EARLY DIAGNOSIS OF *FOREST DECLINE*

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EARLY DIAGNOSIS OF FOREST DECLINE

REPORT OF A ONE-YEAR PILOT STUDY

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COVER ILLUSTRATION
Taking samples from Beech trees in the Black Forest (site D2) (Photograph I S Paterson)

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The **Institute of Terrestrial Ecology (ITE)** was established in 1973, from the former Nature Conservancy's research stations and staff, joined later by the Institute of Tree Biology and the Culture Centre of Algae and Protozoa. ITE contributes to, and draws upon, the collective knowledge of the 14 sister institutes which make up the **Natural Environment Research Council**, spanning all the environmental sciences.

The Institute studies the factors determining the structure, composition and processes of land and freshwater systems, and of individual plant and animal species. It is developing a sounder scientific basis for predicting and modelling environmental trends arising from natural or man-made change. The results of this research are available to those responsible for the protection, management and wise use of our natural resources.

One quarter of ITE's work is research commissioned by customers, such as the Department of Environment, the Commission of the European Communities, the Nature Conservancy Council and the Overseas Development Administration. The remainder is fundamental research supported by NERC.

ITE's expertise is widely used by international organizations in overseas projects and programmes of research.

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SUMMARY

There is need for a predictive capability for the early diagnosis and objective quantification of the recent type of forest decline observed over large areas of central Europe. The aim of this initial study is to investigate possible diagnostic tests using material collected from a number of sites exposed to a wide range of pollution climates.

Sites for sampling Norway spruce, Scots pine and beech were selected in 7 regions, from southern Germany to north-east Scotland. Techniques were developed during spring 1986, with 2 field trials of sampling and storage methods. Laboratory tests were developed in parallel.

Sampling from the selected sites was done from July to the beginning of September. In all, 250 trees were described and sampled. Laboratory tests were performed during autumn 1986.

The results showed large differences between the sites for 8 of the techniques used. The patterns showed marked geographical differentiation in at least one tree species. Data from UK sites were often at one end of the range, with data from German sites (showing visible damage symptoms) at the other. Although this association with visible damage is promising, the observed differences could be attributed to genotype, soil type, climate, etc, and are not necessarily related to pollution.

Further work with potential tests is required to establish the dependence on genotype, sampling conditions and specificity to particular air pollutants. This work will necessitate the experimental exposure of trees to different pollutants under controlled conditions.

1 INTRODUCTION

During the last 10 years, there has been increasing interest in and concern at the decline of forest vitality in many areas of Europe. The problem was identified as a 'new type' of forest decline in West Germany, different from the well-documented declines (particularly in fir) of earlier decades. This realization led to the establishment of a nation-wide survey in West Germany, which had shown a progression from 1982 to the point where half the forests in the country were showing signs of this 'new type' of decline by 1985, with no widespread evidence of recovery.

This 'new type' of forest decline is characterized by marked thinning and change of form in the crowns of coniferous trees, most severe in silver fir (*Abies alba*) and less so in Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Similar decline is also observed in deciduous trees, notably beech (*Fagus sylvatica*) which shows symptoms including leaf curl and dieback of small twigs. The problems identified originally in West Germany have now been observed from southern Sweden to eastern France and Switzerland, often (but not exclusively) at high altitudes. The 'new' characteristic of these observations is the widespread occurrence of similar visible symptoms in several tree species, leading eventually to death of the tree. Affected areas are not necessarily influenced by local air pollution, and are to be found with several different types of soil and bedrock.

Many hypotheses have been proposed to account for these observations, but few have been tested experimentally. Many of them attribute the causal factor to air pollution, often with interactions with other stress factors such as drought or frost. One of the earliest ideas, developed by Ulrich and co-workers, postulated an acidification of soils by wet and dry deposition of acidic pollutants (from sulphur dioxide and nitrogen oxides). This acidification leads in turn to the release of free aluminium ions in the soil, which are toxic to fine roots. Such a mechanism is seen to be operating over a period of years or decades, and is not easily addressed experimentally over short periods. An alternative hypothesis, developed by Prinz and colleagues, is that tree foliage is directly damaged by atmospheric ozone, which is known to exist in many areas of Europe at concentrations likely to cause damage to sensitive plant species. As these large concentrations of ozone are also the result of chemical reactions of gaseous pollutants in the atmosphere, notably nitrogen oxides and hydrocarbons, the ultimate cause of the observations is still seen as combustion of fossil fuels.

These 2 approaches to the problem have been developed, taking account of other stress factors and pollutant inputs. Thus, air pollutants may be seen as pre-disposing factors increasing sensitivity to drought, frost or pathogens. The mode of impact (as rain, mist, particle or gas) also seems to be important in determining any effect, and may involve secondary reactions (eg in soils) and other pollutants such as heavy metals.

The intensive study of the phenomenon over the past few years has led to the establishment of several associations, but not always to a clear mechanism or cause. The yellowing of spruce needles is associated with magnesium deficiency (or potassium deficiency in some soils), but the origin of this symptom remains obscure. Similarly, symptoms of decline have been associated with high altitudes as an effect of acidic cloudwater and/or high ozone concentrations, but experiments have not yet shown unequivocal responses to such exposure.

The well-established national surveys, not only in West Germany, but also in other countries of western Europe, rely on visual observations made from the ground. Although these observations can show the extent of current damage, they give little indication of potential future visible symptoms. They are also to some extent subjective, and there is the clear need to establish objective methods of quantifying the extent of this 'new type' of forest decline. This project was established with a view to addressing these 2 aspects: the identification and development of objective tests which may be used to quantify the extent of damage, before and after visible symptoms have developed.

The project, proposed jointly to the Commission of the European Communities (CEC) by the Institute of Terrestrial Ecology (ITE) and the University of Lancaster, has involved the investigation of a number of techniques, which are discussed later. The objective was as follows: the development of predictive tests which can be used to determine the status of trees and forest ecosystems, in advance of the appearance of symptoms of irreversible damage, the tests being, as far as possible, indicators of specific pollutants. In practical terms, this project involved: the development of appropriate methods of forest survey and field sampling, based on the existing recommended field techniques; and the evaluation and development of techniques for assessing early symptoms of pollutant stress.

In addition to the active participation and collaboration of Dr S Fink of the University of Freiburg, the project attracted considerable interest elsewhere in the UK and in North America. The Canadian Forest Service was represented at planning meetings, and Dr W McIlveen of the Ontario Ministry of the Environment participated in the field work in Britain and West Germany. Dr P Freer-Smith (University of Ulster) also participated fully in the field work, and his results are included later (Section 4.3).

The main sampling strategy was devised after development of the laboratory tests, and exploratory field work in Britain during spring 1986. It took the form of a broad transect from southern Germany to north-east Scotland, encompassing a variety of 'pollution climates'. Foliage samples were taken from 3 tree species: Norway spruce, Scots pine and beech. Experiments were conducted in the field, and samples were preserved for subsequent laboratory analysis.

2 SITES SAMPLED DURING 1986

The idea behind using a broad 'transect' relies on the identification of a number of different 'pollution climates' within western Europe. Seven areas were chosen, based on available data for air pollution, and within each area (with the exception of Scotland) 2 sites were selected which differed in at least one other factor (eg aspect). The sampling sites are shown in Figure 1a, identified by a 2-digit code which is used subsequently to classify results. The classification of regions by pollution climate is summarized in Table 1, where '+', '+ +', and '+ + +' should be regarded as relative terms expressing the importance

Table 1.
Pollution climate of sampling regions

Region	Average SO_2/NO_x concentration	Frequency of O_3 episodes	Frequency of mist	Acidity of rainfall	Amount of deposited acidity
Black Forest (South West Germany)	+	+ + +	+ + +	+ +	+ +
Netherlands	+ + +	+ +	+	+ + +	+ +
Harz Mountains (West Germany)	+	+ +	+ + +	+ + +	+ + +
Fichtelgebirge (West Germany)	+ + +	+ + +	+ + +	+ + +	+ + +
Southern England	+ +	+ +	+	+ +	+
Western Scotland	+	+	+ +	+	+ + +
North-east Scotland	+	+	+	+ +	+

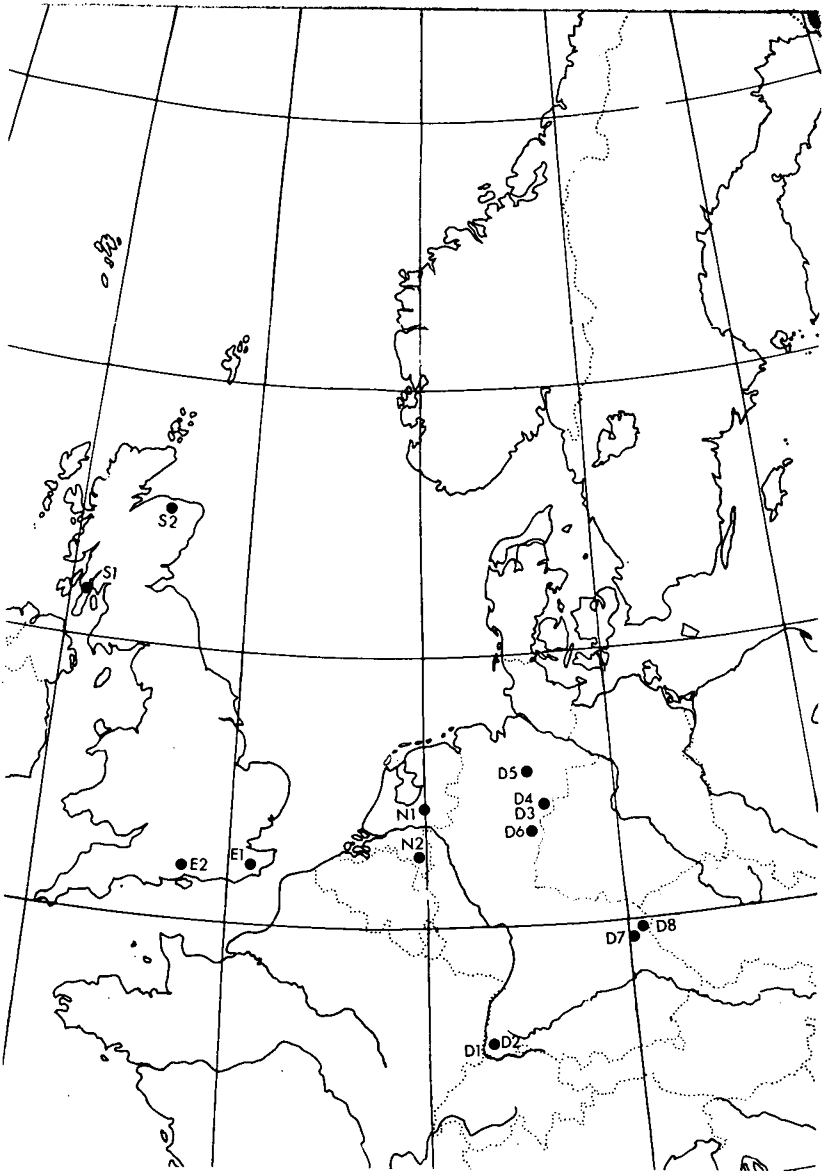


Figure 1a.
Map of western Europe showing locations of sampling sites

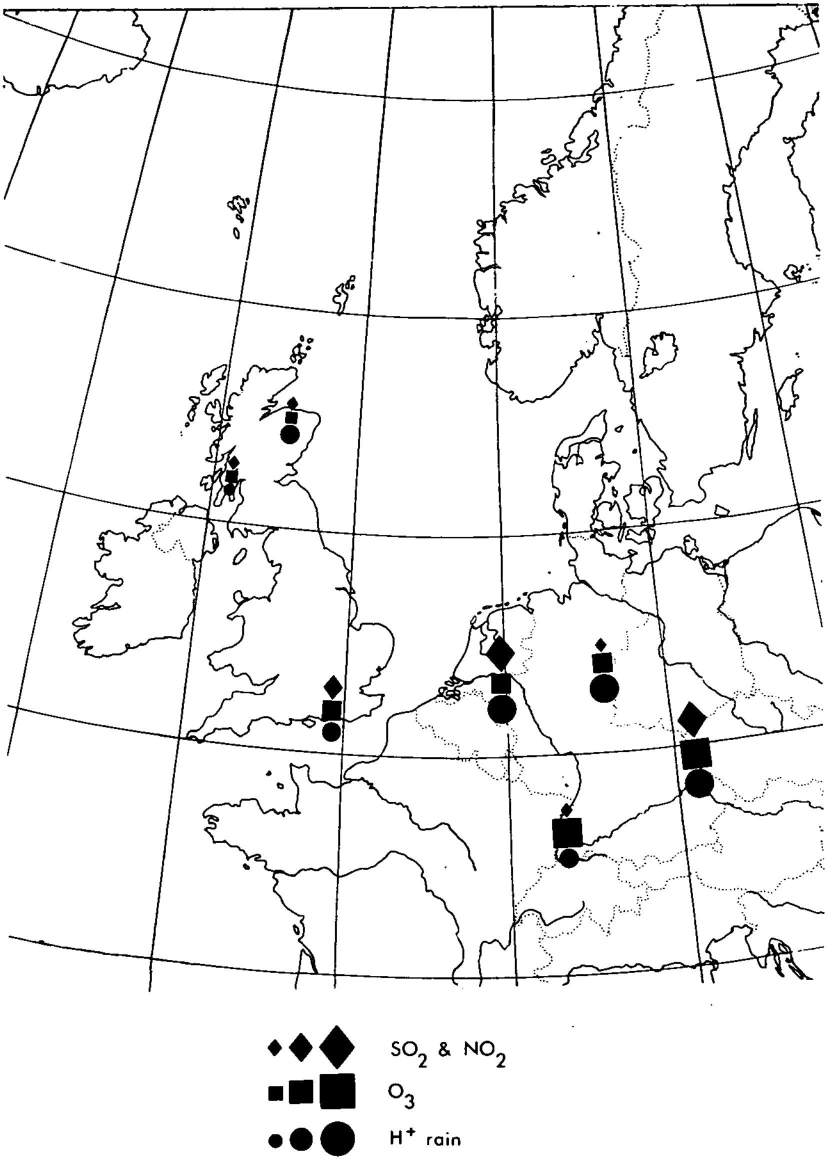


Figure 1b.
Map of western Europe showing range of pollution climates

Table 2.
Timetable for survey

Region	Site	Code	Sampling date
Black Forest	Kälbelescheuer	D1	2–3 July
	Haldenhof	D2	4–5 July
Netherlands	Kootwijk	N1	7 July
	Rips	N2	8 July
Harz Mountains	Lange Bramke (south)	D3	2 August
	Lange Bramke (north)	D4	3 August
	Celle	D5	4 August
	Witzenhausen	D6	5 August
Fichtelgebirge	Fichtelberg	D7	7, 9 August
	Selb	D8	8, 10 August
Western Scotland	Glenbranter	S1	25–27 August
North-east Scotland	Darnaway Forest	S2	28–29 August
Southern England	Birchett Wood	E1	2–3 September
	Parnholt Wood	E2	3–4 September

of a component, and shown schematically in Figure 1b. For each site, a checklist of attributes was compiled (see Appendix) at the time of the site visit. The timetable for site visits is shown in Table 2. In general, most southerly sites were sampled earlier in the year to minimize differences in the stage of growth between sites. The survey was organized in 3 distinct parts: Black Forest and Netherlands (staff from ITE, University of Lancaster, Albert-Ludwigs University at Freiburg, with assistance in the Netherlands provided by the Forest Service); Harz Mountains and Fichtelgebirge (staff from ITE, University of Lancaster, University of Ulster and Ontario Ministry of Environment); and the UK (with staff from ITE, University of Lancaster and University of Ulster).

A brief description of each site follows.

2.1 West Germany, Black Forest (Schwarzwald)

2.1.1 D1: *Kälbelescheuer*, in the Black Forest mountains, is situated 8 km SSW of Münstertal, at an altitude of 950 m. A minor road runs through the site, which is on a steep slope (*c* 40°) and has a northerly aspect. The Norway spruce was growing below the road in a fairly even mixed stand, Douglas fir (*Pseudotsuga menziesii*), beech and rowan (*Sorbus aucuparia*) being the other main species present. They formed a fairly dense canopy and the mean height of the trees was *c* 12 m. Ground cover was <50 % and comprised grasses, mainly wavy hair-grass (*Deschampsia flexuosa*), and fern species, with large areas of scree over the remainder covering a freely drained thin sandy soil.

Beech trees above and below the road were sampled. These stands were more open and their mean height was *c* 16 m. The soil above the road was deeper, and ground cover here was more dense with grass, herbs and bracken (*Pteridium aquilinum*) being the main components of the understorey. Needle and leaf yellowing was prevalent on all tree species, together with thinning of the crowns. Dead and dying trees, mostly Douglas fir, were common throughout the area. We could find no Scots pine in this area.

2.1.2 D2: *Haldenhof*, the second site in the Black Forest, is situated 9 km SSE of Münstertal and 3 km SE of Kälbelescheuer, at an altitude of 1000 m. The Norway spruce and beech stands selected for sampling were located on a steep scree-covered 45° slope which had a south-westerly aspect. The upper edge of the forest block was bounded by a narrow well-used pedestrian track, while a steep herb-rich alpine meadow bounded the lower south-west edge. The Norway spruce was growing in a dense stand, the mean height of the trees being *c* 13 m, with a few Douglas fir and isolated Scots pine scattered throughout the area. The beech trees were located in an adjacent stand also below the track and had at some time been thinned and coppiced. The canopy was, however, quite dense and ground cover here was absent, whereas brambles (*Rubus fruticosus* agg.) and grasses covered 30 % of the ground area under the spruce. The soil was a thin, freely drained, brown earth, with much loose scree on the surface.

Only 4 pines, all apparently healthy, were accessible for sampling. Growth was quite vigorous and their mean height was *c* 10 m. Many of the trees here, particularly Douglas fir, showed signs of leaf and needle yellowing, though there appeared to be rather fewer dead and dying trees compared with Kälbelescheuer.

2.2 Netherlands (central and south)

2.2.1 N1: *Kootwijk* is situated WSW of Apeldoorn on level terrain which has virtually no aspect or altitude. This site was a mixed stand of Scots pine, the predominant species, interspersed with Norway spruce and oak (*Quercus robur*). The stand had already been thinned and was marked for thinning again. The trees were *c* 17 m high and the canopy was fairly open. The pines had thin crowns which did not look healthy, while the spruce tended to lack foliage in the mid-crown but invariably had bushy top crowns. Ground cover was entire, with wavy hair-grass accounting for over 90 % of the cover. The soil comprised a sandy humus layer, *c* 20 cm thick, on top of a pale sand over one m deep.

2.2.2 N2: *Rips* is situated 10 km NW of Helmond and west of the industrial city of Eindhoven. The forest is on flat terrain with no obvious aspect, and the area selected for sampling was a fairly open Scots pine stand with Norway spruce around the perimeter. The mean height of the pine was 13 m but the spruce was much taller, being *c* 20 m. The canopy was fairly open and the ground cover was complete, wavy hair-grass and greater woodrush (*Luzula sylvatica*) being the main species. The beech was growing in a dense avenue on either side of a forest track, with mixed broadleaved species growing behind. The mean height of the trees sampled was 18 m. Many of the Scots pine exhibited discoloration of the needles, ranging from bright pea-green to yellow, and they were also infected by the fungus *Sphaeropsis sapinea*, which affects the branch leaders and eventually causes the trees to die.

The soil profile showed a cultivated sandy humus horizon overlying a deep sandy soil with few stones. During wet periods, groundwater can be found within 70 cm of the surface.

2.3 West Germany: Harz Mountains and north-east

2.3.1 The *Lange Bramke* ridge, 6 km south of Goslar and 3 km north of Schulenberg, provided 2 sample sites. Both were near the brow of the ridge at *c* 600 m, and the predominant species here was Norway spruce. The first site was selected on the south-facing side of the ridge (D3) and the other lay about 2 km west on the north side (D4). The slopes at both sites were very gentle, < 5°. The area had been clearfelled in 1946 and replanted with Norway spruce, but selected stands had been cut and replanted again. The stands were very dense and growth was fairly even, the mean height being *c* 14 m. Most of the trees in the areas sampled had suffered from bark-stripping by red deer (*Cervus elaphus*) but, where possible, trees with undamaged trunks were sampled. The ground flora was mainly wavy hair-grass and bilberry (*Vaccinium myrtillus*) which covered 45 % of the forest floor, the remainder being bare ground and brash. A thin humus layer covered a sandy loam which had a high silica content. The soil was moderately well drained. The spruce trees on the south-facing slopes had quite severe yellowing of the needles, but there was more sub-top death and needle loss on trees growing on the NE-facing slopes.

2.3.2 D5: *Sudbeide* Nature Park, situated 15 km north-east of Celle, contains a fairly open mature stand of Scots pine over 100 years old and a younger adjacent stand aged *c* 30 years. This is one of the

University of Göttingen's experimental sites. The area is flat with an easterly aspect, and a minor public road divides the older plantation from the younger one. The mean height of the mature trees was 30 m and of the young trees 15 m. The canopy was fairly open and ground cover was entire, comprising several fine-leaved grass species, bracken and dog's mercury (*Mercurialis perennis*). Under the mature stand, broadleaved seedlings, including oak, were also growing, while under the younger stand much brash covered the ground.

Some of the trees showed thinning of the crown and discoloration of the needles. The younger trees had lost many needles, particularly on the lower branches. The soil was a compacted sandy soil containing many stones. Four of the older trees, together with 4 young trees, made up the sample number.

2.3.3 D6: *Kaufungerwald* is situated 3 km south-west of Witzenhausen at an altitude of 500 m. This is another of the University of Göttingen's experimental sites and is in a mature stand of beech, 127 years old. The site is below a little-used public road on a steep 30° slope and has a north-easterly aspect. The beech was 31 m high and stem diameter was *c* 40 cm. Canopy cover was quite dense, but ground vegetation covered almost 25 % of the forest floor and comprised ferns, wavy hair-grass, greater woodrush, wood-sorrel (*Oxalis acetosella*) and raspberry (*Rubus idaeus*).

Regenerated oak seedlings were also present. The soil was a thin sandy loam overlying coloured sandstone containing many stones. The trees sampled were selected according to the damage ranking assessed by staff from the University of Göttingen.

2.4 South-east Germany: Fichtelberg Mountains

2.4.1 D7: *Fichtelgebirge*, situated 15 km ENE from Bayreuth and 3 km SE of Oberwarmensteinach, is a mountainous area rising to an altitude of 800 m. The Norway spruce was a dense uniform stand with a mean height of 20 m and was growing on level ground near the ridge with a southerly aspect. Eight Scots pine trees were located in 2 isolated groups on the edge of a mixed stand of Norway spruce and Douglas fir. The slope here was 10° and the aspect was to the north. There was a similar slope at the beech stand, but the aspect was north-westerly. The age of the beech was very variable, with heights ranging between 13 m and 18 m. Ground cover beneath the spruce and pine was almost complete, with wavy hair-grass, bilberry, mosses and ferns present, but there was no ground flora under the beech due to the very dense canopy and thick layer of leaf litter. The trees were all planted between 30 and 40 years ago on a moderately well-drained brown earth soil. Yellowing was obvious on the spruce needles and on some of the beech leaves, and, while the pine trees showed no such discoloration, they did not appear to be growing well.

2.4.2 D8: Steinberg hill rises to 653 m overlooking the Czechoslovakian border near the town of *Selb*, and lies 45 km NE of Bayreuth. It is exposed on the east to Czechoslovakia and on the south, 5 km distant, to a power station at Arzberg. Until recently, the hill was covered by mature forest, but, because the trees were showing signs of severe damage, large areas of older trees have been clearfelled. The 3 species to be sampled were all found at an altitude of 600 m on fairly level ground, the slope being *c* 5°. Beech and Norway spruce were growing in close proximity on a site with a north-easterly aspect, while the Scots pine trees were located one km to the south and had a southerly aspect. The Norway spruce was in a fairly even, dense stand interspersed with occasional beech and other broadleaved species. Mean height of the stand was 18 m and the age was about 35 years. Ground cover was non-existent, with much brash and several dead trees throughout the forest. The spruce trees were inclined to be spindly with thin crowns. As the beech was variable in height, 4 larger trees, with a mean height of 25 m, were sampled in the spruce stand and 4 smaller trees, *c* 12 m, were selected from an adjacent stand close to the forest track. There was no ground vegetation under the beech.

The Scots pine trees were older, with a mean height of 22 m, and these were growing in a narrow strip between a forest track and a hay field. The understorey comprised grasses, herbs and briars with no bare ground.

Soils were brown earths, the upper horizon being a sandy loam and the lower horizons showing signs of gleying. They were, however, free draining.

2.5 Scotland: west and north-east

2.5.1 S1: *Glenbranter*, between Loch Fyne and Loch Eck, was the centre for the 3 sampling areas in Argyll. Norway spruce was located in a wet hollow one km SE of Strachur, at an altitude of 65 m. Although the area is well drained, standing surface water is occasionally present after heavy rain. There is virtually no slope and the aspect is westerly. The trees were planted in 1964 and form a very dense, even stand, the mean height being 12 m. Apart from some windblow, the trees appeared to be in good health. Ground cover was non-existent under the dense canopy and the soil was a very gritty peaty podzol with a disturbed structure due to cultivation and drainage.

The beech stand was situated at an altitude of 35 m on the south side of the river near Glenbranter. It was on a fairly steep slope, *c* 20°, giving an aspect to the north-east. The trees were 44 years old, had a mean height of 13 m and had previously been thinned. Ground cover was sparse and most of the area was covered by leaf litter. A few oak and larch (*Larix decidua*) trees were present, but the beech accounted for 80 % of the dense canopy. The soil was a well-drained sandy brown earth, with a hard layer of sandstone at 25 cm depth. Scots pine was found above the road on the shores of Loch Eck at an altitude of 65 m. The trees were growing on a dry sandy knoll which had a 45° slope and a north-westerly aspect. It was a fairly even-growing healthy stand, the trees having a mean height of 17 m. Western hemlock (*Tsuga heterophylla*) was present behind the pine and also encroached into the stand on the north side, where both species appeared to be susceptible to windblow. Wavy hair-grass and bilberry with occasional clumps of *Sphagnum* moss provided the main ground cover, which was complete. The soil was similar to that under the beech and was very orange in colour.

2.5.2 S2: *Darnaway Forest*, situated 7 km SW of Forres in Morayshire, is at an altitude of 155 m on a northerly facing slope of between 5° and 10°. Both Scots pine and Norway spruce were sampled from a dense mixed stand which also contained larch and Douglas fir. The trees were *c* 30 years old and some were marked for thinning. Only unmarked trees were selected for sampling. The Norway spruce were of even height (about 14 m) but the pines were slightly smaller at 12 m. Ground cover was 80 % and comprised mosses with a few fine-leaved grasses and sedges. Natural regeneration of spruce was also taking place. The soil was a moderately well-drained peaty gley soil.

The beech was selected from 2 sites, both near to a minor public road. One contained a large mixed stand of mature oak and beech and the other consisted of a smaller pure stand of young beech. The ages of the trees in the mature stand ranged from 100 to 150 years old and the height of the trees sampled was 15–20 m. In the younger stand, the trees were only 8 m high. Ground cover at both sites was mainly by beech leaves, but mosses and grasses grew where the canopy was less dense and bracken was abundant in the clearings. The soil under the younger trees was a previously cultivated brown earth, while that under the mature trees was a more orange sandy soil; both were free draining and contained many stones.

This forest was the healthiest and had the most vigorous appearance of all those sampled. It showed no visible signs of any damage.

2.6 South-east England: Kent and Hampshire

2.6.1 E1: *Birchett Wood*, situated south of Ashford, is on level ground with virtually no altitude (40 m) or aspect. Beech, Norway spruce and Scots pine were growing in adjacent stands and the average height of the trees sampled was 15 m. The Scots pine stand was very tall so samples were collected from the more accessible trees along the edge of the stand next to the forest ride.

The plantation had been thinned but the canopy cover was still quite dense, accounting for 75 % cover in the spruce and 90 % in the beech and pine. Brambles, bracken, grasses, nettles (*Urtica dioica*) and mosses formed the ground cover under the pine and spruce, but there was no cover under the beech. All the trees in the area were growing on a very compact clay soil (weald clay) which was very poorly drained.

An infestation of beech scale (*Cryptococcus fagisuga*) was affecting many of the beech trees, and infected trees were not sampled where possible.

2.6.2 E2: *Parnholt Wood*, 10 km west of Winchester and north of the village of Ampfield, is comprised of blocks of mixed tree species planted mainly between 1951 and 1952. Beech planted in 1951 was nearest to the public road and was on fairly level ground (altitude 125 m) with no aspect. The trees were in a mixed stand with Douglas fir, which was in the process of being thinned. The mean height of the trees was 17 m and the canopy was very dense. Ground cover was less than 5 % but ivy (*Hedera helix*) was growing on the trunks of many of the trees, which also showed signs of beech scale infestation. Where possible, infected trees were not sampled. The soil was a brown earth overlying a flinty clay. Scots pine was growing at the lowest point of the forest (altitude 100 m) on an area with a 5° slope. The trees planted in 1952 had just been thinned, leaving the stand very open and exposed to the north. The mean height of the remaining trees was *c* 17 m and they lacked lower branches. Ground cover had been destroyed by extraction vehicles and a brown muddy clay formed a layer on top of a compact chalk subsoil which was poorly drained.

Norway spruce was located growing in a narrow strip (*c* 20 m wide) between a hay field and a mixed broadleaved plantation. The slope was *c* 5° and had a westerly aspect. The mean height of the trees was 15 m but the stand was uneven in growth. A very dense canopy prevented any ground cover developing, but creepers and brambles intertwined among the trees. Cultivation of the soil until 1959, when the trees were planted, had caused destruction of the soil profile in the upper layers, but at 60 cm there was a very pronounced compacted chalk layer. The soil was freely drained.

3 SAMPLING METHODS

A Ford Transit minibus (owned by ITE) was converted for use as a small mobile laboratory. This vehicle conveyed personnel to all sites. A petrol-driven electrical generator was used to provide power on site for balances, lamps and heaters (where necessary) and to run auxiliary equipment.

At each site, a number of trees was assessed visually and scored for height, form and visible damage (see Appendix for scoresheet). The number of each species sampled at each site is shown in Table 3. A

Table 3.
Number of trees sampled at each site

Site	Norway spruce	Species Scots pine	Beech
Kälbelescheuer (D1)	12	—	8
Haldenhof (D2)	8	4	6
Kootwijk (N1)	6	6	6
Rips (N2)	6	6	6
Lange Bramke S (D3)	8	—	—
Lange Bramke N (D4)	8	—	—
Celle (D5)	—	8	—
Witzenhausen (D6)	—	—	8
Fichtelberg (D7)	8	8	8
Selb (D8)	8	8	8
Glenbranter (S1)	8	8	8
Darnaway (S2)	8	8	8
Birchett Wood (E1)	8	8	8
Parnholt Wood (E2)	8	8	8
Total number of trees	96	72	82
Total number of samples	288	216	82
Total number of 3 species			
Trees	250		
Samples	586		

'squirrel' pruner, which extended up to *c* 11 m, was used to take 2 branches from the central third of the crown. This limitation to sampling technique necessarily constrained the sampling to relatively young trees (<40 years). At 2 sites, Celle and Witzenhausen, samples were obtained from older trees by a professional tree climber.

Individual branches were then scored for needle (or leaf) loss and discoloration. Note was also taken of any recognizable insect damage or pathogen. The branches were then processed for analysis and storage.

3.1 Scots pine

3.1.1 Histological examination (Freiburg)

A section of branch with current year (1986 growth), one-year-old (1985) and two-year-old (1984) needles, was cut, labelled and sealed in a black polythene bag. Where necessary, these bags were despatched by express post to Freiburg.

3.1.2 Contact angle measurements (ITE)

Ten needle pairs from each year class (current, one year, two years) were removed using tweezers, and mounted on a specially designed card holder to prevent surface abrasion. The card holders were then sealed in a polythene bag and sent by express post to ITE's Bush laboratories for immediate analysis.

3.1.3 Härtel test (ITE)

From each year class (0, 1, 2), 5 grams (fresh weight) of needles were sealed in polythene bags before sending by express post to ITE's Bush laboratories.

3.1.4 Biochemical tests (Lancaster)

Approximately 3 g fresh weight of needles from each year class were sealed in small polythene bags, which were further sealed in labelled paper envelopes before immersion in liquid nitrogen. These samples were then transported in liquid nitrogen to Lancaster University in the 'mobile laboratory'.

3.1.5 Hydrocarbon emission (Lancaster)

Three replicate one g samples of fresh needles were removed for incubation and subsequent sampling (see 5.2.2).

3.1.6 Cuticular wax analysis (ITE)

Five g (fresh weight) of needles from each year class were accurately weighed and shaken with 50 ml chloroform ($CHCl_3$, analytical grade).

The wax solution was then decanted through a small Buchner funnel under gravity into a sealable polyethylene bottle. The washed needles were placed in paper bags for drying. Where possible, preliminary drying was carried out to avoid mould formation. (These samples were used for calculating fresh weight/dry weight ratios.) The sealed bottles containing the chloroform solutions were transported back to ITE's Bush laboratories for further processing.

3.1.7 Nutrient analysis (ITE)

At least 5 g of each year class were placed in paper bags for subsequent drying, milling and analysis.

3.2 Norway spruce

Most of the techniques for handling spruce samples were similar to those for Scots pine. The differences are noted below.

3.2.1 Histological examination

Samples were taken exactly as for Scots pine.

3.2.2 Contact angle measurements

Needles for these measurements were taken from the material sent to ITE's Bush laboratories for the Härtel test (see below).

3.2.3 Härtel test

Four or 5 shoots from each year class were placed on moistened cotton wool in a black polyethylene tube (*c* 5 cm ×20 cm), sealed with flexible plastic film, and sent by express post to ITE's Bush laboratories.

3.2.4 Biochemical tests

Samples were as for Scots pine, except that spruce needles were removed from shoots by plunging the whole shoot into liquid nitrogen for a few seconds. Needles could then be stripped off at the needle base without fracturing.

3.2.5 Hydrocarbon emissions

Samples were taken exactly as for Scots pine.

3.2.6 Cuticular wax analysis and nutrient analysis

Samples were taken as for Scots pine except that spruce needles were removed from shoots using liquid nitrogen (as above).

3.3 Beech

The tests performed on beech were often different from those performed on conifers.

3.3.1 Modulated fluorescence

Twigs (20–40 leaves) were cut from the main branch and sealed in black polythene bags for dark conditioning prior to analysis (see 4.2.1).

3.3.2 Biochemical tests

Samples were taken and stored as for pine needle samples.

3.3.3 Nutrient analysis

At least 10 g (fresh weight) leaves were accurately weighed and placed in a paper bag for subsequent drying. These samples also gave dry weight/fresh weight ratios.

3.3.4 Hydrocarbon emissions

Samples were taken and treated as for Scots pine.

3.3.5 Water relations (Ulster)

Twigs were cut from the branch and cut ends placed in water (see 4.3).

The necessity for speed in processing the samples involved the development of methods suitable for use in the field, remote from mains services. These techniques were tested by sampling visits to Devilla Forest (central Scotland) and Gisburn Forest (NW England) during the spring of 1986. Team work in the field was essential for efficient operation, with everyone involved willing and able to take part in the processing of any of the samples listed above. In this respect, the continuity provided by the survey team leader (I S Paterson) was invaluable. On all occasions, at least 2 members of the team of 4 had taken part in earlier sampling exercises, which also provided continuity.

4 ANALYTICAL TESTS AND RESULTS

Although the main objective of this year's pilot study was to identify potential diagnostic tests related to air pollution, it was evident that differences between sites would arise from differences in soil, nutrient status, climate, genetic composition, management practices, tree age, sampling date and time. Nevertheless, it was felt that, at the least, the survey might show gross changes worthy of further examination, and, at best, a clear suggestion of the effects of one or more types of air pollution (ie gases or wet deposition of acidity). Where visibly damaged trees (assessed by crown thinning or foliage discoloration) were present, apparently undamaged tissue was sampled. In general, no significant differences were found between 'damaged' and 'undamaged' trees growing at the same site, but this finding is hardly surprising in view of the small sample numbers involved. The greatest utility in comparing site data may be obtained by:

i. comparing adjacent sites which differ only in respect of a single major factor (eg the north- and south-facing slopes at Lange Bramke);
ii. the grouping of site data (eg all UK sites showing similar behaviour);
iii. comparison with (relatively) unpolluted sites (eg the Scottish sites).

Some or all of these methods of examining the results are used below. Although differences may be expressed primarily in terms of potential effects of air pollution, in many cases the observed site differences will have no dependence on pollution levels.

4.1 Institute of Terrestrial Ecology

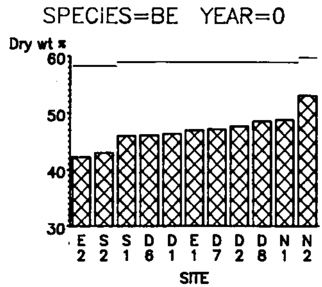
In all the results presented below, sites which appear under the same horizontal bar in the histograms are *not* significantly different from each other (based on Duncan's multiple range test) at the 5% level.

4.1.1 Dry weight/fresh weight ratios

For beech, there was little variation (Figure 2) but an interesting difference between the 2 Dutch sites which were sampled on consecutive days at approximately the same time. The site at Rips (N2) is considered to be more polluted than that at Kootwijk (N1). The older beech trees sampled at Witzenhausen (D6) do not appear significantly different from the younger trees elsewhere. The significantly smaller ratio at 2 of the British sites (S2 and E2) may be related to the later sampling date, but that does not explain the difference between Parnholt Wood (E2) and Birchett Wood (E1), which were sampled on consecutive days.

The relatively large variation in current year spruce needles is probably related to time of sampling, with smallest ratios for those sites sampled in early July and largest for those sampled in late August/early September. Variations in older needles are much smaller, but again there is a significant difference between the 2 Dutch sites (N1 and N2).

Leaf dry weight as % fresh weight



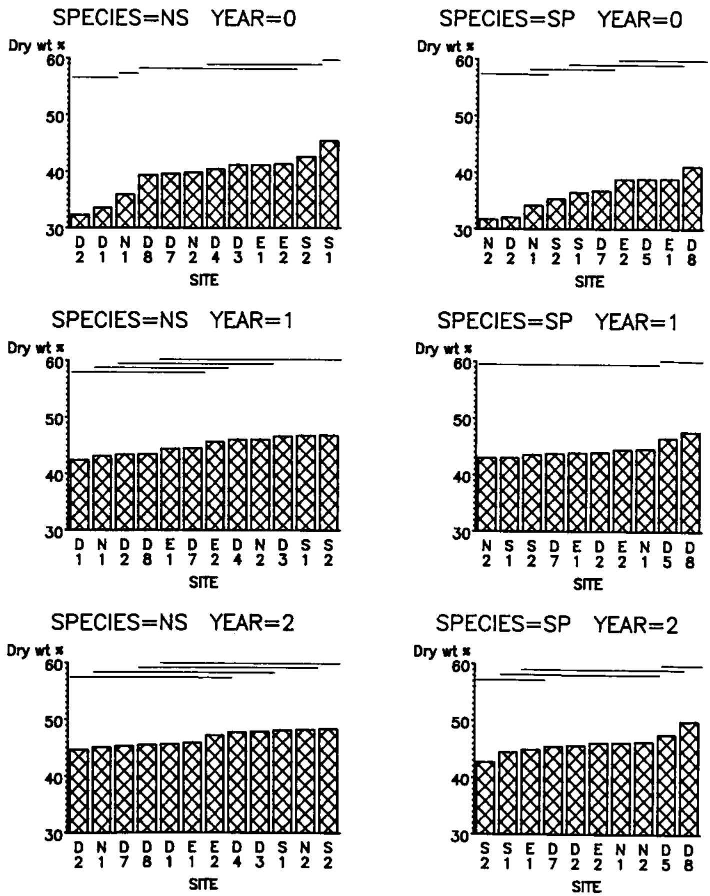


Figure 2.
Ratio of dry weight to fresh weight for leaf or needle samples
BE = beech, NS = Norway spruce, SP = Scots pine
Year 0 = 1986 foliage, 1 = 1985 foliage, 2 = 1984 foliage
Values under the same horizontal bar are not significantly different (P = 0.05, Duncan's multiple range test)
Site codes are given in Table 3

In current year needles of Scots pine, the effect of sampling time is not apparent. In all year classes, the trees from Selb (D8) near the Czechoslovakian border have the highest ratio (for which there may be many reasons). For 2-year-old needles, the British sites have consistently smaller ratios. This fact could be interpreted in terms of air pollution but is more likely to be related to climate and water availability.

4.1.2 Chloroform-extractable wax

Method: chloroform extracts (as described in 4.1.6) were filtered (0.2 μm PTFE membrane) and the solution reduced under vacuum. The concentrated solution was transferred with washing to a pre-weighed aluminium foil dish, and the remaining solvent was allowed to evaporate in a fume cupboard. Samples were weighed after 24 h and 48 h to ensure complete evaporation of solvent. Amounts of wax from each 5 g (fresh weight) sample are expressed in terms of percentage dry weight (Figure 3).

In spruce, there was a wide variation among the sites in all 3 year classes, with the smallest values consistently at the British sites. In current year needles, there was again a significant difference between the 2 Dutch sites (N1 and N2) which is presumably related to the rate or amount of wax production. Subsequent years' wax reflects the influences on production and subsequent erosion or removal.

For Scots pine, no significant differences were observed, except for current year needles, the 2 Dutch sites showing a pattern similar to that observed in spruce. This is not simply the result of expressing the data relative to dry weight, as the dry weight/fresh weight ratio was significantly greater at Kootwijk (N1) than at Rips (N2) (Figure 2). The large difference between the 2 Scottish sites may reflect the severe attack of the needle cast fungus (*Lophodermium seditiosa*) which occurred in 1985–86 at Glenbranter (S1). The site in the Netherlands at Rips (N2) also showed signs of fungal attack, by *Sphaeropsis sapinea*.

4.1.3 Chloroform-insoluble material

When leaves are washed with chloroform, particulate material on the leaf surface is brought into suspension. This chloroform-insoluble material may be soil-derived dust, plant or micro-organism debris, inorganic salts or carbonaceous material (soot) from the atmosphere. In filtering the chloroform extracts (4.1.2), the amount of chloroform-insoluble material retained by the filter was weighed, and expressed as a fraction of sample dry weight (Figure 4). Although termed 'dust', this

Surface wax as % dry weight

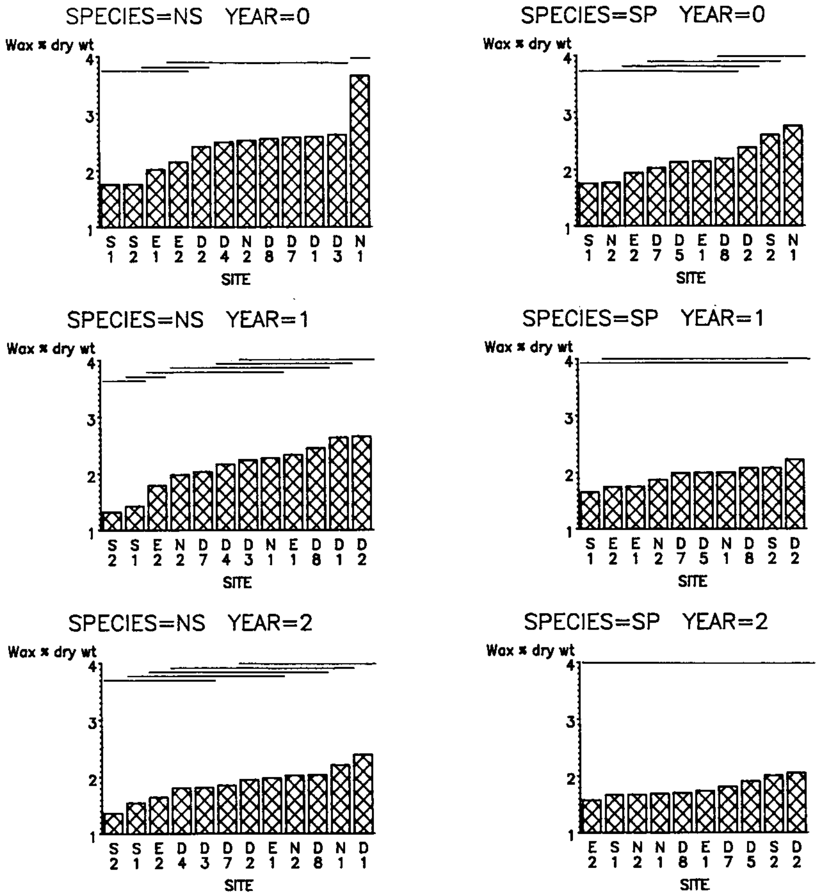


Figure 3.
Amounts of leaf surface wax extracted by a single 10 second wash in chloroform, expressed as percentage sample dry weight
Key as in Figure 2

Insoluble surface material as % dry weight

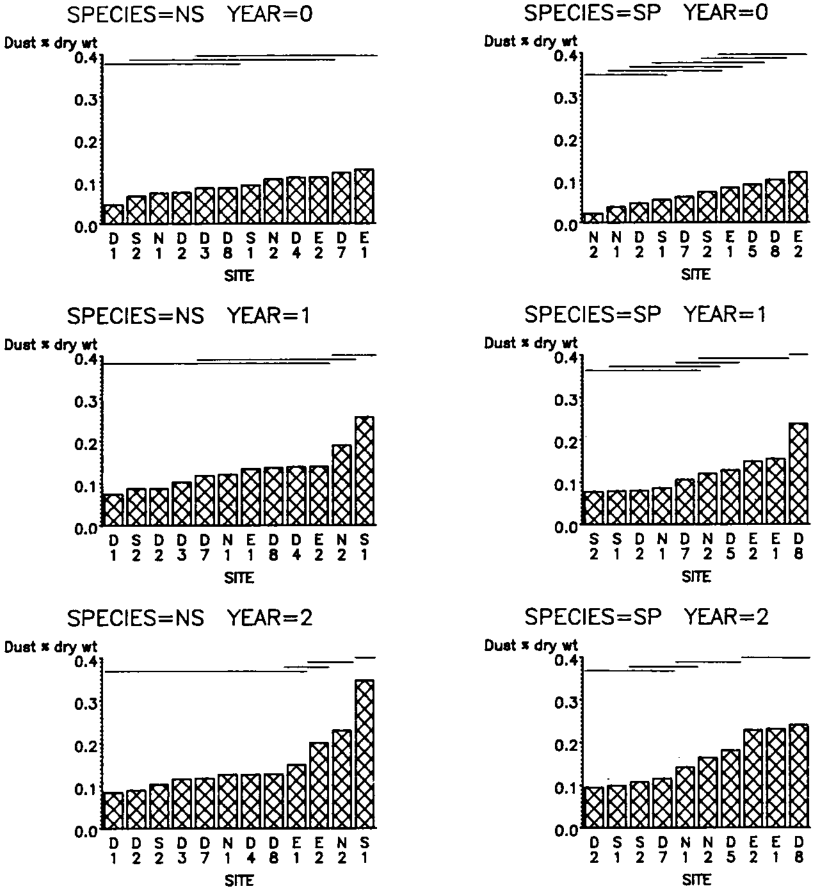


Figure 4.
Amounts of chloroform-insoluble material derived from leaf surface washings, expressed as percentage sample dry weight
Key as in Figure 2

material is black in colour, and probably comprises all the components listed above.

There was a significant increase with time for both spruce and pine at all sites, as might be expected, as most of this 'dust' is likely to be insoluble in water and will therefore accumulate on the leaf surface.

In spruce, there are few significant differences, although in the older needles the more polluted Dutch site (N2) does show a significantly greater amount than site N1. The very large values for Glenbranter (S1) are surprising; we can only suggest an unidentified local source of particles. This hypothesis is borne out by the very small values for pine at Glenbranter, from a site *c* 10 km distant.

In Scots pine, the differences are more marked, although any differences between the Dutch sites disappear. There is no obvious pattern in the measurements, except for the relatively large values for the sites in southern England (E1 and E2) and near the Czechoslovakian border (Selb, D8). These were also sites with large dry weight/fresh weight ratios, so the amount of 'dust' expressed in terms of fresh weights would be even greater. In view of the potential for local contamination, these data may not be particularly useful, but do point to significant differences between species growing at the same site, which presumably reflect the aerodynamic properties of the tree crowns.

An alternative method of expressing the data is in terms of the 'dust'/wax ratio for each sample. This method removes variations in fresh weight/dry weight ratios but includes variations in wax amount.

For spruce, the Glenbranter site stands out as before, and the conclusions are similar. The same applies for pine, with the marked separation of the 2 Scottish sites (S1 and S2) from the 2 English sites (E1 and E2).

4.1.4 Contact angles

The use of contact angle measurements to characterize the physical and chemical nature of pine needle surfaces has been developed by Cape (1983), and the methods used there have been extended to Norway spruce, by using water droplets of 0.2 μl rather than 1 μl. The results shown in Figure 5 are the mean values of 10 determinations for each sample (ie each bar of the histogram is an average of up to 80 measurements).

In spruce, site differences increased with age of needle, although the

Contact angle of water droplets

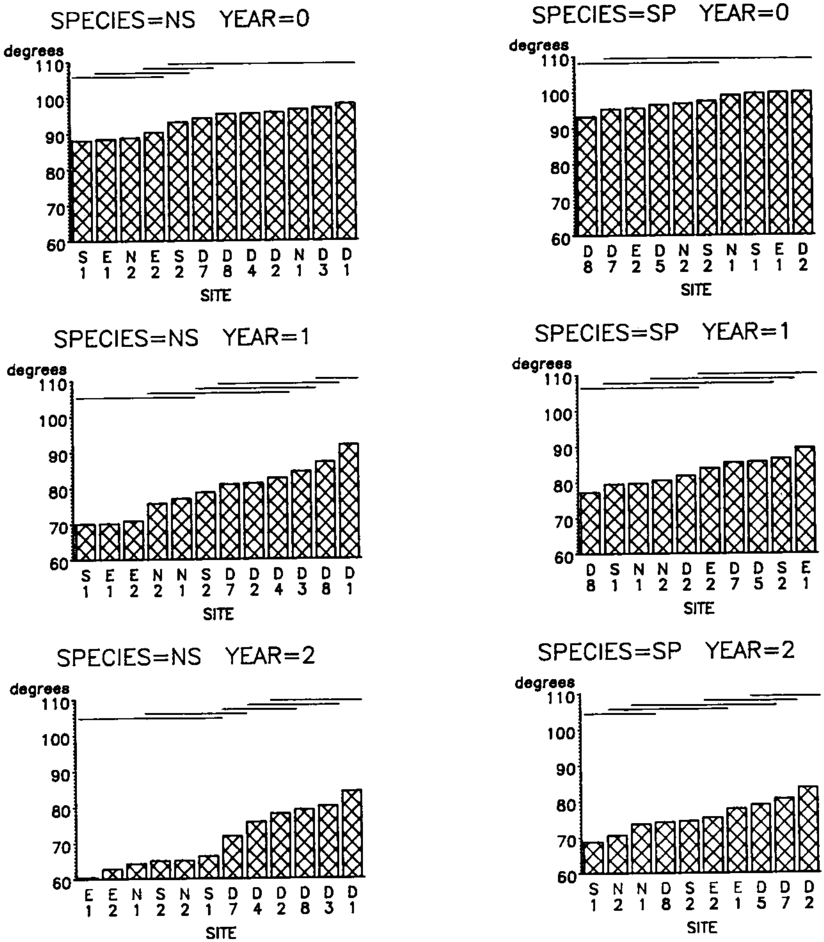


Figure 5.
Contact angle of deionized water droplets on abaxial needle surface
Key as in Figure 2

ranking of sites was similar for all ages. The data on 2-year-old needles suggest a separation into 2 groups: British and Dutch (with small values) and German (large values). As this separation presumably involves changes from an initially more uniform pattern (current year needles), it reflects smaller changes in leaf surface properties at those sites where the 'new' type of forest damage is observed. Insufficient is known of the variation of contact angles with age on Norway spruce for any further interpretation.

In contrast, data for Scots pine show no clear geographical separation of this measurement. The very small values for Glenbranter (S1) may be related to the fungal attack noted earlier, while those for Selb (D8) may be attributable to large winter SO_2 concentrations, insofar as this is a common factor with earlier measurements of contact angles on Scots pine (Cape 1983).

As year-to-year differences are likely to be important if contact angle changes are the result of external influences, these have been calculated.

In spruce, the largest differences are for the British and Dutch sites, and are much greater in the first year of growth. The greater change between current and first year needles is also shown for pine, but here the changes in the first year are least at the British sites and greatest at the Dutch sites. In the second year, the pattern is reversed (as contact angles approach an asymptote at each site with time).

This behaviour is consistent with that observed for Scots pine (referred to earlier) at 4 sites in Britain (2 polluted, 2 unpolluted) which showed a larger change in contact angle in the first year in polluted areas, and a correspondingly smaller change in the second year.

4.1.5 Härtel turbidity test

This test, devised by Härtel (1953) and used over the past 30 years (see, eg, Fuchshofer & Härtel 1985), has been related to the SO_2 concentration to which trees were exposed. The methods used were as defined by Härtel (1953), except that a thermostatted block-heater was used in place of a brine heating bath. Initial experiments showed that fresh material could be stored for up to one week without significant changes, and in all cases duplicate samples were analysed. Great care must be taken when removing spruce needles from the shoot that the needle is not 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.

The turbidity measurements (taken after 2 hours' cooling to room temperature) are shown in Figure 6, expressed as the percentage absorption of light. According to the test, increased exposure to SO_2 should increase the absorption. Later studies (Fuchshofer & Härtel 1985) indicated that this reaction is related to calcium concentrations in the extract, with less light absorption at higher calcium concentrations. The test was originally applied to older needles, but each year class was used in this analysis. The results show the most pronounced differences with the older (2-year-old) needles.

In spruce, turbidity was greatest at British sites and least at the 2 Dutch sites. There is no obvious relationship with ambient SO_2 concentrations as might be expected. The Härtel test, while applicable to areas around point sources, does not appear to be useful on a regional scale. However, within a site, 'damaged' trees gave greater turbidity values than 'undamaged' trees.

For Scots pine, the large values for 2-year-old needles from Selb (D8) may reflect exposure to SO_2, but, in general, the results are not useful on a regional scale.

As an additional measurement, the conductivities of the extracts were determined, and these show a similar pattern. For spruce, the largest conductivities are from British sites, and the smallest from the Dutch, ie similar to the turbidity results. There was a smaller range in the results and fewer significant differences. This relationship was less clear for pine, but the sites with smallest conductivities were also those for which the conductivities of spruce extracts were small.

4.1.6 Nutrient analyses

Analyses for major nutrients (N, K, Ca, Mg, S) were made only for Norway spruce samples after drying (3 days at 80°C) and milling. All analyses were performed by the ITE Analytical Chemistry Service Section at the Merlewood Research Station. All elements except S were determined after acid digestion using standard methods. Total sulphur was measured using X-ray fluorescence.

The results for total sulphur and total nitrogen are shown in Figure 7. The relative ranking of the sites is fairly constant for all 3 year classes. As might be expected, sulphur contents are highest for those sites in the east of West Germany (D3, D4, D7, D8) which are exposed to the largest concentrations of SO_2, and least at Scottish sites. In contrast, the influence of NH_3 on the nitrogen content of needles is clearly shown for the 2 Dutch sites, of which Rips (N2) is regarded as the more polluted.

Härtel turbidity test

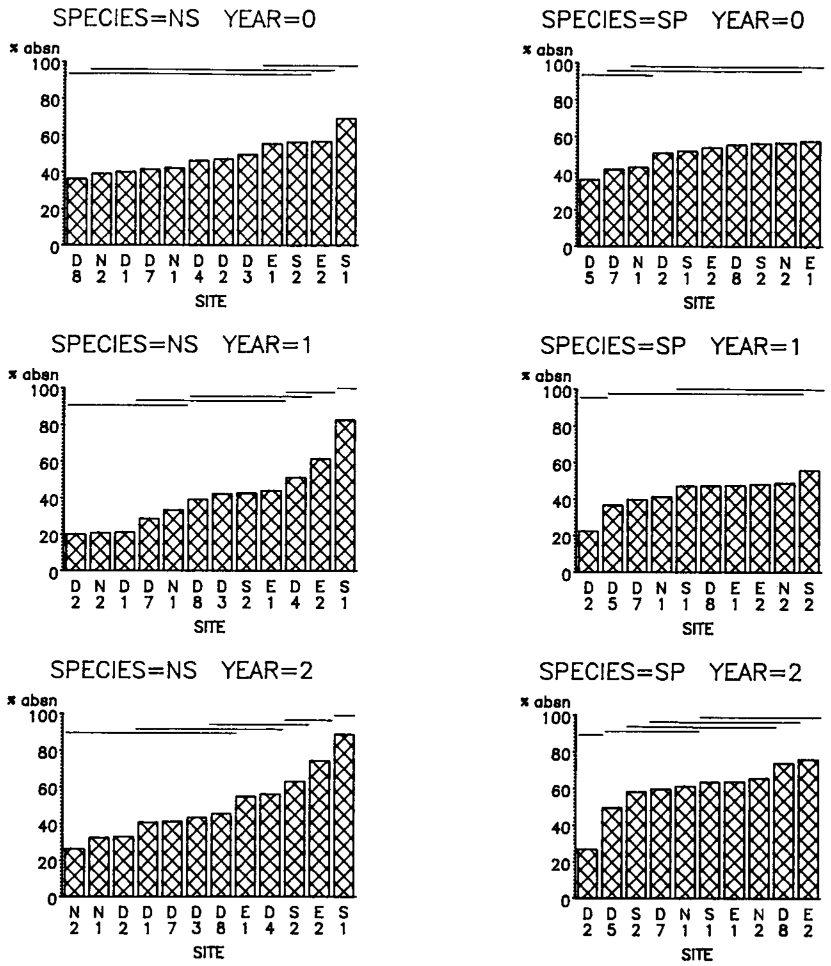


Figure 6.
Light absorption (%) at 590 nm of boiling water extract of conifer needles
Key as in Figure 2

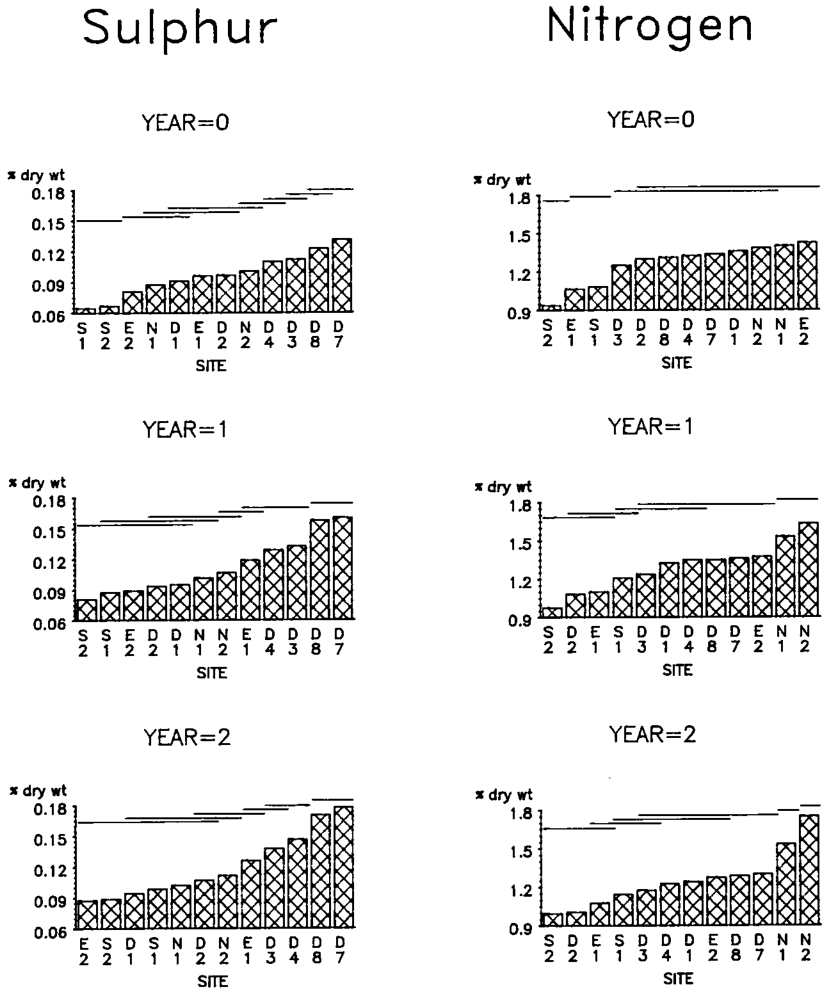


Figure 7.
Total sulphur and nitrogen content of Norway spruce needles, expressed as percentage dry weight

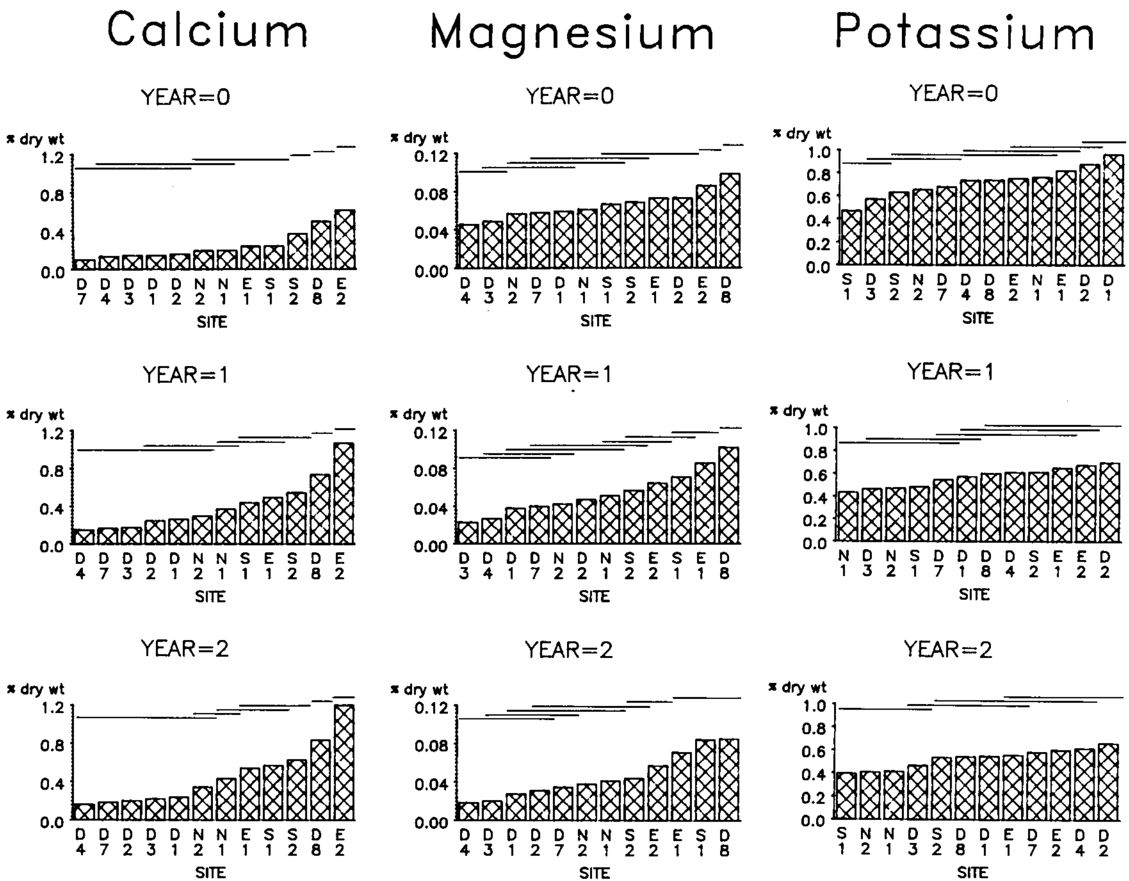


Figure 8.
Calcium, magnesium and potassium content of Norway spruce needles, expressed as percentage dry weight

The 3 metals (K, Ca, Mg) show rather contrasting patterns (Figure 8). There is relatively little variation in potassium content, and no clear geographical effect. For calcium, however, there is a very clear indication of relatively small concentrations at all the German and Dutch sites, with the exception of D8 (Selb). The very large values at site E2 reflect the chalk bedrock. Magnesium concentrations followed a similar, but less extreme, pattern, with smallest values at those sites where foliar yellowing (attributed to Mg deficiency) has been observed. These foliar element concentrations perhaps reflect most accurately our preconceived ideas on the probable influence of the environment on tree health.

4.2 University of Lancaster

4.2.1 Modulated fluorescence

The measurement of fluorescent emissions by chlorophyll a during photosynthesis has been established as a useful and non-destructive method for assessing the state of the photosynthetic apparatus and the rate of CO_2 assimilation when plants are exposed to environmental stress (Smillie & Nott 1982; Öquist & Ögren 1985). A recent advancement in this field has been the development of a portable instrument capable of generating modulated light signals, and measuring the responding chlorophyll fluorescence from leaves in continuous white light (Ögren & Baker 1985). Using this instrument, we hoped to obtain fluorescent signals from beech leaves under field conditions, and preferably when still attached to the tree. Preliminary investigations showed, however, that the fluorescent response depended largely on the light conditions which the leaf had experienced prior to and during the measurement, and, for this reason, it was not possible to compare leaves from different parts of a tree, or from different sites, or at different times of day. If, however, the leaves were preconditioned in the dark for a period before measurement and were all measured under the same background light conditions, these differences were eliminated.

Needles from conifers were not investigated in this study, because of technical problems. The fluorescent signal obtained from spruce and pine varies greatly depending on the orientation of the needles to the light source and photodetector, and no quick and effective method of controlling this factor has yet been devised.

Materials and methods

The apparatus for fluorescent measurement (Ögren & Baker 1985) consists of a sample holder into which the leaf is clamped, adaxial side

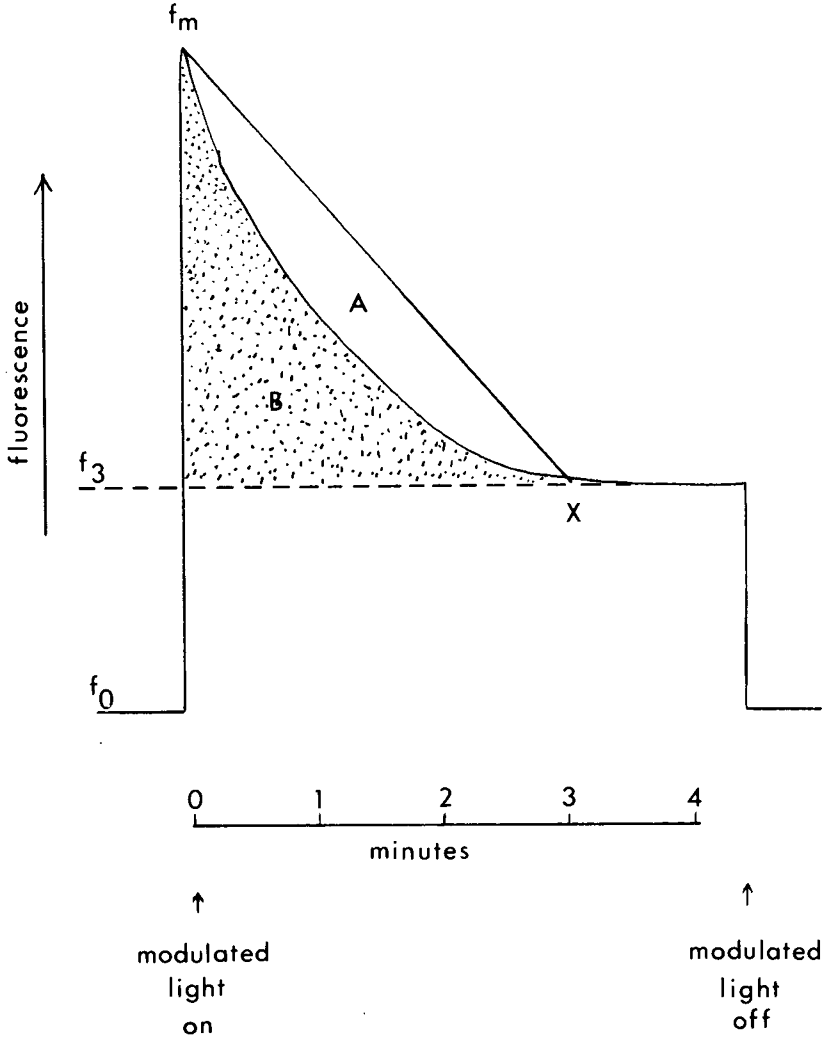


Figure 9.
Schematic response of fluorescence to modulated light
f_m =maximum (peak) fluorescence
f_3 =steady-state fluorescence after 3 minutes
f_0 =background fluorescence
For explanation of areas A and B, see text (5.2.1)

uppermost. This holder is fitted with a light source to provide 1000 μE $m^{-2} s^{-2}$ white light to the leaf surface, and with a modulated light source and photodetector which responds to fluorescence emitted only at the frequency of the modulated light source. The modulated light and fluorescent signal are controlled by a portable battery-operated fluorescence meter which is connected to a chart recorder.

Branches of beech were placed in black plastic bags for one hour to precondition the leaves. Individual leaves were removed and placed immediately in the sample holder, with the white light source on. After establishing zero background fluorescence, the modulated light was switched on and the trace recorded for 3 minutes. Triplicate leaves were sampled from each tree.

Results

The response of the leaf to modulated light is shown in Figure 9. When the modulated light is switched on, the fluorescence rises quickly to a maximum (f_m) and then falls gradually to a constant rate, f_3. The initial rise and the rate of fluorescence quenching to f_3 are considered useful indicators of the leaf's photosynthetic efficiency. Because fluorescence is an energy-wasting process, it might be expected that higher levels of fluorescence, or a reduced ability to recover from the initial rise in fluorescence, would indicate leaf damage.

Several parameters were measured in order to find which would provide the most interesting differences between the sites. The height of f_m and the constant rate, f_3, followed similar trends between sites (Figures 10a & b), indicating generally high fluorescence at sites S1, S2 and E2, compared with sites D2 and N1. The ratio of these 2 values, f_m/f_3, showed a different trend, with Scottish sites and site E1 having the highest values whilst sites E2 and N2 were relatively low (Figure 10c).

The rate of fluorescence quenching was measured by the ratio of the shaded area (B) over the triangular area, $f_m - f_3 - X$ (A). This ratio was high in sites S1, S2 and E2 and lowest in the Black Forest and Netherlands sites (Figure 10d).

Assuming that Scottish sites were unpolluted compared with the Black Forest and Netherland sites, it appears from these results that high initial fluorescence followed by a slow recovery rate to a relatively high constant level is a feature of healthy plants, and that in polluted areas initial fluorescence is lower, and quenching more rapid. This finding is difficult to interpret in terms of our present understanding of

Fluorescence – beech

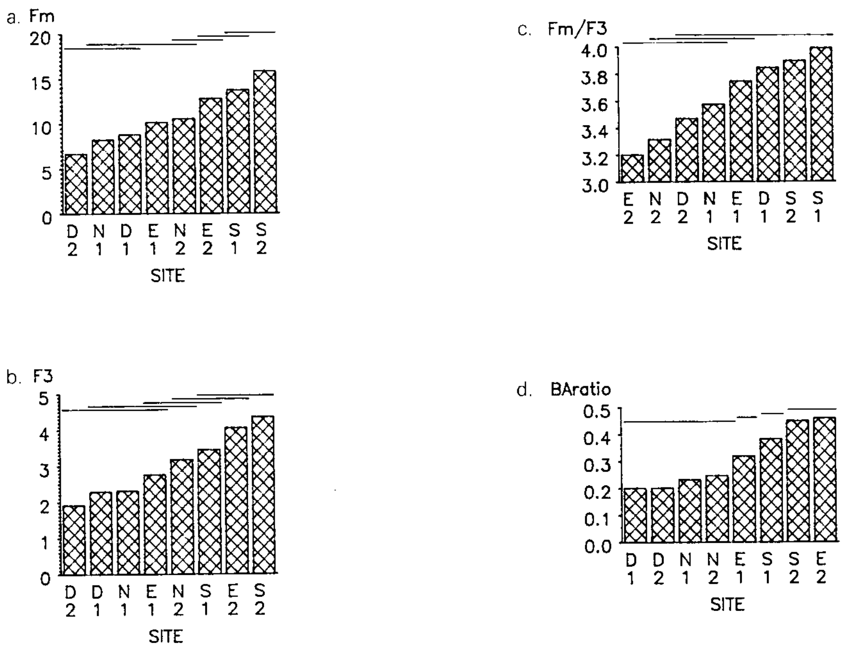


Figure 10.
Fluorescence of beech leaves from selected sites

a. f_m: peak fluorescence
b. f_3: fluorescence after 3 minutes
c. ratio $f_m : f_3$
d. area ratio (area B over triangle A + B in Figure 9) representing rate of fluorescence quenching

fluorescence, and it seems likely that outside factors other than pollutant damage are confusing the issue. The differences between sites cannot be related to any of the pigment analyses made in this study. Nevertheless, clear and significant differences have been found and these may prove of interest when compared with results of other tests. This technique is still in the early stages of development and requires further investigation before its usefulness as an indicator of pollutant damage is understood.

4.2.2 Hydrocarbon emissions from needles

A range of hydrocarbons has been shown to indicate stress and injury in plants. Ethylene (C_2H_4), produced by all plants, is widely known as a stress indicator in living cells, its production increasing in proportion to the degree of stress. Other simple organic compounds such as acetaldehyde and ethanol may also result from stress, and indicate a switch in metabolic pathways away from normal aerobic respiration (Kimmerer & Kozlowski 1982). Ethane, propane and pentane, on the other hand, are produced from free-radical mediated peroxidation of membrane lipids, and are thus indicative of irreversible cell damage and death which can occur in response to atmospheric pollutants (Zhou Rong-Ren *et al.* 1982).

Kimmerer and Kozlowski (1982) showed that SO_2 fumigation of red pine (*Pinus resinosa*) resulted in the production of ethylene, acetaldehyde and ethanol, all of which increased with SO_2 fumigation up to the point of cellular necrosis. Ethane was not produced until the onset of necrosis. It has, therefore, been suggested that the measurement of all these compounds in the gas phase might give a useful indication of the physiological and metabolic states of the plant.

The original intention in this survey was to measure as many hydrocarbons and related compounds as possible. However, because of the extremely erratic observations of ethanol, acetaldehyde, propane and methanol, we have concentrated only on ethylene and ethane (C_2H_4 and C_2H_6).

Methods

Spruce and pine needles were removed carefully from their twigs to avoid damaging the needles. Beech leaves were picked together with their petioles. Triplicate one g samples of this fresh material were sealed in 25 ml glass bottles with perforated screw-capped lids holding rubber liners. The bottles were incubated for 24 h in a water bath at a temperature of 20–25°C, either in the shade outside or with an artificial light source. After incubation, gas was transferred from each bottle using a double-ended needle to an evacuated 5 ml bottle, and the leaf material was discarded. The gases were stored in these small bottles in a cool box or refrigerator until the time of analysis.

Gas analysis was carried out using a PYE 104 gas chromatograph with a flame ionisation detector. The column (1.5 m) was packed with 80/100 Poropak Q and run at a temperature of 100°C with N_2 carrier gas flow of 450 ml min^{-1}. A standard gas mixture containing 16.8 ppm C_2H_6 (Alltech Ltd, Carnforth) was used as calibration.

Results

i. Norway spruce (Figures 11a & 11c)

There were large differences in ethylene evolution between sites, trees from both Scottish sites giving consistently low values for all needle ages. There was no consistent change in ethylene evolution with age.

The highest C_2H_4 concentration came from trees in the Harz mountains. Of the 2 sites in this area, the north-facing slope (site D4) showed much higher values, significantly above those of the nearby south-facing slope (site D3) and about 9 times higher than the Scottish sites. Despite large variations between trees within each site, the Black Forest also showed significantly higher C_2H_4 production than Scottish sites, as did site N2 (Rips), but not site N1 (Kootwijk) in the Netherlands.

In the Fichtelgebirge, C_2H_4 production at site D7 was only just above that in the Scottish sites, whilst at site D8 it was significantly higher for all needle ages.

The 2 English sites were very different from each other in terms of C_2H_4 production. In the Kent site (E1), only current year needles gave significantly higher C_2H_4 than those of Scottish sites, whilst the Hampshire site (E2) was around 6 times higher than Scottish sites for all needle ages.

Ethane was detected only in very small concentrations at most sites and showed greater variability within sites. The highest values were found at site E2. In both Black Forest sites, small concentrations of C_2H_6 were produced, which increased with age. No C_2H_6 could be detected at sites S2 or E1.

ii. Pine (Figure 11b)

C_2H_4 was generally higher than in spruce and showed a greater variability within sites. At English and Scottish sites, pine produced

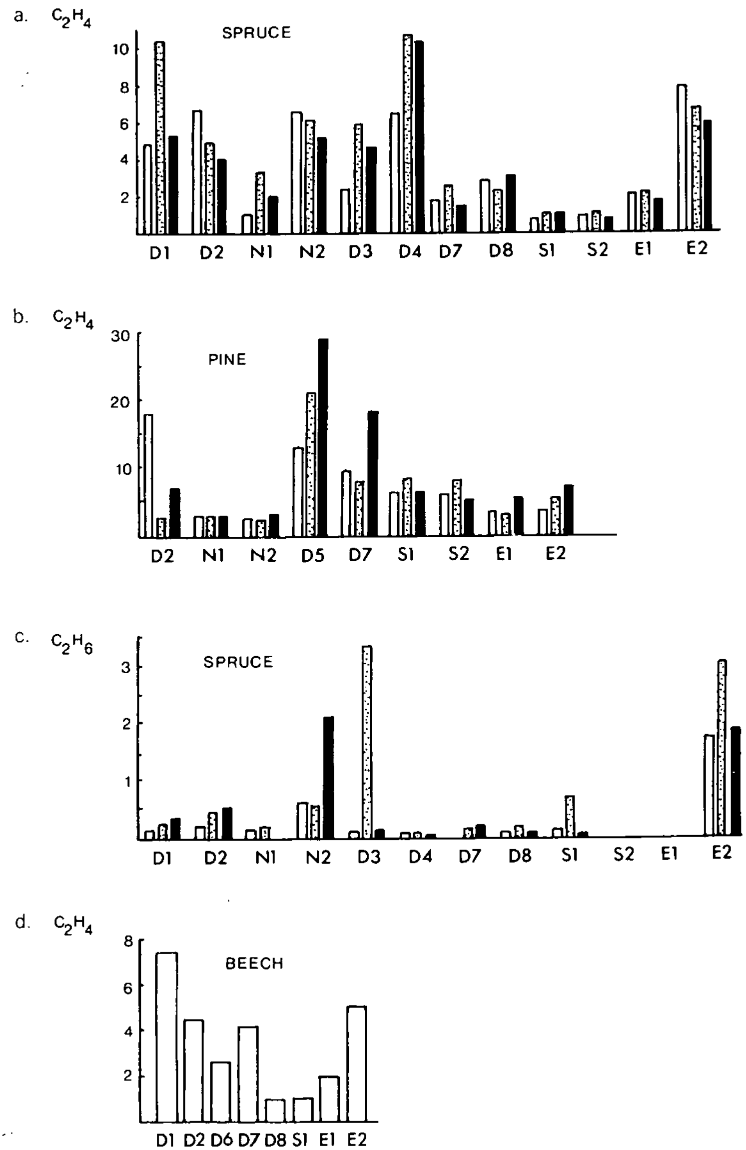


Figure 11.
Emissions of hydrocarbons from foliage. Units on y-axis are arbitrary
Open columns =year 0 (1986), shaded =year 1 (1985), solid =year 2 (1984)

about 6 times more C_2H_4 than Scottish spruce. The highest concentrations of C_2H_4 were found in pine from Celle (site D5) and Fichtelgebirge (site D7). However, due to the high within-site variation, only second year needles were significantly different from those of Scottish trees. Unlike spruce, pine did not produce large amounts of C_2H_4 at site E2.

C_2H_6 was not detected in pine samples from any site, apart from 2 trees from site D5 and one 2-year-old sample from Fichtelgebirge (site D7).

iii. Beech (Figure 11d)
The amounts of C_2H_4 produced were similar to those from spruce, and followed roughly the same trend between sites. Site S2 was not sampled. The results for sites D7 and D8 were the reverse of those for spruce, with site D7 producing 4 times as much C_2H_4 as site D8, but this difference was not statistically significant. As in spruce, C_2H_4 production at site E2 was relatively high.

The production of different levels of C_2H_4 at different sites cannot in itself be used as an indicator of plant stress resulting from atmospheric pollution. C_2H_4 is produced by plants in response to many kinds of stress and also during the natural senescence of leaves. Nonetheless, it is interesting to note the relatively low C_2H_4 production by all 3 species from the Scottish sites, which are considered to be unpolluted, compared with most other sites. This factor might be useful if correlations can be established between C_2H_4 production and other injury responses.

C_2H_6 concentrations were disappointingly low and difficult to interpret. The Black Forest sites (D1 and D2), which experience high O_3 pollution and might therefore be expected to show indications of lipid peroxidation, both increased their C_2H_6 production with needle age. In other sites, one year group of needles produced much higher quantities of C_2H_6 than other needle ages on the same tree. The high concentrations of C_2H_6 at site E2 cannot yet be explained.

4.2.3 Buffer capacity

The buffering ability of plant tissue against acidic changes is of obvious importance when considering acidic air pollutants. It has been suggested (Scholtz & Reck 1977) that buffering capacity in conifers is an important genetic factor, giving rise to resistance or susceptibility to acidic precipitation. Several reports have indicated a decline in buffering

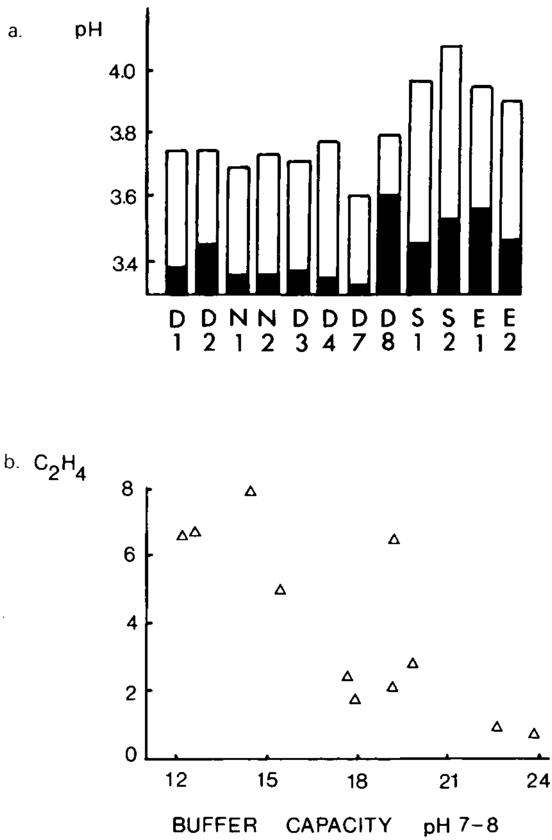


Figure 12.

a. pH of macerated spruce needles
Solid columns =year 0 (1986), open =year 2 (1984)

b. Relationship between ethylene emission and buffer capacity in range pH 7–8 for current year spruce needles

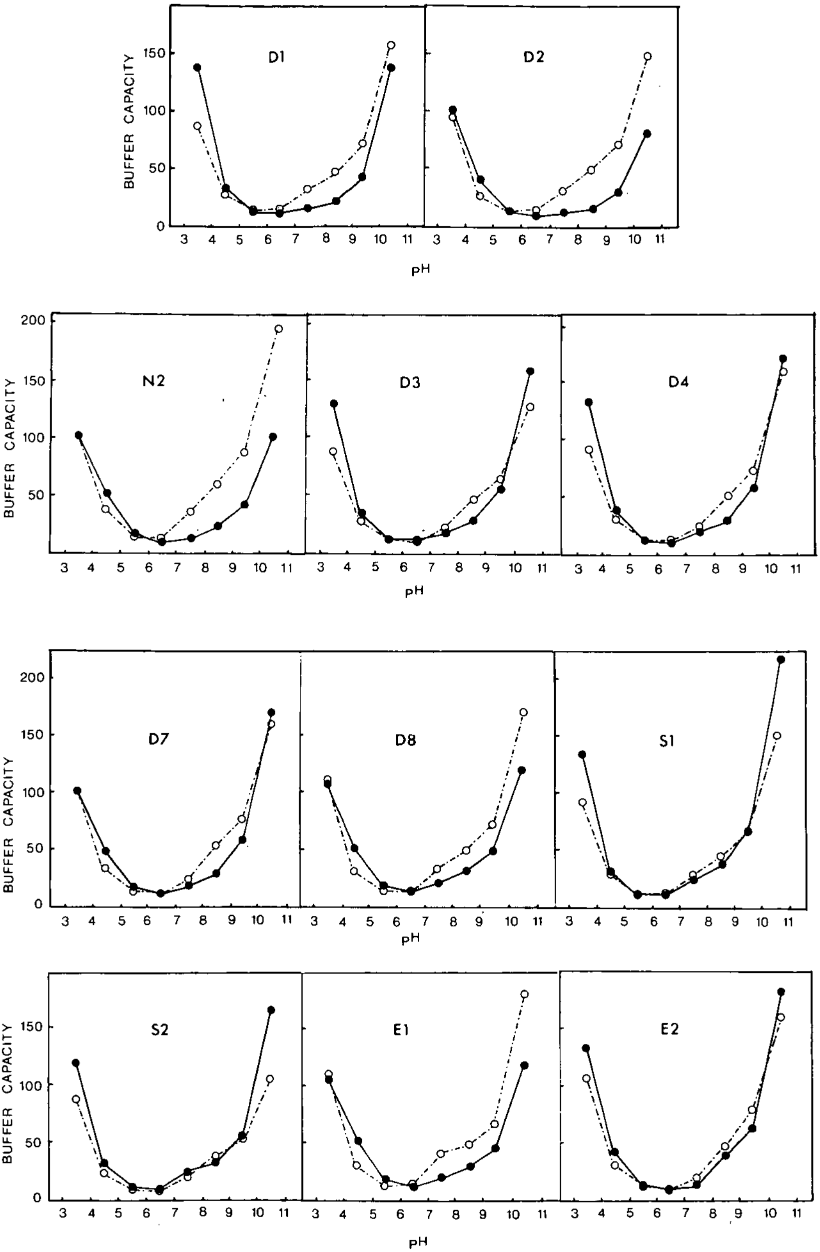


Figure 12.

c. Buffer capacity of macerated spruce needles as a function of pH (units μmol H^+/pH g^{-1} fresh weight).
Solid circles = year 0 (1986), open = year 2 (1984).

capacity after exposure of plants to SO_2 and acid mists. Grill (1971) compared the buffering ability of Norway spruce growing at sites with different pollution climates, and found that trees in urban areas and those fumigated with SO_2 showed lower buffer capacity than those in control 'clean air' areas. This reduction in buffer capacity correlated with lower concentrations of organic acids in needle sap.

Methods

Samples of 2 g of frozen needles or beech leaves were homogenized in 80 ml water. The homogenate was halved and 40 ml titrated against either 0.05 N HCl or 0.05 N NaOH. This method was carried out by stepwise additions of 0.5 ml acid or alkali, the pH being noted after each addition. Buffer capacity between consecutive pH units was calculated as μmol (pH unit)$^{-1}$ g^{-1}.

Results

The buffering capacity and pH of homogenates of spruce showed considerable variation between sites. In general, tissue pH increased with age and was highest in British sites. However, contrary to this trend, the highest pH of current year needles was found in the Fichtelgebirge (site D8) (Figure 12a).

Buffer capacity can be divided into 3 main sections within the pH range 3–11 (Figure 12c). Between pH 3 and 5, buffering was high and showed large variations between needles of different ages and between sites. In general, younger needles were better buffered than 2-year-old needles in this region. Between pH 5 and 7, buffering was low in all samples, with little difference between age groups or sites. The largest variations came in the pH range 7–10. In Scottish sites (S1 and S2) and site E2, there was little difference between current and 2-year-old needles, whilst in all other sites buffering of current year needles was significantly less, and that of 2-year-old needles significantly more, than in sites S1 and S2.

The difference in buffer capacity between current and 2-year-old needles is considered of interest, as this difference, rather than buffer capacity itself, appears to be a feature which distinguishes the sites. Sites showing a large rise in buffer capacity between pH 7 and 10 from current to 2-year-old needles (especially sites D1, D2, N2 and E2) also tended to show the biggest decreases with age in buffering of the acid region. In addition, such sites showed a smaller rise in pH with needle age. If age-related pH and buffer capacity changes can be interpreted as an indication of the plants' adjustment to environmental conditions, these results could mean that, in damaged sites, the normal increase in tissue pH with age is prevented by the interference of acidic pollutants, which the plant is unable to buffer. The differences in buffer capacity from pH 7 to 10 are most apparent between current year needles which have a significantly reduced buffering ability in most sites compared with Scottish sites. This finding suggests that, in polluted sites, young needles maintain a higher buffering capacity towards acid at the expense of alkaline buffering. As the needles age, this acidic buffering capacity is not maintained, and the buffering capacity in the alkaline region is similar at all sites.

Increases in buffering capacity and the maintenance of constant cell pH must constitute a drain on the energy resources of the plant, and might therefore be expected to affect the growth rate and/or cause stress. It is interesting to note that, in current year needles, a significant negative correlation was found between buffer capacity in pH 7–8 and C_2H_4 evolution of needles (Figure 12b). This did not occur in older needles, and no significant correlations were found between C_2H_4 evolution and tissue pH or acid buffering capacity.

These measurements are being extended to beech and pine samples, but the results have not yet been compiled.

4.2.4 Pigment analysis

Plant pigments have often been suggested as diagnostic parameters (see Darral & Jäger 1984) but have never been successfully applied routinely to field surveys of forests or crops. Chlorophyll a/b ratios and levels of chlorophyll have been used to examine the effects of SO_2 on a variety of conifers (Katz & Shore 1955; Müller 1957; Börtitz 1964), but only Arndt (1971) has investigated the possible use of carotenoids, especially beta-carotene. The techniques of separation and measurement of individual pigments have been improved, and these improvements have been assessed in a preliminary manner in this survey.

Methods

Leaf material for pigment analysis was stored in liquid N_2 until immediately before extraction, as storage at higher temperatures failed to prevent pigment deterioration.

Samples of 0.5 g of frozen material were homogenized in 5 ml of 50 % methanol. The homogenate was shaken with 15 ml chloroform to extract the pigments into the chloroform layer. This mixture was centrifuged at 3000 rpm for 3 min, and the chloroform layer filtered through Whatman no. 1 filter paper and then through a Millipore filter. Ten μl of the resulting sample were applied neat to a high-performance liquid chromatograph column for pigment separation and the remainder was diluted 1:5 for determination of total chlorophyll a and b, and total carotenoid content in a spectrophotometer.

Results

The pigment ratios for 2-year-old spruce and pine needles at all sites and for beech at 4 sites are shown in Figures 13a–13c. Compared with the Scottish sites (S1 and S2) which are considered as unpolluted controls, the chlorophyll a/b ratio was low at all sites. For most of the other ratios, trends between sites differed in the 3 species. The a/b ratio was high in the Harz spruce but not in pine. These sites also show reduced beta-carotene/chlorophyll a ratios for spruce. Site D7 in the Fichtelgebirge differed most from the Scottish sites in its low beta-carotene/xanthophyll and beta-carotene/chlorophyll a ratios. These trees also had high violaxanthin/lutein ratios. Pine at site D7 showed none of these characteristics.

The violaxanthin/antheraxanthin ratios in spruce were high at continental sites compared with Scottish trees, whilst the violaxanthin/lutein ratios were generally lower than at Scottish sites. In pine, these 2 ratios were almost the reverse.

Occasional unidentified peaks occurred, notably a compound just before lutein, and the incidence of these unusual peaks was higher in continental sites than in British ones.

Discussion

The suggested role of carotenoid pigments as protective agents against photosensitized oxidants (Krinsky 1966) is now starting to attract more attention from those interested in carotenoid biochemistry, and we are engaged in active discussions with Dr G Britton (Liverpool) on developing this topic further. Our results show that, although beta-carotene changes in spruce samples from the Fichtelgebirge were unduly low, our most promising indications of significant change were shown in (i) the ratio of violaxanthin/antheraxanthin in Norway spruce being higher on the continent than the UK sites, (ii) the ratio of violaxanthin/lutein in Scots pine being higher on the continent than UK sites, (iii) chlorophyll/carotenoid ratios in Norway spruce being generally higher in sites showing damage, and (iv) a higher frequency of unusual carotenoids in damaged sites. Of these indications, we regard (i) and (iv) as the most significant, and certainly (i) as the most notable, especially as the chlorophyll a/b ratios show a slight but definite trend. They would appear to support the idea that possible

Pigment ratios — Norway spruce

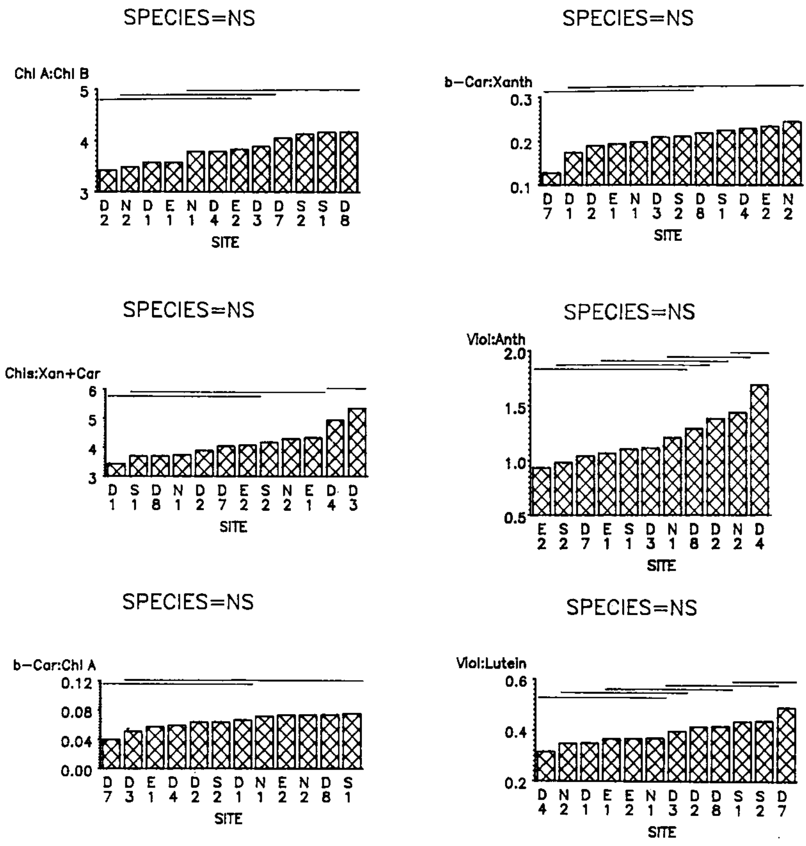


Figure 13.

a. Pigment ratios for 2-year-old Norway spruce needles
Chl A::Chl B =chlorophyll A:chlorophyll B
Chls:Xan +Car =total chlorophylls:xanthophylls +carotenoids
b-Car : Chl A =β –carotene chlorophyll A
b-Car : Xanth =β –carotene total xanthophylls
Viol:Anth =violaxanthin:antheraxanthin
Viol:Lutein =violaxanthin:lutein

Pigment ratios – Scots pine

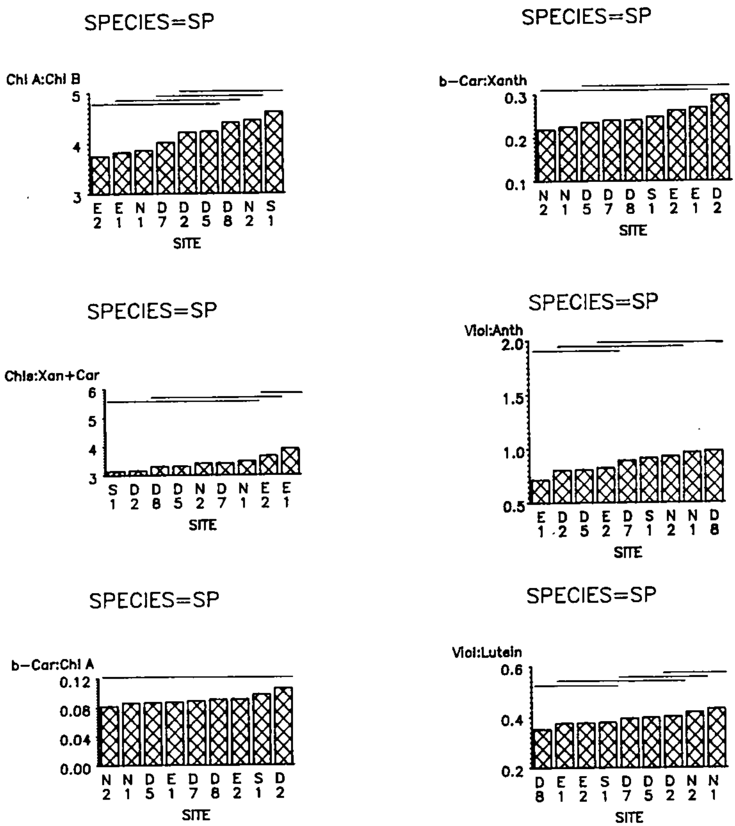


Figure 13.

b. Pigment ratios for 2-year-old Scots pine needles
Key as in Figure 13a

Pigment ratios – beech

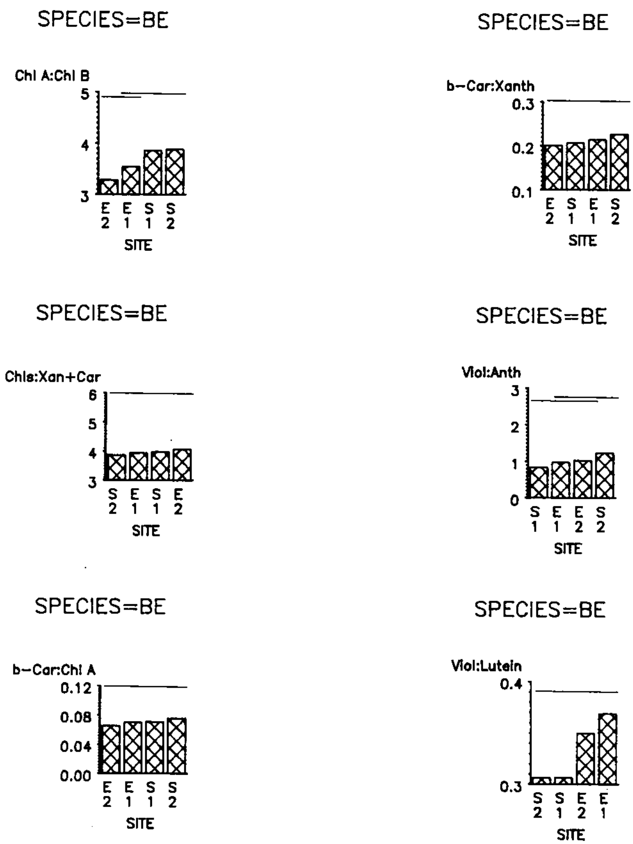


Figure 13.

c. Pigment ratios for beech leaves
Key as in Figure 13a

extra oxidation events are absorbed by the pool of xanthophylls, which is correspondingly altered in the direction of extra epoxidation taking place, turning antheraxanthin into violaxanthin, as originally proposed by Yamomoto *et al.* (1962). Very obviously, this interconversion should be closely examined in the future in a series of experimental fumigations to define it further.

4.2.5 Alpha-tocopherol analysis

Enhanced senescence and premature needle loss have been observed as symptoms of forest decline. Alpha-tocopherol is an anti-oxidant present in plant membranes and is known to increase during senescence. Recently, we have shown that alpha-tocopherol in current year needles increases after a 2-year fumigation with SO_2. This response was promoted by O_3, although O_3 alone had no impact on the alpha-tocopherol content (Mehlhorn *et al.* 1986). In 2-year-old needles, this response was even more pronounced, but here O_3 alone also significantly increased the alpha-tocopherol content. It has been suggested that an increase in alpha-tocopherol content provides a protection against oxidation of polyunsaturated fatty acids by free radical attack, and alpha-tocopherol might therefore be an indicator of exposure to atmospheric pollution.

Method

Frozen needles or leaf material were macerated on ice in 80 % ethanol and extracted into hexane. Samples were applied to an HPLC column, eluted in 95 % methanol, and the absorption was measured at 290 nm.

Results

With the exception of sites D3 and D4 (Harz), the alpha-tocopherol content in current year needles was significantly higher at all sites than at site S2 (NE Scotland). In 2-year-old needles, this response was even more pronounced, and sites D3 and D4 also had significantly higher alpha-tocopherol contents (Figure 14). These results are interesting with respect to higher SO_2 and O_3 concentrations in all these areas compared to northern Scotland. The higher alpha-tocopherol content indicates that SO_2 and O_3 may enhance senescence in Norway spruce trees. This response appears to be caused primarily by SO_2 (current year increases in alpha-tocopherol) and may be further promoted by O_3.

4.3 University of Ulster – physiological analysis

A survey of the literature suggests that tissue/water relations, stomatal function, gas exchange and photoassimilate transport are all influenced early on in the decline sequence, when trees are grown under

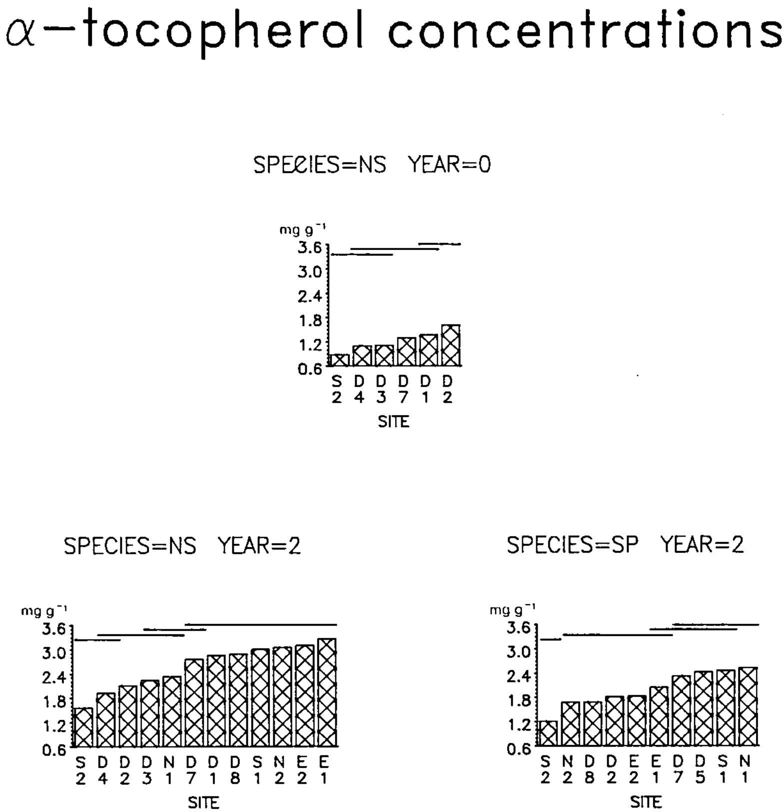


Figure 14.
Alpha-tocopherol content of Norway spruce needles, expressed as mg g^{-1} dry weight

unfavourable environmental conditions. Of these factors, needle/water relations of Norway spruce and Scots pine and leaf gas exchange in beech were selected as the most appropriate for analysis during the 1986 survey.

4.3.1 Shoot/water relations

Preliminary work was conducted during the winter of 1985–86, using 15 cm lengths of shoot cut from mature Norway spruce and then sealed into a portable pressure chamber of the Scholander type. This method showed that vertical gradients of xylem water potential could be determined using this technique in the field (Figure 15). As in Sitka spruce (Hellkvist *et al.* 1974), xylem potentials were greater at the top of the canopy, showing gradients of between 0.03 and 0.09 MPa m^{-1}. Weather conditions were shown to influence the xylem potentials and

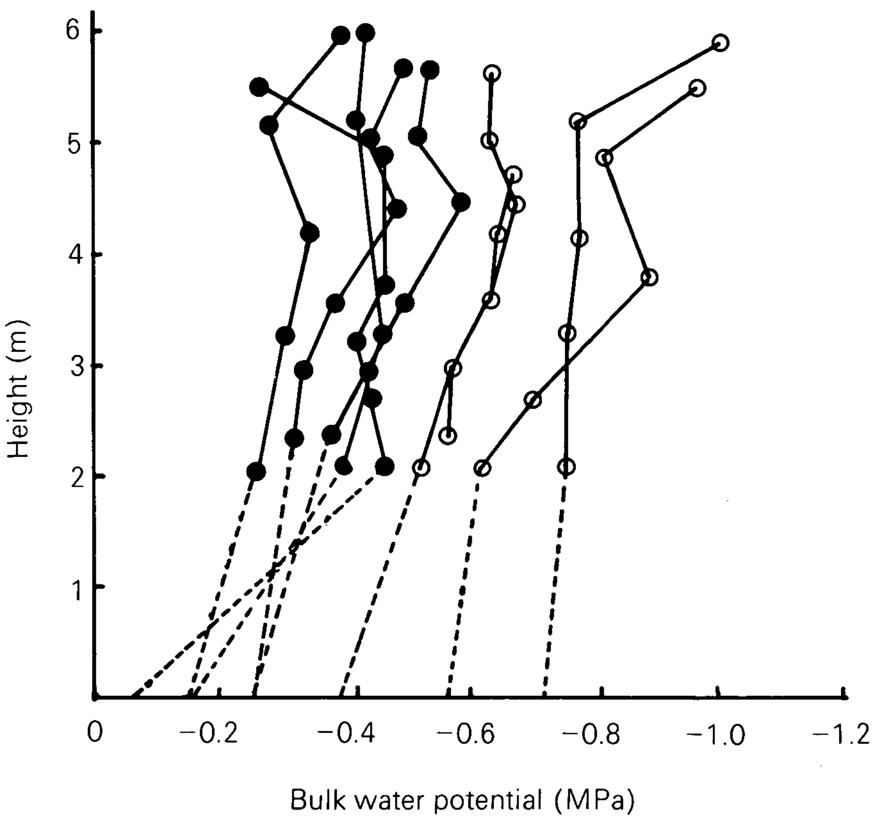


Figure 15..
Vertical profiles of xylem water potential of current year shoots of Norway spruce on 16 December 1985 (●), which was a cloudy day with mean air temperature 8°C and continuous rain, and on 3 January 1986 (○), which was a clear winter's day after a night frost with air temperature *c* 2°C

possibly also the gradients observed, suggesting that the profiles of xylem potential were related to the physiological activity of the tree. However, this approach required measurements to be made of 5 or 6 shoots from different heights up each tree, and it was clear that time would not be available for a thorough analysis of this kind during the 1986 survey.

A small pressure chamber for use with individual needles (Roberts & Fourt 1977) was therefore connected to conventional pressure chamber controls and a binocular microscope. The xylem potentials of individual needles were measured at 2 sites for Norway spruce (Lange Bramke in the Harz Mountains on the south- and north-facing slopes) and at 2 sites for Scots pine (Oberwarmensteinach in the Fichtelgebirge and Birchett Wood in SE England). Three needles of 2 or 3 year classes were removed from the same branches as were sampled for biochemical and surface characteristics. For some year classes, both green and chlorotic needles were sampled. Table 4 shows the means for each category of needle sampled at each site.

The data were highly variable, with large differences between trees, but with less variability between individual needles from the same

Table 4.

The mean xylem potentials (MPa) of Norway spruce and Scots pine needles sampled in the summer of 1986

i. Norway spruce

Site D3 (Trees 91–98, south-facing slope)				Site D4 (Trees 99–106, north-facing slope)			
Needle year class	n	$\bar{x}$	SD	Needle year class	n	$\bar{x}$	SD
0	8	−2.96	1.05	0	7	−2.46	0.63
2	4	−3.02	0.70	2*	8	−3.39	0.85
2*	4	−3.43	0.87				

ii. Scots pine sampled in the Fichtelgebirge and in SE England

Site D7 (Trees 123–128, Oberwarmensteinach)				Site E1 (Trees 234–242, Birchett Wood, Kent)			
Needle year class	n	$\bar{x}$	SD	Needle year class	n	$\bar{x}$	SD
0	6	−1.07	0.42	0	8	−0.79	0.56
1	6	−1.08	0.45	2	8	−1.02	0.67
2	6	−1.01	0.45	2*	4	−1.49	0.39

*Needles showing yellowing or flecking, see text for details branch. The needle xylem potentials were consistently more negative for Norway spruce as compared to Scots pine, and for Norway spruce the second year needles also showed larger negative values than the current year needles, a difference which was less apparent in Scots pine. At Lange Bramke, the 2 sites were selected because the southern aspect showed extensive needle yellowing, while the northern aspect showed greater needle loss and hence crown thinning. At site 1, half the second year needles sampled showed yellowing, while at site 2 all the second year needles were mottled, discoloured or had poor growth. In view of the known diurnal fluctuations of canopy water potential (Hellkvist *et al.* 1974; Jensen & Salisbury 1984), measurements were made on the southern slope between 1230 and 1630 hours on 2 July 86 and on the northern slope the following day between 1200 and 1500 hours. The most striking feature of these data is that, where yellow or otherwise discoloured needles were sampled, they showed large negative xylem potentials. There were, for example, large negative potentials recorded for tree no. 106 (−4.0 and −3.98 MPa for current and second year needles respectively) and the record sheet for this tree (scored independently) shows the comment 'needles dry and ready to fall off'. Where yellow or bleached needles were deliberately selected for analysis on the pines at Oberwarmensteinach and at Birchett Wood, large negative potentials were consistently shown by such needles. At Oberwarmensteinach, insect, fungal and mechanical injuries were not apparent, although needle yellowing and flecking were recorded, while at Birchett Wood insect or fungal damage was recorded, and white fungal mycelium was seen on the needles of some trees.

The correlation between severe yellowing, needle cast and large negative xylem potentials is perhaps not surprising and does not imply a causal relationship, but it does suggest that a closer examination of how needle water potential alters prior to needle loss could be of value. Needles from each branch sampled (for some trees both yellow and green needles) were preserved in FAA (formalin-aceto-alcohol) at the time the potentials were measured, and these needles will be sectioned to allow examination of the cell dimensions and inspection for mechanical injury to the cells. It would be anticipated that cell dimensions would differ for needles with differing water relations. It is clear that more detailed analysis of needle/water relations would be required, if this factor is to be developed as an early indicator of decline. Day-to-day and diurnal variations need to be allowed for, and a more thorough analysis of single trees would be required. The use of small chamber psychrometers would enable a number of needles to be incubated at one time (up to 20 depending on facilities), and could give the value of solute potentials as well as xylem potential so that needle turgor at the time of sampling could be calculated. This technique would be more accurate and repeatable for the needles of spruce, and would supply more information. The development of this approach using plant material after controlled pollutant exposure and the imposition of other environmental stress is now favoured.

4.3.2 Photosynthesis

The rate of net photosynthesis (A) was measured on individual beech leaves (3 per branch) from the same branches sampled in the biochemical tests. Measurements were made using a portable CO_2 infra-red gas analyser (ADC LCA2) and air supply unit connected to a 'Parkinson' leaf chamber suitable for broadleaves. Measurements were made at 3 sites: Witzenhausen (Solling area), trees 115–122; Selb, trees 145–152; and Oberwarmensteinach, trees 153–160 (the latter 2 sites both in the Fichtelgebirge).

At Witzenhausen, mature trees (127 years old) were sampled by tree climbing, and measurements of A were made within 20 min of cutting on leaves still attached to the branches. Values of A were highly variable (coefficient of variation of 78 % between trees) and trials indicated that A declined rapidly (within 5 min) after cutting the branches. As a result, subsamples of second order laterals were re-cut under water from the main samples taken at Selb and Oberwarmensteinach. Values of photosynthesis were then recorded for healthy (green), yellow and curled leaves on these subsamples with the re-cut stems in water. This method gave consistent and steady values of A, and the mean values for each category of leaf at each site are shown in Table 5.

Table 5.
The rates of net photosynthesis (μmol CO_2 m^{-2} s^{-1}), in beech leaves of different condition (green, yellow and curled) at 2 European sites with known pollution stress

Tree numbers and sites		Leaf descriptions: Green (healthy)	Yellow	Curled
Numbers 145–152,	n	6	5	2
sampled at Selb	$\bar{x}$	34.2	21.7	7.3
(Site D8)	SD	6.7	13.7	10.3
Numbers 153–160,	n	5	8	3
sampled at Oberwarmensteinach	$\bar{x}$	32.4	7.8	8.0
in the Fichtelgebirge	SD	16.3	8.3	9.3
(Site D7)				

The green leaves showed rates of photosynthesis in the range which would be expected for beech and, as anticipated, yellow leaves at both sites showed a decline in net CO_2 uptake. Leaves exhibiting the distinctive leaf curl associated with environmental stress in this species also showed a large decline in net photosynthesis. The appearance of such leaves suggests that analysis of xylem water potential could show that leaf water deficit was also occurring with the curl symptom. Analysis of A and of xylem potential during the development of injury in controlled exposure conditions might indicate the chronological sequence in which these 2 factors are influenced. It is interesting to note that more detailed and extensive analysis of the gas exchange of Norway spruce has been made at the Oberwarmensteinach site, and that there were no differences in A until needles showed yellowing (Zimmermann 1987). The extensive studies at this site have correlated the yellowing of spruce needles with loss of magnesium and, because other nutrients (N, P, K) usually become deficient before Mg on nutrient-poor sites or where root damage occurs, it has been suggested that the loss of Mg with accompanying yellowing is a shoot-mediated effect.

4.4 University of Freiburg – histology and histochemistry

Single twigs from each of the conifer samples were first placed for 24 h in the dark in order to allow dissolution and translocation of assimilation starch in the chloroplasts. Segments of each year's needles were then fixed, and embedded in Spurr's epoxy resin; semi-thin sections were prepared, pre-treated with tannic acid and ferric chloride, and finally stained with toluidine blue. By this method, cell walls, starch and tannins were visualized, and the sections were microscopically evaluated with regard to pathological accumulation of starch and cell necrosis.

According to the present results of these histological and histochemical evaluations of the needles from Norway spruce and Scots pine collected on the different sites in summer 1986, the following microscopical appearances can be distinguished (compare with Plate 1).

Type I: This is the normal appearance of a healthy needle with no damage. The mesophyll cells are intact without any starch accumulation. The vascular bundle shows no alterations, and the phloem consists of open, functional sieve cells. (In older needles, there is, of course, an increasing number of non-functional sieve cells.) This type was most prevalent in the samples from Scotland (S1, S2), though it

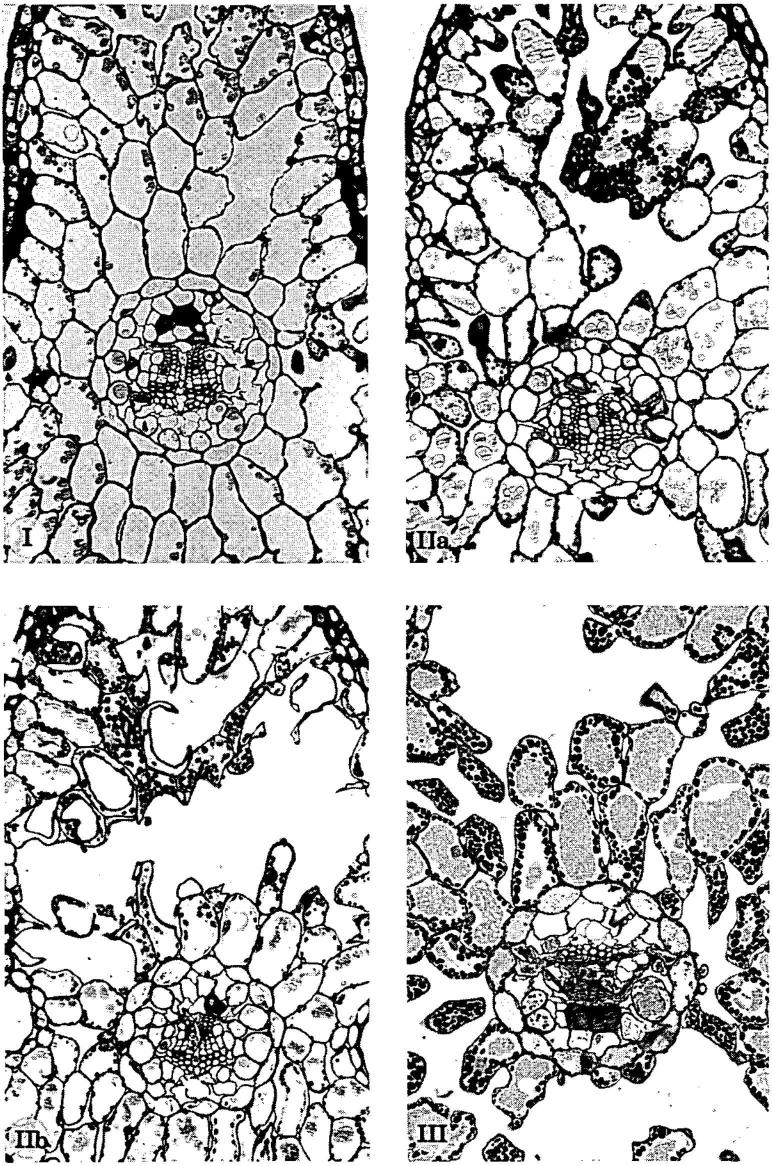


Plate 1.

Semi-thin stained sections of Norway spruce needles

Type I.	Normal, healthy
Type IIa.	Localized starch accumulation and mesophyll damage, but vascular bundle intact
Type IIb.	Advanced stage IIa, with some mesophyll cells totally collapsed
Type III.	Uniform starch accumulation in all mesophyll cells which remain intact. Collapse of phloem in vascular bundle with hypertrophy of adjoining cells

was also frequently found in some of the samples from the other sites which showed no external sign of damage (discoloration).

Type II: In this case, the vascular bundle is not affected either, but the mesophyll cells show various degrees of damage. The first signs of damage are localized accumulations of starch (black dots) in some mesophyll cells (IIa). This symptom demonstrates that assimilation is still functioning, but the enzymatic dissolution and the transport of the assimilation products are in some way prevented. Frequently, this localized starch accumulation appears to be only the first step in the damage process, as such mesophyll cells may become necrotic and collapse in more advanced stages (IIb). This type of damage was only found in some of the samples from England (E1, E2) and the Netherlands (N1, N2) and in very few needles from the Fichtelgebirge (D7, D8). According to our present knowledge, this pattern of mesophyll damage and intact vascular bundle is typical for the direct impact of gaseous pollutants.

Type III: Here, no necrosis of mesophyll cells can be found, but a specific necrosis and collapse of the sieve cells occur in the phloem of the vascular bundle. Cambial cells and adjacent parenchyma cells, on the other hand, show hypertrophy and hyperplasy. The mesophyll exhibits a general and uniform accumulation of starch. This symptom was always found in combination with yellowing symptoms of the older needles, though it also occurred in needles which were still green. It was most pronounced on the 6 German sites (D1–8). With our present knowledge, we can attribute these pathological changes to disturbances of mineral nutrition (especially lack of magnesium) rather than to a direct impact of gaseous air pollutants. The phloem necrosis blocks the translocation of assimilates from the needles to the stem, and thus induces a pathological starch accumulation in the chloroplasts of the needles. This starch accumulation also occurs in needles which are still green, but may be on the edge of turning to yellow.

Because different forms of pathological starch accumulation occur in damaged needles of type II as well as of type III, monitoring of the starch accumulation could perhaps be a criterion for early diagnosis of damage. Accumulated starch was found in needles from the 6 sites in Germany, to a lesser extent in the needles from the Netherlands and southern England, and virtually not at all in the Scottish samples.

Besides these general types of microscopical appearance, 2 special phenomena were noted in some of the sections.

The 'flecking'-type of damage appeared, especially, in needles from the higher elevations, and less in those from lower elevations. Originally thought to be caused by ozone, there is now considerable doubt. Histologically, these flecks differed from those occurring in ozone-fumigated needles: whereas the latter are always related to stomata and consist of totally collapsed cells, the former occur mainly along the edges of the upper side of the needle; the cells are dead, though not collapsed, and thus appear 'freeze-dried'. The dead cells are in most cases packed with starch. With such observations and knowledge from the literature, it appears most probable that at least some of these flecks are climatically caused ('winter fleck injury') and not necessarily related to the impact of atmospheric pollution.

Somewhat outstanding was the material from the Netherlands, which was characterized by signs of internal late-frost damage, relatively large cells and thin walls, and different staining patterns. These observations so far sustain the theory of an increased impact of atmospheric N compounds. Similar phenomena were not observed in the other material, however.

In summary, there is some evidence of a direct impact of atmospheric pollutants on needles in England and the Netherlands and in some samples from the Fichtelgebirge. Most other damage, especially from the 6 German sites, appears to be much more clearly related to imbalances of mineral nutrition, though increased input of nitrogen seems to be an additional factor, especially in the Netherlands.

4.5 Statistical analysis

In order to investigate the relationships between sites using the data obtained in the pilot survey, statistical analyses were performed using the SAS computer package (SAS Institute Inc. 1985). For illustration, the results below refer to 2-year-old Norway spruce.

Initially, a simple average linkage cluster analysis was performed using mean values for each site of all the variables which had been measured at all sites. The average distance tree (in normalized units) is shown in Figure 16. It is immediately obvious that the pairs of sites geographically adjacent (eg D1 and D2, D3 and D4) are well discriminated, and there appears to be a separation along a line from south-east to north-west. The only anomaly is the apparent close relationship between site D8 (on the Czech border) and the 2 English sites. This relationship may be caused by the high calcium content of the needles at all these sites (see Figure 8).

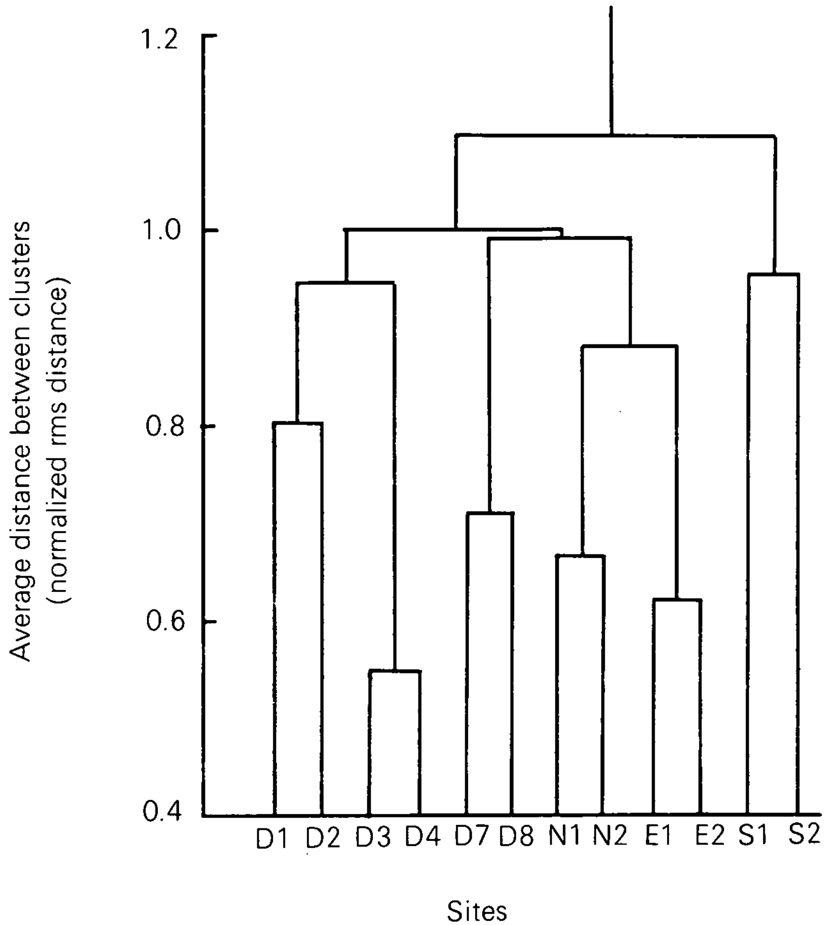


Figure 16.
Average linkage cluster analysis using 15 significant (P =0.05) variables from discriminant analysis

Table 6.
Discriminant analysis for 2-year-old Norway spruce. Variables making significant (5 %) contribution

Variable	Partial r^2	F-static	Probability$\geq$ F
Calcium	0.79	20.9	0.0001
Sulphur	0.75	17.1	0.0001
Nitrogen	0.73	15.3	0.0001
Tissue pH	0.65	10.0	0.0001
Contact angle	0.62	8.8	0.0001
Magnesium	0.60	7.9	0.0001
Dust as % dry weight	0.55	6.5	0.0001
Violaxanthin/antheraxanthin	0.51	5.2	0.0001
Chlorophylls/xanthophylls	0.46	4.2	0.0002
Chlorophyll a/b	0.62	8.0	0.0001
Potassium	0.43	3.6	0.0007
Violaxanthin/lutein	0.41	3.3	0.0019
α-tocopherol	0.38	2.8	0.0062
Dry weight as % fresh weight	0.37	2.6	0.0094

Which variables are responsible for this clustering? The raw data (ie measurements for each tree) were used in a stepwise discriminant analysis to discover which made a significant contribution to the observed clustering. A variable was only included if its contribution was significant at the 5 % level. The rankings are given in Table 6. The importance of Ca explains the linkage of site D8 with the English sites. It is interesting that nutrient content alone is a good site discriminant, with only tissue pH and contact angle giving a partial r^2 greater than 0.6.

Table 7.
Site means for the first 3 canonical variables using the 14 discriminant variables in Table 6 for 2-year-old needles of Norway spruce

Canonical variable	1		2		3	
	S1	6.2	D8	4.1	N2	4.4
	S2	4.2	D7	4.0	N1	2.5
	E2	3.7	E1	1.1	S1	1.6
	E1	2.8	S1	1.0	D1	1.4
	D8	0.7	D3	−0.2	D8	1.2
	N1	−0.9	D2	−0.5	E2	0.4
	N2	−1.3	D4	−0.8	D7	−0.2
	D3	−2.3	N1	−1.1	E1	−0.9
	D2	−2.6	N2	−1.5	D4	−1.9
	D1	−3.0	E2	−1.5	D2	−1.9
	D4	−3.2	S2	−2.1	D3	−2.1
	D7	−3.4	D1	−4.0	S2	−2.9

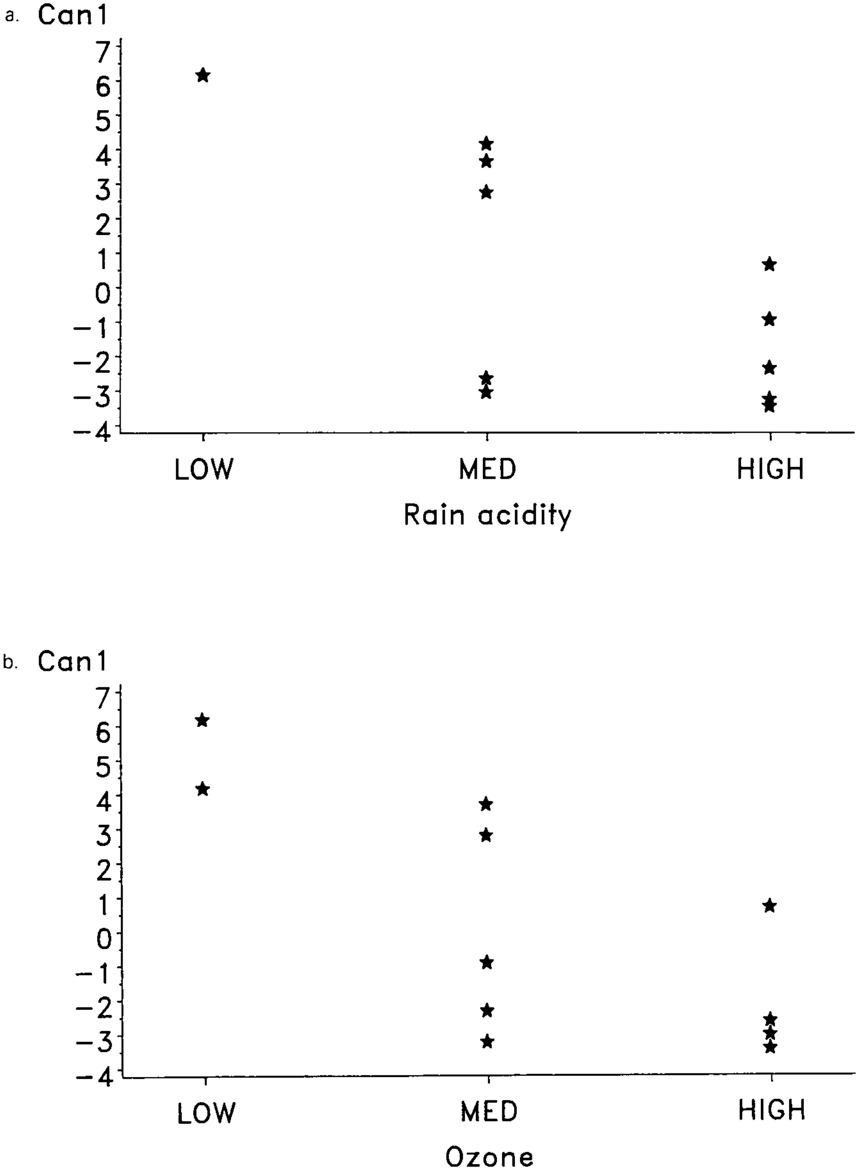


Figure 17.

a. Variation of first canonical variate as a function of rainfall acidity at each site

b. Variation of first canonical variate as a function of ozone exposure at each site

These 14 variables were then used in a canonical discriminant analysis, and the site means for the first 3 canonical variables are given in Table 7, with the relative site rankings. In terms of the pollution climates of the sites (Table 1), the first canonical variable correlates significantly only with mean rainfall pH and ozone, while the second canonical variable correlates weakly ($P = 0.06$) with deposited acidity, and the third ($P = 0.07$) with primary pollutant gases (Figure 17). The ranking of the sites by the first canonical variable is very similar to the clustering produced using the average linkage method (Figure 16).

Although these statistical analyses are preliminary, they indicate the type of correlations which may be extracted from the measured data.

5 DISCUSSION

The difficulties in interpreting the results of this study have been described already (Section 4), but in terms of the objectives there has been a measure of success. In 8 of the tests employed, significant differences between sites were found for at least one tree species. These differences also 'grouped' the sites geographically, with the UK sites at one end of the distribution and continental sites (especially those showing visible damage) at the other end. The nature of some of the tests, particularly those measuring biochemical information, also indicates potential causes of the changes in terms of known reactions to air pollution.

The usefulness of such tests depends to a large extent on their sensitivity to sampling date, weather and time of day, which is likely to be more important for the biochemical tests. Differences between sites in leaf surface properties, while not so greatly influenced by short-term variations, are in general smaller. Both the physiological and histological studies provide useful information on probable mechanisms, but may be difficult to quantify. However, the observation of starch accumulation at West German sites suggests the further development of chemical methods to quantify this accumulation.

To summarize, the following tests are worthy of further consideration:

i. Amounts of surface wax
   Large site differences for Norway spruce (but not pine) with a consistent behaviour over 3 year classes, all UK sites having smaller amounts than continental sites.

ii. Contact angles
Large site differences, especially for older spruce needles, with British and Dutch sites showing consistently smaller values than German sites. Absolute values for Scots pine were less useful, but year-to-year changes showed a marked geographical pattern.

iii. Härtel turbidity test
Large site differences for spruce, with the largest values in Britain and smallest in the Netherlands. The response of this test appears to depend upon factors other than air pollution.

iv. Modulated fluorescence
Large differences for beech, with high excitation maxima and slower quenching at UK sites.

v. Hydrocarbon emissions
Large site differences for spruce and beech, with low emissions of ethylene (and ethane) at Scottish sites. Emissions for pine appear to be related more to tree age.

vi. Buffer capacity
Large site differences in leaf pH, with largest values at UK sites. Current year needles show a large buffering capacity in pH range 7–10 at British sites.

vii. Pigments
Large site differences in ratios of pigments, with low violaxanthin/antheraxanthin ratios in spruce, and low violaxanthin/lutein ratios in pine in the UK. Total chlorophyll/carotenoid ratios were higher in spruce from sites showing visible damage, and unusual carotenoids were also identified at these sites.

viii. Alpha-tocopherol
Large site differences, especially in older spruce needles, with smallest values at Darnaway Forest (S2).

One of the major benefits of this year's study was the close collaboration in the field among the participating institutions, which meant that a very wide range of techniques could be applied to the same plant material. The correlations and interactions observed have still to be analysed in detail, but a much more complete picture has been obtained than by the application of individual tests in isolation.

In order for any of these techniques (or any other) to be of value as diagnostic tests, they must be studied in greater detail under controlled conditions. The criteria for successful development are:

i. small variation between genotypes,
ii. small (or controllable) variation with conditions (eg weather),
iii. small (or predictable) variation with leaf/needle age,
iv. small (or predictable) variation with tree age,
v. specific response to individual pollutants.

In addition, the influence of other stresses (eg frost, drought or nutrient deficiency) should be recognized, although they may interact with pollutants.

Plans are already well advanced to grow young trees and rooted older shoots in controlled environments for use in further development. Ultimately, the refined tests will need to be examined by extensive surveys in the field.

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7 REFERENCES

Arndt, U. 1971. Konzentrationsänderungen bei Blattfarbstoffen unter dem Einfluss von Luftverunreinigungen, Ein Diskussions-beitrag zur Pigmentanalyse. *Environ. Pollut.*, **2**, 37–48.

Börtitz, S. 1964. Physiologische und biochemische Beiträge zur Rauchschaden-Forschung. 1. *Biol. Zbl.*, **83**, 501–513.

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

Darrall, N. M. & **Jäger, H. J.** 1984. Biochemical diagnostic tests for the effect of air pollution on plants. In: *Gaseous air pollutants and plant metabolism*, edited by M. J. Koziol & F. R. Whatley, 333–349. London: Butterworths.

Fuchshofer, H. & **Härtel, O.** 1985. Zur Physiologie des Trübungstests, einer Methode zur Bioindikation von Abgaswirkungen auf Koniferen. *Phyton, Horn*, **25**, 277–291.

Grill, D. 1971. Pufferkapazität gesunder und rauchgeschädigter Fichtennadeln. *Pflanzenkrankheiten*, **78**, 612–622.

Härtel, O. 1953. Eine neue Methode zur Erkennung von Raucheinwirkungen an Fichten (Trübungstest). *Zentbl. ges. Forst- u. Holzw.*, **72**, 12–21.

Hellkvist, J., Richards, G. P. & **Jarvis, P. G.** 1974. Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure bomb. *J. appl. Ecol.*, **11**, 637–667.

Jensen, W. A. & **Salisbury, F. B.** 1984. *Botany*. 3rd ed. Wokingham: Wadsworth.

Katz, M. & **Shore, V. C.** 1955. Air pollution damage to vegetation. *Air Pollut. Control Ass.*, **5**, 144–150.

Kimmerer, T. W. & **Kozlowski, T. T.** 1982. Ethylene, ethane, acetaldehyde and ethanol produced by plants under stress. *Pl. Physiol., Lancaster*, **69**, 840–847.

Krinsky, N. I. 1966. The role of carotenoid pigments as protective agents against photosensitized oxidations in chloroplasts. In: *Biochemistry of chlorophasts*, Vol. 1, edited by T. W. Goodwin, 423–430. London; New York: Academic Press.

Mehlhorn, H., **Seufert, G.**, **Schmidt, A.** & **Kunert, J.** 1986. Effects of SO_2 and O_3 on production of antioxidants in conifers. *Plant Physiol.*, **82**, 336–338.

Müller, J. 1957. Spezifischer Nachweis von SO_2-Rauchschäden an Pflanzen mit Hilfe von Blattpigmentanalyse. *Naturwissenschaften*, **44**, 453.

Ögren, E. & **Baker, N. R.** 1985. Evaluation of a technique for the measurement of chlorophyll fluorescence from leaves exposed to continuous white light. *Plant Cell Environ.*, **8**, 539–547.

Öquist, G. & **Ögren, E.** 1985. Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics. *Photosynthesis Research*, **7**, 19–30.

Roberts, J. & **Fourt, P. F.** 1977. A small pressure chamber for use with plant leaves of small size. *Pl. Soil*, **48**, 545–546.

SAS Institute Inc. 1985. *SAS users' guide, version 5*. Cary, NC: SAS Institute Inc.

Scholtz, F. & **Reck, S.** 1977. Effects of acids on forest trees as measured by titration *in vivo*. Inheritance of buffering capacity in *Picea abies*. *Water Air Soil Pollut.*, **8**, 41–45.

Smillie, R. M. & **Nott, R.** 1982. Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo*. *Plant Physiol.*, **70**, 1049–1054.

Yamomoto, H. Y., **Nakayama, T. O. M.** & **Chichester, C. O.** 1962. Studies on the light and dark interconversions of leaf xanthophylls. *Archs Biochem. Biophys.*, **97**, 168–173.

Zhou Rong-Ren, Li Zhen-Guo, Yang Xie-Rong, Yu Zi-Wen & **Yu Shu-Wen.** 1982. Evolution of propylene and propane from tobacco leaves exposed to HSO_3 – or SO_2. *Acta Phytophysiol. Sin.*, **8**, 413–416.

Zimmermann, R. 1987. Transpiration and photosynthesis – a comparison of two sites with different proton inputs in North Bavaria. In: *Direct effects of dry and wet deposition on forest ecosystems*. Brussels: Commission of the European Communities.

APPENDIX

Sheet 1

Forest Decline Survey (1006)

Site data Date ______

Site ______________ Sample Nos. [ ]

Precise location					
Elevation		Aspect		Slope	
Geology					
Soil type					

Soil characteristics

Humus type						
Depth						
Water regime (drainage)						

Annual means	sunshine	temperature	rainfall	windspeed		
	SO_2	NO_x	O_3	NH_4		
Gas concentrations						
Liability to cloud/mist						

Stand data

Age class	
Yield class	
History	
Recent damage from identified causes	
Damage classification (from previous nat mon. prog.)	
Stand structure ground vegetation	

Other comments

Sheet 2

Tree data

Site | Date .86

Species	Norway Spr./Scots pine/Beech			Sample No.			
Height	M.	Diameter mm		Sample Point	Top crown Mid crown Lower crown		N side S " E " W "
Situation in stand	Edge Near Edge Centre						
Dominance in stand	not dominant	equal	just dominant	clearly dominant			
Crown development	Good Moderate Poor		Dense Bushy Thin/Spindly		Vigorous One sided Subcrown shade		
Crown form (shape)							
Branching type	plate	bush	comb			normal	
Fructification							
	0-10%	>10-25%	>25-60%	>60%		0-100%	
Discolouration							
Defoliation							
Damage							

Needle data

	Current	1 yr	2 yr	3 yr	4 yr
Needle/leaf size					
Needle/leaf necrosis					
Needle/leaf loss					

Insect/fungus damage	
Presence or absence of lichens/fungi	
Mechanical injuries	

Any other comments

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