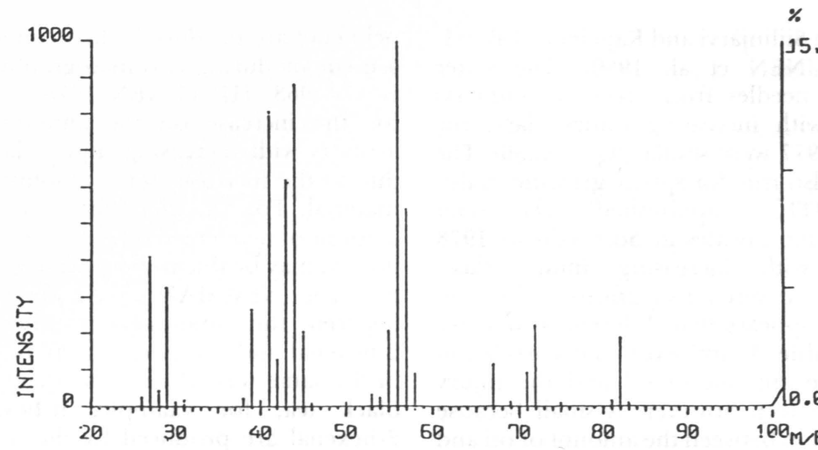

Fig. 3. Mass spectrum of n-hexanal
Kuva 3. n-Heksanaalin massaspektri

DISCUSSION

Poltavchenko (ZAVARIN et al. 1971) reported decreasing quantity of volatile oil with increasing age of pine needles. JUVONEN (1966) observed that the content of pine oil in emerging needles is greater than in older needles and that the oil content in older needles fluctuates less in mature (ca. 80 years old) trees than in younger ones. The increase in the total amount of oil, especially that in trees growing at Siilinjärvi, may be caused by emission of fertilizer compounds from the fertilizer factory. In previous studies the total concentration of oil in fertilized sample trees has been greater than in the controls (HILTUNEN et al. 1975). To the amount of volatile oil is expressed as a percentage of dry or fresh weight of the needles, the water content of the needles was also studied. The water content (percentage dry weight) was significantly different in needles of differing

Table 4. The water content (% dry weight) of needles of various ages (years) and trees of various injury classes.
Taulukko 4. Neulasten vesipitoisuus (% kuivapainosta) neulasten iän ja vaurioluokan mukaan ryhmiteltynä.

Study area *Tutkimusalue*	Collection date *Keräysaika*	Age of needles – *Neulasten ikä* Water content – *Vesipitoisuus, %*				Test variable *Testitunnus*		
		1	2	3	4	N[1])	F[2])	r_s
Kapeinen	1977-06-21	108	99	96		60	7,4**	
Kuopio	1977-06-14–16	95	90	87		30	3,0	
Sorsasalo	1977-06-2–8	93	87	84		48	2.9	
Siilinjärvi	1977-06-22–29	102	93	89		60	18.2***	
		Injury class – *Vaurioluokka*						
Sorsasalo	1978-06-7–13	92	94	93	79	72	5,9**	–0.25**
Siilinjärvi	1978-06-13–21	88	94	99	–	70	4.9**	0.47**

[1]), [2]) and [3] see Table 2. – *Vertaa taulukko 1*

ages at both Siilinjärvi and Kapeinen (Table 4, cf. HUTTUNEN et al. 1980). The water content of needles from trees at Siilinjärvi increased with increasing injury class; the results in 1977 were similar ($r_s = 0.236$). The same was also true for spruce growing in this area (LEHTIÖ, unpublished). The water content of the needles at Sorsasalo in 1978 decreased with increasing injury class, although there were no significant differences in 1977. It appears that differences in water content (Table 4) influence the correlation between the amount of oil and the injury class. This effect, however, is small because the correlation between the amount of oil and injury class is almost the same regardless of the way in which oil content is expressed (Table 2).

Most of the compounds (n-hexanal, α-terpinene, 2-hexenal, cis- and trans-β-ocimene) are produced when conifer needles are cut or during chromatography (MAJOR et al. 1963, HILTUNEN 1976). The reason for the increase on the amount of these artifacts with increasing injury class may be due to the increase in the amount of source material. For example, the increase in the amount of myrcene in low-carene trees at Siilinjärvi may be due to thermal degradation of β-pinene (NEWMAN 1972). The correlation between the amounts of n-hexanal and 2-hexenal and injury class may be explained in the same way. During the manufacture of black tea, for example, n-hexanal and 2-hexenal are produced by the degradation of lipids in leaves that have been damaged in the presence of oxygen (SELVENDRAN et al. 1978). In pine needles, changes in lipid biosynthesis occur in trees exposed to sulphur dioxide (MALHOTRA and KHAN 1977).

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SELOSTE:

ILMAN EPÄPUHTAUKSIEN VAIKUTUS MÄNNYN NEULASTEN HAIHTUVAN ÖLJYN MÄÄRÄÄN

Vuosina 1976–78 tutkittiin 1–3 -vuotisten männyn (*Pinus sylvestris L.*) neulasten haihtuvan öljyn määrää ja monoterpeenikoostumusta Kuopion kaupungin alueella sekä Savon Sellu Oy:n selluloosatehtaan ja Kemira Oy:n lannoitetehtaan ympäristössä kasvavista keskimäärin 80-vuotiaista puista. Pääasiallisimmat ilman epäpuhtaudet ovat näillä alueilla rikkidioksidi ja fluoridi.

Haihtuvan öljyn määrässä havaittiin lisääntymistä lannoitetehtaan ympäristössä kasvavilla puilla niiden vaurioitumisasteen kasvaessa. Selluloosatehtaan ympäristössä eriasteisesti vaurioituneiden puiden öljymäärät erosivat toisistaan merkitsevästi. Nuoremmissa neulasissa todettiin olevan enemmän öljyä. Eri vaurioluokkien välillä olivat monoterpeenikoostumuksessa suurimmat erot kamfeenin, β-pineenin, myrseenin ja trisykleenin määrissä.