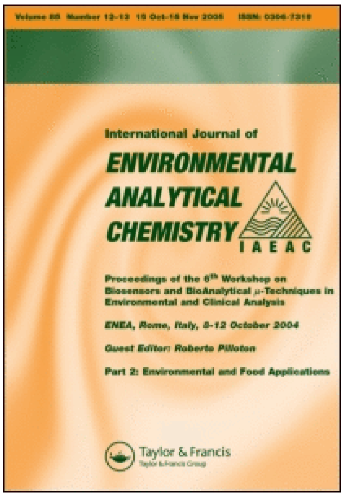

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Forest Damage: Characterization of Spruce Needles by Pyrolysis Field Ionization Mass Spectrometry†

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Needle tips of Norway spruce are investigated by direct probe pyrolysis field ionization mass spectrometry and Curie-point gas chromatography/mass spectrometry. The obtained results are used to characterize the thermally stable plant constituents and major macromolecular structures of spruce needles. The analyses of green, damaged, ozone-fumigated and acidic fog water treated spruce needles reveal the major differences between the different samples. It is shown that ozone-fumigation degrades flavones and the lignin structure whereas acidic precipitation changes the polysaccharides in damaged spruce needles. However, the damage pattern in nature cannot be explained by the patterns of experimentally damaged spruce needles alone.

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†Preliminary results have been presented at the Symposium "Ozone and Biologie" organized by the Association Internationale de l'Ozone, Rennes, France, April 17–19, 1984.

These results suggest that other, probably combined additional causes lead to the observed damage.

KEY WORDS: Spruce needles, forest damage, field ionization MS, pyrolysis-FIMS, Curie point GC/MS, ozone, acidic fog (rain).

INTRODUCTION

In the past few years a severe and rapidly increasing decline of forests has been observed in Europe.[1] While for the most affected silver fir (*Abies alba*) tree damage has been reported since 1830,[2] in recent years the other major tree species Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and common beech (*Fagus sylvatica*) have also been severely affected. The present forest dieback occurred almost simultaneously at various locations and the trees show new symptoms of damage which in most cases are different from the well-known fume damages.[3] Up to now this environmental problem could not be linked to any specific cause. Hence many hypotheses have been proposed to explain the new type of tree damage. Besides the arguments that the observed forest damage is caused by climatological changes,[4] microwaves,[5] radioactivity[6] or virus diseases[7] etc., in the F.R.G. chemical air pollution is nowadays generally considered as the prevailing reason for the present forest decline.[8]

Air pollutants can affect plant tissues *directly* by their deposition on above-ground plant surfaces. On the other hand by their deposition on soils, they can affect plants *indirectly* by air pollutant-induced changes of the soil chemistry leading to acidification[9] and accumulation of nitrogen[10] in soils. It is known that direct impact of car exhaust gases,[11] sulphur dioxide,[12] volatile chlorinated hydrocarbons,[13] H_2O_2,[14] PAN,[15] ozone[16] and acidic precipitation[17] can damage above-ground plant tissues severely. Furthermore, it has been suggested that triethyl-lead from the combustion of lead-containing gasoline[18] and photochemically formed dinitrophenols could have phytotoxic effects.[19] All these air pollutants are present in the ambient atmosphere and are potentially detrimental over a wide concentration range. However, due to synergistic effects of various gases[20] and interactions of gaseous and dissolved air pollutants with heavy metal-containing aerosols, which are deposited on plant surfaces,[21] it seems not yet possible to extract any specific reason for the observed damages.

The best analytical approach for deducing characteristic damage reactions would be to analyse all chemical changes within the damaged plant tissues and then to reach conclusions on potential causes. Recently, many leaf constituents such as nutrients, phytohormones, enzymes and metabolic products of photosynthesis of damaged plants have been thoroughly investigated and compared with materials from macroscopically undamaged control trees. The vast variety of alterations in the chemical composition, the morphological and physiological states suggests that the forest damage has to be considered as a multi-factor problem.[22] In particular, for the chemical analysis of plant material, the presumption of multivariability requires analytical methods which provide maximal and universal information about composition and structure of plant materials.

The methodology of choice seems to be analytical pyrolysis (Py) because it is nowadays widely and successfully used for the rapid characterization of synthetic macromolecules[23] and complex biomaterials.[24,25] In combination with soft ionization mass spectrometry (MS), which produces almost exclusively molecular ions of the volatile thermal degradation products such as low molecular weight volatiles and primary building blocks of biopolymers, it is possible to gain a great deal of significant information about the chemical composition and structure of complex bio-macromolecules.[26,27] Hence this methodology has been recently applied to the problem of forest dieback and shows that Py-field ionization (FI)MS is a very suitable soft ionization technique for the comprehensive characterization of ozone-fumigated beech leaves.[28] The application of this method provided information about the chemical alterations in the structure of thermally stable plant constituents, polysaccharides and lignin. These preliminary results encouraged the use of Py-FIMS to analyse conifer needles.

The aim of this study is to compare Py-FI mass spectra of green, yellowish, ozone-fumigated and acid fog water treated spruce needles in order to establish the major chemical differences. From these results changes in the macromolecular structure of spruce needles might be seen and perhaps detrimental reactions or specific causes deduced.

MATERIALS AND METHODS

Spruce needles

In December 1985 one-year-old needles were taken from an eighty-year-old Norway spruce growing at the Kleiner Feldberg (800 m a.m.s.l., Taunus mountains, F.R.G.). In contrast to the green, shaded spruce needles, the upper sunshine exposed needles showed yellowish needle tips. This well-known phenomenon indicates the destruction of chlorophyll and consequently the disturbance of photosynthesis. The ambient air in the Taunus mountains is contaminated and mean concentrations of gaseous air pollutants are shown in Table I.

In February 1986, twigs were taken from a twelve-year-old Norway spruce at the same site in the Taunus mountains and supplied with water in the laboratory. At room temperature some of the shaded, light-green half-year-old needles were treated with fog water. This acidic solution was allowed to evaporate overnight each time. The fog water with its original pH of 2.9 was sampled at the Kleiner Feldberg in October 1985 and acidified with H_2SO_4 to pH 0.5. After 5 days the spruce needles became brown and dropped from the twigs. In addition, needles from five-year-old Norway spruce trees, grown in a fumigation chamber, were provided by Dr. B. Prinz, Landesanstalt für Immissionsschutz in Essen (F.R.G.). Besides the control trees grown in purified air (38 KLux), spruce needles were taken from trees subjected to ozone fumigation (150 μg/m^{-3}, 38 KLux) for 42 days. Comparison with Table I shows that these conditions almost approached the natural conditions. All spruce needle samples were air-dried and the analyses of damaged

TABLE I

Monthly (M) and daily (D) mean values of gaseous air pollutants at the Kleiner Feldberg, F.R.G. (μg/m^3).[29]

1984	M	D
SO_2	27	73
NO_2	20	39
NO	3	10
O_3	49	100

needles always compared with the analysis of the respective control needles.

Methods

Direct probe temperature-programmed/time-resolved Py-FIMS was carried out with whole needle tips cut from the spruce needles. The samples, about 400 μg, were transferred into a commercially available aluminium crucible which was mounted orthogonal to the FI emitter of a Finnigan MAT 731 mass spectrometer equipped with a combined EI/FI/FAB ion source. The ion source was kept at a pressure below 10^{-7} Pa and at a temperature of 250 °C. The samples were heated linearly from 50 to 500°C at a rate of 1°C/s. Between the magnetic scans the emitter was flash-heated to 1,500 °C in order to avoid condensation. In general, 45 spectra were recorded electrically in the selected temperature range which allows plotting of the total ion counts against time and/or temperature (thermogram). The FI signals of all these spectra were integrated and the summed spectrum normalized to its total ion count using the Finnigan MAT Spectro-System SS200. The integrated and normalized spectra of the three specimens were averaged. For comparison the averaged spectra of the control needle tips were subtracted from the averaged spectra of the treated needle tips. The elemental composition of some selected molecular ions was determined by high resolution and peak matching. In general, between 70 and 80% of the sample material volatilized in the mass spectrometer. No significant differences in the mean sample residue (23%) could be detected between green and damaged needle tips. The weight averaged molecular weights, $\bar{M}_w$, of the pyrolysates were calculated from the FI mass spectra according to Lattimer and Schulten.[30]

In addition, Curie-point-Py coupled with GC-EI/FIMS was carried out to identify some pyrolysates of the needle tips.[31] In the pyrolysis oven, which was connected directly to the injector (280 °C) of the Varian gas chromatograph model 3700, the needle tips were heated from room temperature to 500°C (10 s) with a Fischer 0310 Curie-point pyrolyser. The only modification made to the commercially available apparatus was the inclusion of a leak to enable rapid pressure release after Py and sample injection.[32] After Py, the pyrolysates were flashed with helium carrier gas onto the DB1-30W

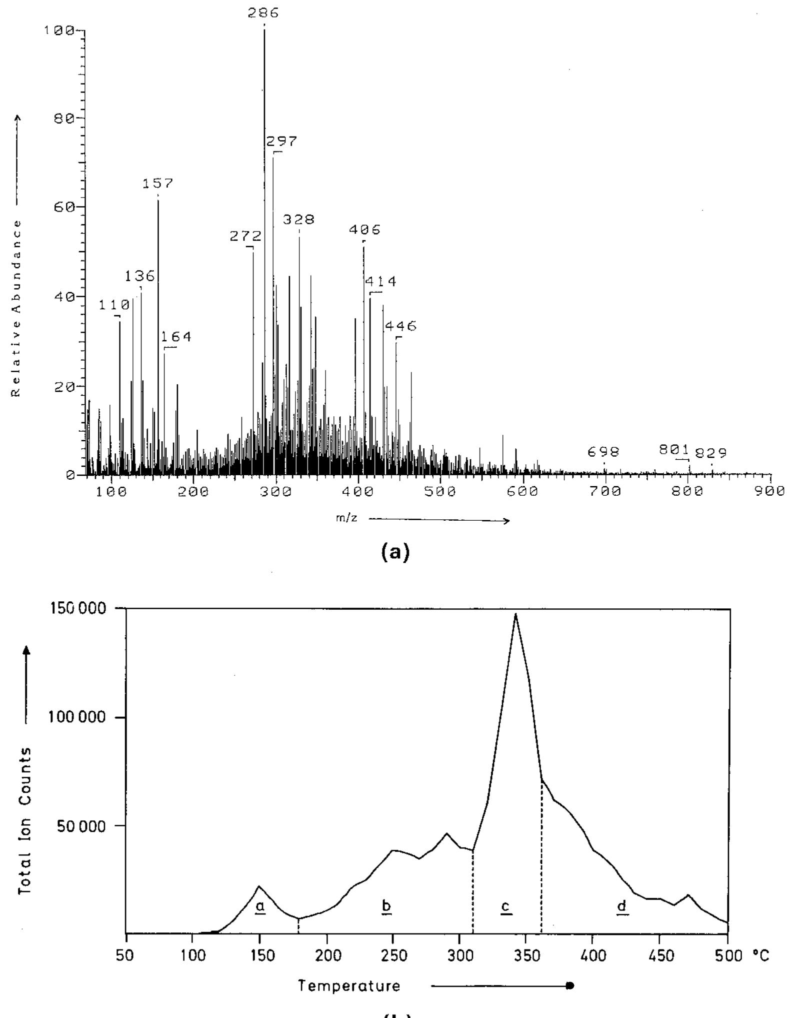
(25 m) capillary column which then was heated linearly from 40 to 300 °C with a rate of 10 °C/s. The separated pyrolysates were detected with a Finnigan MAT 212 mass spectrometer and their molecular weights established by their FI mass spectra (voltage and emitter potential 3 kV, counter electrode −8 kV, multiplier 2.2 kV, scanning speed 1.1 s/mass decade, mass range m/z 70–600).[33] In an additional GC run with EI (accelerating voltage 3 kV, ionization energy 70 eV, multiplier 2.2 kV, scanning speed 1.1 s/mass decade, mass range m/z 40–500) tentative identification of the volatile pyrolysates could be achieved by the comparison of their EI mass spectrum with the spectra of the NBS library.

RESULTS

Pyrolysis field ionization mass spectrum of spruce needles

The averaged Py-FI mass spectrum of green spruce needles is shown in Figure 1(a). More than 700 signals of nominal masses represent the mass spectrometric pattern of a spruce needle. Almost all of these FI mass signals are due to molecular ions of the pyrolysates. Applying Curie-point-Py and GCMS 32 compounds could be identified and are listed in Table II. In combination with the results of former Py-FI studies of pure biopolymers[34] such as polysaccharides, lignins and chitin,[35] it is possible to give a thorough interpretation of the presented Py-FI mass spectrum.

Molecular ions of thermal degradation products derived mainly from mono- and polysaccharides are seen with their typical distribution and relative abundances at m/z 72, 74, 84, 85, 96, 98, 110, 112, 114, 126, 144 and 162. Characteristic signals derived from aldohexose subunits of cellulose can be seen at m/z 126 and 144,[36] whereas aldopentose subunits of hemicellulose produce the typical mass signals at m/z 114 and 132.[37] While most of the above-mentioned molecules could be identified by GCMS (Table II), under the applied experimental conditions, it was not possible to chromatograph levoglucosenone and levoglucosan which occupy the mass signals at m/z 126 and 162 because of their thermal lability.[38] Besides the neutral polysaccharides characteristic peaks can be seen at m/z 71 derived from phospholipids and at m/z 99 and m/z 117 derived from proteins.[34] Both thermally labile biopolymers produce most of their



(a)

(b)

FIGURE 1 (a) Integrated, averaged and to one million total ion counts normalized Py-FI mass spectrum of one-year-old, green spruce needle tips (50–500 °C, 1 °C/s, base peak: 27,200 counts; $\bar{M}_w$:372). (b) Thermogram showing the total ion counts during the time-resolved pyrolysis of a green spruce needle tip.

TABLE II

List of tentatively identified compounds in the Py-FI mass spectra of spruce needles

Direct probe Py-FIMS m/z (mol. ion)	Curie-point-Py-GC-EIMS compound	Origin[b]
44	acetaldehyde	P
58	acetone	L
60	acetic acid	P
74	1-hydroxy-2-propanone	P
94	phenol	L
96	2,5-dimethylfuran[a]	P
98	furfuryl alcohol[a]	P
98	5-methyl-2-furanone[a]	P
110	5-methyl-2-furaldehyde	P
112	5-methyl-furfurylalcohol[a]	P
112	3-methyl-2,5-furandione[a]	P
116	1-methylethoxy-2-propanone[a]	P
120	2,3-dihydrobenzofuran[a]	L
124	guajacol[a]	L
136	*p*-hydroxy-acetophenone	L
138	4-methyl-guajacol[a]	L
138	4-ethyl-1,3-benzenediol[a]	L
144	2-methyl-3,5-dihydroxy-2,3-dihydro-4H-pyran-4-one	P
150	*p*-ethenyl-guajacol	L
152	vanillin	L
152	4-ethyl-2-methoxy-phenol[a]	L
164	2-methoxy-4-(1-propenyl)-phenol	L
178	coniferyl aldehyde	L
180	coniferyl alcohol	L
182	4-hydroxy-3-methoxy-benzene-acetic acid[a]	L
208	sinapyl aldehyde	L
210	sinapyl alcohol	L
214	2-phenoxy-benzoic acid	
256	palmitic acid	V
414	*β*-sitosterol	V
424	10-nonacosanol[a]	V
430	*α*-tocopherol[a]	V

[a] Molecular weight confirmed by Curie-point-Py-GC-FIMS.

[b] L: thermal degradation product of lignin;[41-49] P: pyrolysate of polysaccharides;[36,37] V: volatile, thermally stable plant constituents.

abundant degradation products as molecular ions in the mass range below m/z 70 which has not been recorded in the present investigation. In addition to these common biopolymers, typical fragments of aliphatics are also found in the FI mass spectrum of spruce needles at m/z 73, 87, 101, 115 and 129 (Figure 1(a)). As examples, palmitic acid, a very common constituent of plants, and 10-nonacosanol could be detected by Py-GCMS (Table II) and as their molecular ions by Py-FIMS at m/z 256 and at m/z 424. Recently it was shown that 10-nonacosanol is a characteristic component in the epicuticular wax of conifers.[39] Even under FI condition in the recorded temperature range, this secondary alcohol produces very abundant fragment ions at m/z 157 and m/z 297 by α-cleavage and at m/z 406 by the loss of a water molecule.[40] These three fragment ions can be seen in Figure 1(a) with relative abundances above 45%.

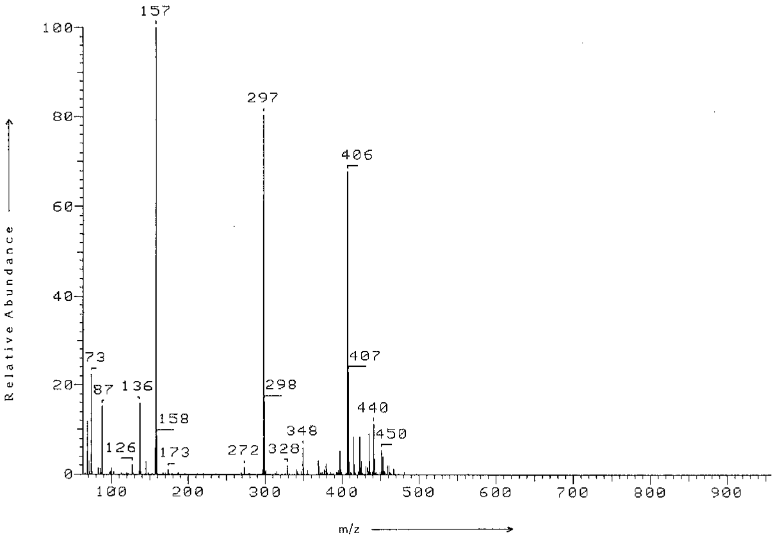
Conifer needles also contain at least 5 to 10% lignin[41] and thus lignin-derived FI signals have been detected at m/z 94, 108, 120, 124, 136, 138, 148, 150, 152, 154, 164, 178, 180, 194, 208 and 210.[42] Table II shows that these mass signals are occupied by monolignol building blocks of spruce lignin with its major components guajacol at m/z 124, 4-methyl-guajacol at m/z 138 and coniferyl alcohol at m/z 180. Former Py-GCMS studies of lignins[43–46] agree very well with our results, although in the present experiment the whole spruce needle was pyrolysed. Analogous to beech lignin, the mass signals at m/z 272, 284, 298, 300, 302, 312, 314, 316, 326, 328, 330, 332, 344, 358, 372 and 418 are due to molecular ions of dilignol building blocks.[28] The mass signals between m/z 272 and m/z 418 represent a plurality of dimeric guajacyl and syringyl building blocks being indicated by homologous series of ions at m/z 272, 302, 332, 362, 392 and at m/z 298, 328, 358, 388, 418. These homologous series of ions can be explained by stepwise addition of methoxy groups to basic dilignol building blocks.[47] Obviously nearly all other mass signals in this mass range indicate these dilignol building blocks altered by progressive side-chain substitution. In analogy, the homologous series of molecular ions at m/z 446, 476, 506, 536, 566 and 596 can also be interpreted by stepwise addition of methoxy groups to basic trilignol building blocks of spruce lignin. The same building blocks with a double-bond lead to the mass signals at m/z 444, 474, 504, 534, 564 and 594 with the molecular ion of dehydrotriconiferyl alcohol at m/z 534.[48]

However, not all mass signals in the higher mass range are derived from lignin. Py-GCMS shows that some thermally stable plant constituents can be detected after Curie-point-Py of spruce needles (Table II) and hence the abundant mass signals at m/z 286, 414 and at m/z 430 are due to molecular ions of occluded volatiles. High resolution mass spectrometric analysis of the base peak at m/z 286 revealed that this signal is mainly due to a $[C_{15}H_{10}O_6]^{+\cdot}$ ion (measured: 286.0480, calculated: 286.0477) which is tentatively assigned as the molecular ions of tetrahydroxyflavones such as kaempferol which has been reported to occur in needles of *Picea abies.*[49] In the higher mass range above m/z 500 no estolidic compounds which occur in the epicuticular wax of spruce needles have been found.[39] However, homologous $(CH_2)_2$-series of odd-numbered ions indicate the occurrence of unsaturated and saturated mono- and diglycerides which form abundant $[(M+H)—H_2O]^+$ fragment ions under FI conditions.[40]

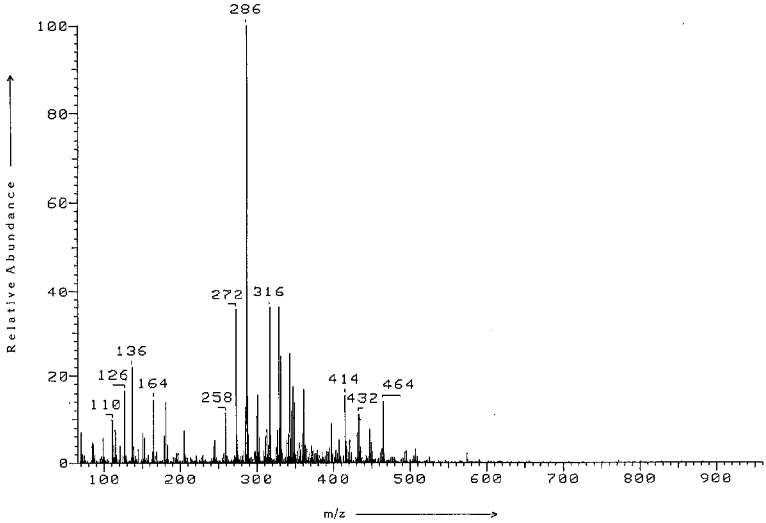
Temperature-programmed Py-FIMS of spruce needles

The thermogram corresponding to the Py-FI mass spectrum of a green spruce needle (Figure 1(a)) is shown in Figure 1(b). Obviously, the relative maximum of the total ion counts at 150 °C indicates relatively volatile constituents of spruce needles. The corresponding summed spectrum of the temperature range 80 to 180 °C (*a* in Figure 1(b)) is shown in Figure 2(a). This spectrum looks very similar to the FI mass spectrum of epicuticular wax of Norway spruce previously reported.[40] The most prominent mass signals at m/z 157, 297 and at m/z 406 are due to 10-nonacosanol. The molecular ions of *n*-fatty acids are seen at m/z 340 (C_{22}), m/z 368 (C_{24}) and m/z 396 (C_{26}). Furthermore, molecular ions of 10-nonacosanone, methylenecyclolanostanol, octyl docosanoate and octyl tetracosanoate are seen at m/z 422, 440, 452 and 480. All these long-chain compounds contribute to the aliphatic fragment ions at m/z 73 and m/z 87. In addition some other molecular ions of volatiles are detected at m/z 136 (carene) and m/z 414 (sitosterol).

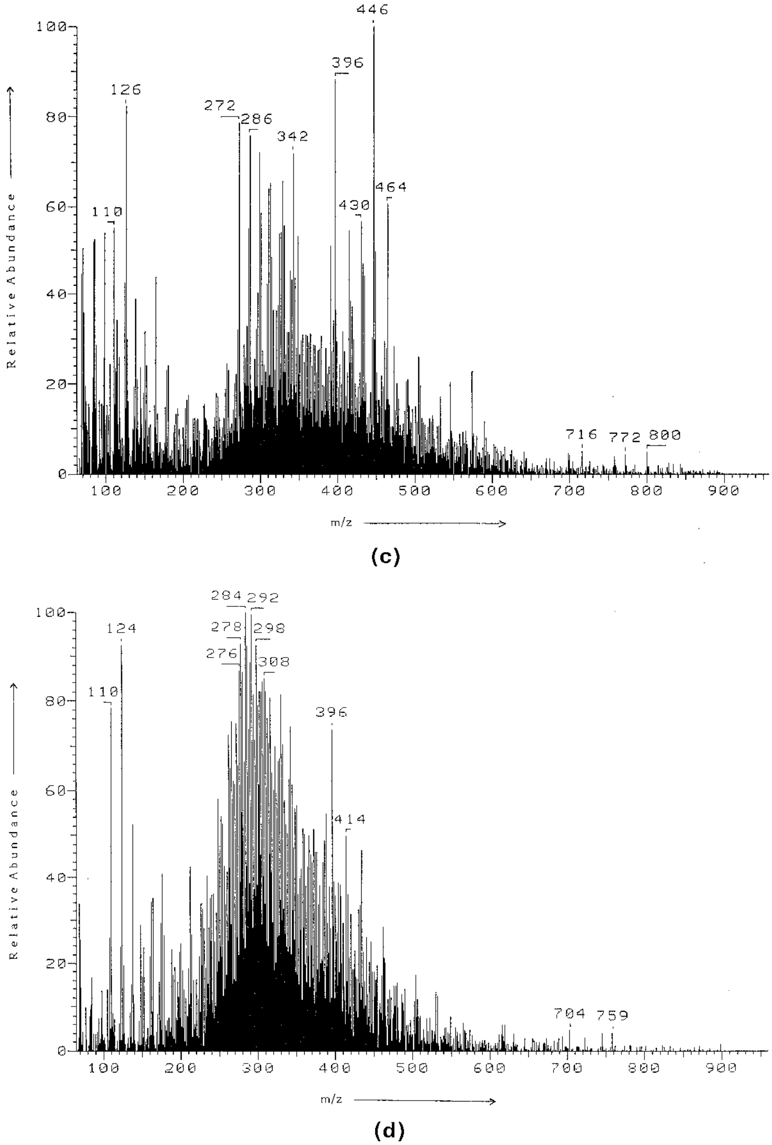
The summed FI mass spectrum in the temperature range from 180 to 310 °C (*b*) is shown in Figure 2(b). Now, characteristic mass signals of thermal degradation products derived from mono-, oligo-, polysaccharides and glycosidic lignin moieties already can be seen.


157
297
406
407
73
87
136
298
158
440
348
450
126
173
272
328
100
80
60
40
20
0
Relative Abundance
100 200 300 400 500 600 700 800 900
m/z

(a)


286
272
316
136
126
164
110
258
414
432
464
100
80
60
40
20
0
Relative Abundance
100 200 300 400 500 600 700 800 900
m/z

(b)



(c)

(d)

FIGURE 2 For selected temperature intervals (see Figure 1(b)) integrated FI mass spectra of green spruce needles: (a) 80–180 °C; base peak: 144,700 counts; $\bar{M}_w$: 337. (b) 180–310 °C; base peak: 326,800 counts; $\bar{M}_w$: 337. (c) 310–360 °C; base peak: 67,400 counts; $\bar{M}_w$:397. (d) 360–500 °C; base peak: 30,400 counts; $\bar{M}_w$: 359.

However, this spectrum is dominated by the mass signal of the thermally stable, polyphenolic plant constituents at m/z 286 which highest ion count is observed at 240°C indicating the thermal cleavage of the glycosidic bond of tetrahydroxyflavones. Furthermore, the molecular ion of another polyphenolic compound, the flavonol $C_{22}H_{22}O_{13}$ (measured: 446.1051, calculated: 446.1060) at m/z 446 and of sitosterol at m/z 414 with its fragment ion due to water elimination at m/z 396 are observed. In addition, molecular ions of sesquiterpene hydrocarbons lead to the typical mass signals at m/z 204. The highest total ion count is achieved in the temperature range from 310 to 360°C (*c*). As can be seen in Figure 2(c), this maximal ion count is mainly caused by ions of thermal degradation products of polysaccharides and by ions of mono-, di- and trilignol building blocks of spruce lignin. Furthermore, thermally stable plant constituents such as α-tocopherol at m/z 430 and various polyphenols appear as major mass signals. It is interesting that high mass signals prevail in this temperature range leading to the maximal weight averaged molecular weight of pyrolysates $\bar{M}_w$ observed during temperature-programmed FIMS (Figure 2). At higher temperatures between 360 and 500 °C the $\bar{M}_w$ decreases again and the summed spectrum over the temperature range *d* (Figure 2(d)) indicates the high degree of thermal conversion of biomaterial to char. Except for the monolignol mass signals below m/z 200, this FI mass spectrum looks very similar to FI mass spectra of low rank, bituminous coal.[50]

DISCUSSION

Py-FI mass pattern and composition of spruce needles

The approximate composition of conifer needles is shown in Table III. Polysaccharides and lignin already contribute 50% to the dry weight of coniferous needles. Without elaborate and tedious sample preparation, the corresponding bio-macromolecules cannot be investigated with conventional analytical techniques. However, as shown above, the molecular ions of thermal degradation products of these biopolymers are detected by Py-FIMS. In addition, the Py-FI mass spectrum of spruce needles also shows mass signals derived from flavones and other polyphenolic plant constituents, sesquiterpenes,

TABLE III

Approximate composition of conifer needles (Pinaceae)

Compound class	Percentage dry weight	References
Neutral polysaccharide constituents	35.0	51, 57
Lignin	15.0	41, 51, 57
Free sugars and glycosides	8.0	52, 56, 57
Resinous material	5.0	53
Crude protein	5.0	51, 54, 55, 57
Acid polysaccharide constituents	3.4	51, 57
Glycolipids	3.4	53
Major nutrient elements (ash)	2.5	51, 57
Phospholipids	1.8	53
Cuticular wax	1.7	53, 58[a]
Free acids	1.6	51, 57
Diglycerides	1.4	53
Hydrocarbons	1.2	51, 57
Polyprenols	1.1	59
Monoglycerides	0.9	53
Sterols	0.7	51, 53, 57
Diterpene alcohols and aldehydes	0.7	57
Mono- and sesquiterpenes	0.5	51, 57
Steryl esters	0.5	51, 53, 57
Volatile leaf oil	0.5	60
Triglycerides	0.2	53
Sum of major constituents	90.1	

[a] Mainly estolides (Reference 39).

proteins, free acids, hydrocarbons, phospholipids, waxes, sterols and glycerides. Although the Py-FI mass pattern gives only a semi-quantitative measure of the composition of spruce needles, the comparison with Table III demonstrates that the results obtained by Py-FIMS reflect quite well the complex structure of the biomaterials analysed and therefore is suitable for the rapid characterization of biomass. Physical pretreatment of the plant material such as drying or pulverizing has no essential effect on the Py-FI mass pattern in the investigated range.

Differences between shaded, green and unshaded, yellow spruce needles

The Py-FI mass spectrum of green spruce needles (Figure 1(a)) looks very similar to the spectrum of yellow spruce needles. In order to demonstrate the major differences the spectrum of the green needles was subtracted from the spectrum of the yellow needles. The resulting subtraction spectrum is shown in Figure 3. Obviously the spectrum of the yellow needles shows much less abundant signals of 10-nonacosanol at m/z 157, 297 and at m/z 406 as the spectrum of the green needles. In contrast, some volatiles such as α-tocopherol at m/z 430 are enriched in yellow spruce needles. Furthermore, the spectrum of green needles is, besides the epicuticular wax related mass signals, characterized by more abundant dilignol mass signals at m/z 300, 302, 314, 332, 334, 342, 344 and the more abundant sitosterol signals at m/z 396 and at m/z 414.

It seems that a sunshine-promoted impact on air pollutants changes and/or removes the epicuticular wax of these spruce needles. Furthermore, the structure of lignin is also degraded. These findings agree well with the observation that in the same yellow needles more

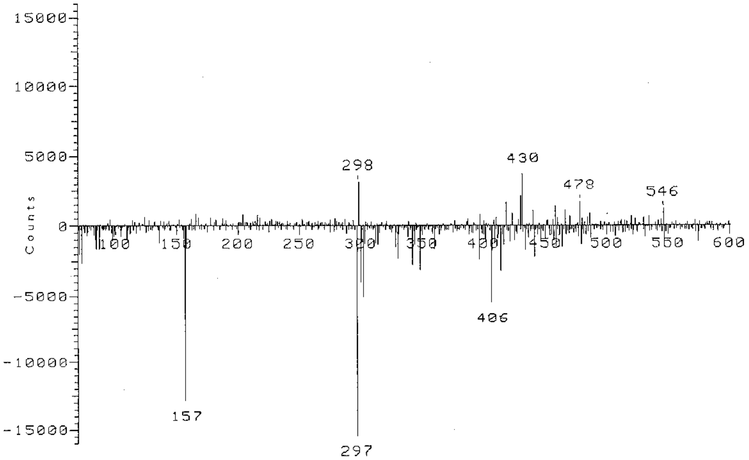


FIGURE 3 Difference spectrum obtained by subtracting the integrated, averaged and normalized Py-FI mass spectrum of green, shaded spruce needle tips ($\bar{M}_w$: 372) from yellow, sunshine-exposed needle tips ($\bar{M}_w$: 372). Both samples of one-year-old spruce needles were taken from one twig of an 80-year-old *Picea abies* tree growing in the Taunus mountains, F.R.G.

plant cells are collapsed as in the corresponding green needles.[61] The fact that yellow needles are more damaged can also be seen in the more abundant mass signal at m/z 430. This signal is mainly due to the antioxidant α-tocopherol and recently it has been shown, that an increased formation of this substance is caused by the impact of sulphur dioxide.[62]

This brief overview of preliminary Py-FI mass spectrometric results makes clear that yellow spruce needles from the Taunus mountains are damaged on three levels:

1) *outer surface* or epicuticular wax layer;
2) *cell structure* caused by the chemical degradation of lignin; and
3) *metabolism* indicated by the biochemically induced augmentation of vitamin E (α-tocopherol).

Surprisingly no difference in the polysaccharide composition can be seen.

Spruce needles fumigated with ozone

In Figure 4(b) the Py-FI mass spectrum of ozone-fumigated spruce needles is shown. In comparison to the spectrum of the control needles (Figure 4(a)), the major difference can be easily seen. While the spectrum of green needles shows abundant signals of polyphenolic volatiles at m/z 286 and m/z 446, both these mass signals are reduced very much in the spectrum of the fumigated needles indicating the oxidative destruction of flavonic plant constituents. In addition, most of the di- and trilignol related mass signals are also diminished (Figure 4(b)) indicating again the chemical degradation of lignin macromolecules. Hence, mass signals derived from thermal degradation of polysaccharides, from the mass spectrometric fragmentation of 10-nonacosanol and from the molecular ion of sitosterol prevail in the Py-FI mass spectrum of ozone-fumigated needles. In contrast to the control spectrum (Figure 4(a)), in the spectrum Figure 4(b), the mass signals below m/z 100 are of very high abundance indicating strong thermal degradation of ozone-treated plant material. In general, all lignin-related mass signals are less pronounced demonstrating an oxidative degradation of the lignin structure in the plant cells. Recently, this was also found for ozone-fumigated beech leaves[28] and seems to be a general observation.

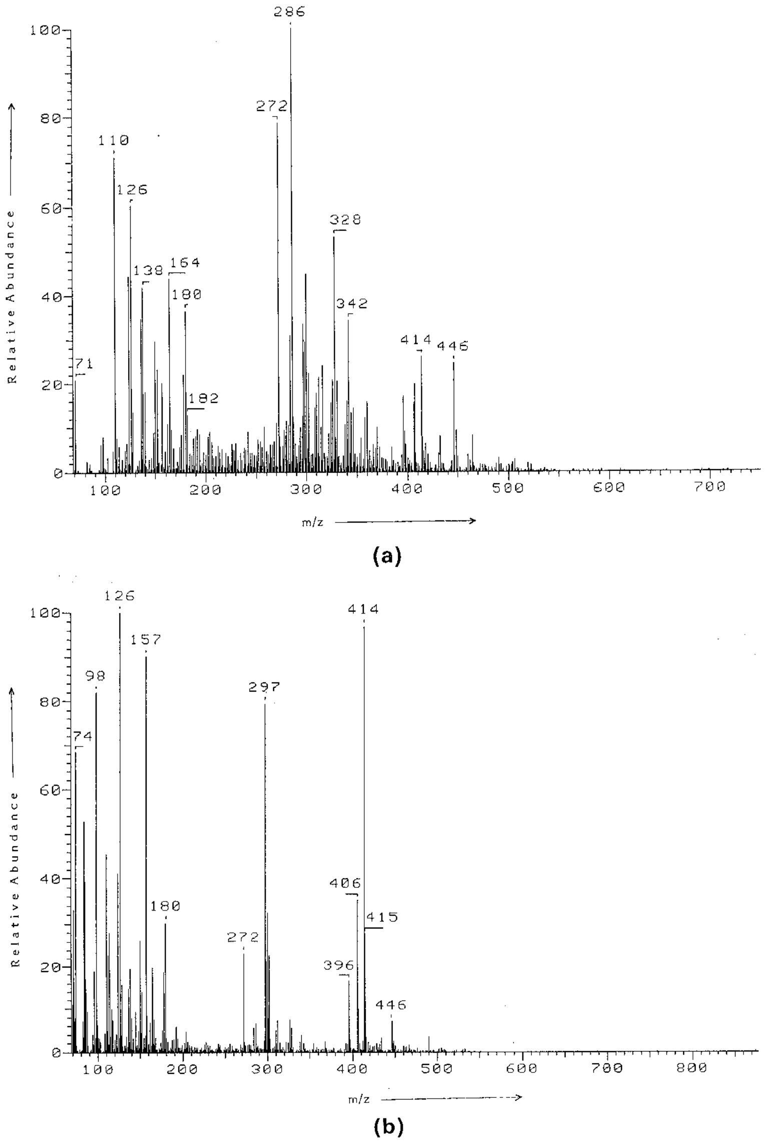


FIGURE 4 Averaged and to the total ion count normalized Py-FI mass spectra of spruce needles. (a) Control trees, 38 KLux (base peak: 44,700 counts; $\bar{M}_w$:306). (b) Fumigation with 150 μg O_3/m^3 for 42 days, 38 KLux (base peak: 60,500 counts; $\bar{M}_w$: 275).

However, these structural changes do not appear to reduce the lignin content of needles because Tingey *et al.*[63] reported, that ozone treatment had no effect on the hydrolyzable lignin content of pine seedlings. Furthermore, the signal of the antioxidant vitamin E at m/z 430 is also decreased in the ozone-treated needles demonstrating its consumption by ozone-induced hydroxyl radicals.

Spruce needles treated with acidic fog water

In Figure 5 a typical Py-FI mass spectrum of a green, half-year-old spruce needle is shown. In comparison with older needles (Figure 1(a)), the biosynthesis of lignin and the flavone at m/z 286 is not fully completed. Therefore, the cellulose and hemicellulose related mass signals dominate the spectrum. In comparison with the spectrum of the acid treated spruce needles in Figure 5(b) drastic changes concerning the mass signals at m/z 74, 110, 126, 138, 144, 204, 414, 430 and 446 can be seen indicating that the treatment with acids alters the polysaccharide composition, reduces the content of sitosterol and vitamin E and increases the content of sesquiterpenes, which are typical "waste products" of plant metabolism. In contrast to the ozone-fumigation, the flavones and the lignin structure do not appear to be damaged severely. At present, distinct chemical changes of the epicuticular wax layer cannot be deduced from the obtained spectra. However, new reaction products increase the mass signals at m/z 204, 406 and m/z 446 and their identification needs further studies.

CONCLUSION

In general, ozone fumigation of spruce needles results in a degradation of the lignin structure and the destruction of hydroxyflavonoid glycosides whereas the evaporation of water and concentration of sulphuric acid results mainly in a degradation of cellulose and hemicellulose components. Both bio-macromolecules are essential building blocks of plant cells. Thus, the often observed collapse of plant cells can be explained by the impact of atmospheric pollutants on leaves and can be related to the destruction of either lignin or cellulose. Concerning the Py-FI mass spectra of the damaged needles

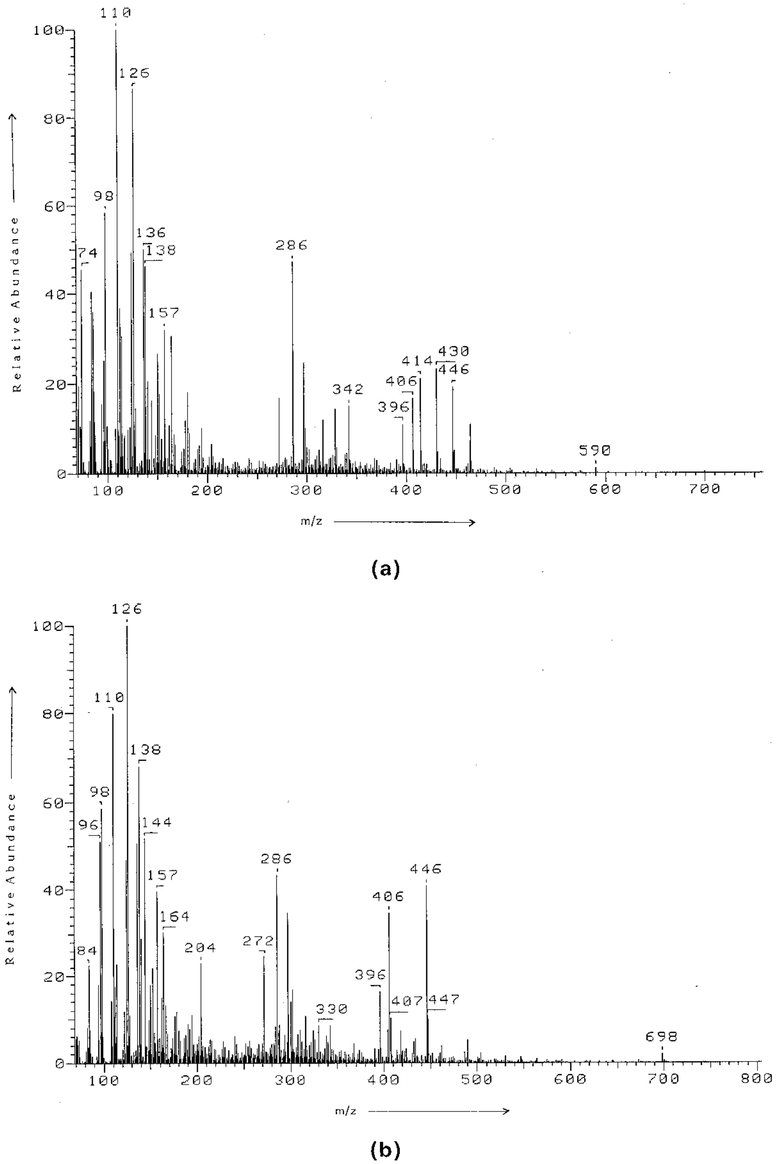


FIGURE 5 Averaged and to the total ion count normalized Py-FI mass spectra of half-year-old spruce needles. (a) Control needles (base peak: 54,800 counts; $\bar{M}_w$: 264). (b) With acidic fog water (pH 0.5) treated needles (base peak: 51,300 counts; $\bar{M}_w$: 283).

from the Taunus mountains, no general conformity with the Py-FI patterns of treated needles can be seen. The spruce needles treated in the laboratory, or in the fumigation chamber, show drastic differences in comparison to the mass pattern of their control needles. This is not true for the needles from the Taunus mountains, indicating a less severe impact of the ambient mixtures of gaseous air pollutants (Table I) or the impact of other stress factors. Concentrating on various details, no single cause for the differences of the Taunus needles can be deduced. These needles show no change in the structure of polysaccharides. Therefore the action of acidic rain in this respect could be excluded. Furthermore, their lignin structure is altered. This would indicate the action of ozone. However, then the antioxidant α-tocopherol should not be increased in the damaged needles. Thus, as a reason for the discolouring of the Taunus needles the impact of ozone alone and of sulphuric acid alone can be excluded. This is supported by macroscopic observations of the colour of damaged needles. Ozone-treated needles showed only a slight bleaching of its green colour, whereas acid-treated needles became brown. By contrast, the spruce needles from the Taunus mountains are yellowish. Recently, Krause *et al.*[64] studied the additive impact of ozone fumigation and acidic precipitation. They concluded that the often observed yellowish colour of coniferous needles is not derived from the direct photooxidation of chlorophyll by air pollutants, but could be explained by nutrient deficiencies which are caused by the oxidative destruction of biomembranes and the successive leaching of nutrients.

Thus, synergistic or additive action of various gaseous and dissolved air pollutants causes the chemical changes reflected in the mass spectrometric pattern observed. In order to find single phytotoxicologically effective substances and reaction mechanisms, however, the comparison of spectra gained from control and untreated needles does not appear to provide convincing results. Therefore the only possibility of tackling this problem is the application of pattern recognition techniques. With these multivariate, statistical methods Py-FI mass spectra of a large data base can be evaluated in combination with data gained from other chemical and physical analyses.[65] The integrated interpretation of results from macroscopic and microscopic observations, nutrient analyses, physiological investigations and pyrolysis studies seems to provide a promising approach to detect and to identify significant and characteristic key substances, which can be either used as "biomarkers" or which help to explain the current tree damages.

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