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Early Effects of Acid Mist on Sitka Spruce Planted on Acid Peat

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With 1 Figure

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Summary

SHEPPARD L., CROSSLEY A., CAPE J. N., HARVEY F., PARRINGTON J. & WHITE C. 1999. Early effects of acid mist on sitka spruce planted on acid peat. – *Phyton* (Horn, Austria) 39 (1): 1–25, 1 figure. – English with German summary

Ten year old Sitka spruce [*Picea sitchensis* (BONG.) CARR.], growing in a commercial plantation, on a drained, acid peat were sprayed with simulated mist providing a 'no spray', N alone, S alone and a combination of N, S and acidity at pH 5

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and 2.5, (single and double dose) treatments. Approximately twice weekly from May (budburst) to December, six treatments (equivalent to 2 mm precipitation) Na_2SO_4 , NH_4NO_3 , $\text{H}_2\text{SO}_4 + \text{NH}_4\text{NO}_3$ at pH 2.5 (single and double dose), and pH 5 and an unsprayed control were applied, to four replicate chambers of 10 trees. The treatment 'chambers' comprised a framework of poles, supporting 2 full cone sprayers per tree mounted within 0.5 m of the tops of the trees, without wall structures. This paper reports some sensitive indicators of treatment effects, stemwood growth, foliar chemistry and frost hardness, together with measurements of soil chemistry after one and two treatment seasons.

In the first year of treatment no significant effects on growth were found although stem basal area increment increased by approximately 50%. After one year of treatment there was a tendency for shoots receiving N/acidity to be least frost hardy when tested in December (by approximately 4 °C). Foliar S, N and Mg concentrations were significantly increased in response to S and N additions. In the second year, no biologically significant effects were found. Two years of treatment with N and S, both with and without acidity, still failed to affect growth measured as relative stem basal area increment. However, relative increments in stem volume indicated a significant positive response to the acidified S + N treatments after two treatment seasons. Initial findings are discussed in relation to results from similar studies in controlled environments and the field. Early indications are that the soil nutrient supply is dominating the tree response to N, S and acid treatment. and that acidified S and N inputs are not damaging the trees but have stimulated growth over the first 2 years of treatment.

Zusammenfassung

SHEPPARD L., CROSSLEY A., CAPE J. N., HARVEY F., PARRINGTON J. & WHITE C. 1999. Frühwirkungen von saurem Nebel auf in saurem Torf gepflanzte Sitkafichten. – *Phyton* (Horn, Austria) 39 (1): 1–25, mit 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Zehn Jahre alte Sitkafichten [*Picea sitchensis* (BONG.) CARR.], welche in einer Anpflanzung auf entwässertem sauren Torf wachsen, wurden mit simuliertem Nebel besprüht, wobei Behandlungen „nicht gesprüht“, nur N, S alleine und eine Kombination von N und S sowie Säurebehandlung bei pH 5 und 2,5 (in einfachen und doppelten Dosen) angewandt wurden. Ungefähr 2mal wöchentlich ab Mai (Austrieb) bis Dezember wurden 6 Behandlungen (entsprechend 2 mm Niederschlag) in Kammern mit 10 Bäumen und 4 Wiederholungen durchgeführt: Na_2SO_4 , NH_4NO_3 , $\text{H}_2\text{SO}_4 + \text{NH}_4\text{NO}_3$ bei pH 2,5 (einfache und doppelte Menge) und bei pH5 sowie eine unbesprühte Kontrolle. Die Anordnung „Kammern“ bestand aus einem Gestell aus Stangen, auf die 2 Kegelsprüher pro Baum innerhalb 0,5 m in der Krone der Bäume montiert waren. In dieser Arbeit wird über einige empfindliche Indikatoren zu Behandlungseffekten berichtet, wie Holzzuwachs, Blattchemie und Frosthärte, aber auch Messungen der Bodenchemie nach 1 und 2 Untersuchungsperioden.

Im ersten Untersuchungsjahr konnten keine signifikanten Einflüsse auf das Wachstum beobachtet werden, obwohl der basale Stammflächenzuwachs um rund 50 % zunahm. Nach einem Jahr Behandlung zeigte sich eine Tendenz bei den Sprossen, welche N und Säure erhielten, daß sie weniger frosthart waren, als sie im Dezember bei ungefähr 4 °C getestet wurden. S, N, und Mg-Konzentrationen in den Blättern waren als Antwort auf S- und N-Gaben signifikant erhöht. Im zweiten Jahr

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konnten keinerlei biologisch signifikante Reaktionen gefunden werden. Zwei Jahre Behandlung mit N und S, beide mit und ohne Säure, hatten nach wie vor keinen Einfluß auf das als relative Stammflächenzunahme gemessene Wachstum. Die relative Zunahme im Stammvolumen jedoch wies auf eine signifikante positive Antwort auf die sauren S- und N-Behandlungen nach zwei Behandlungssaisonen hin. Die ersten Befunde werden im Zusammenhang mit Ergebnissen von ähnlichen Studien unter kontrollierten Bedingungen und dem Freiland diskutiert. Frühe Anzeichen sind, daß die Bodennährstoffversorgung die Reaktion der Bäume auf N, S und saure Behandlungen dominiert und daß saure S- und N-Einträge die Bäume nicht schädigen; hingegen haben sie das Wachstum während der ersten zwei Jahre gefördert.

Introduction

Effects of acid rain and mist on spruce trees can be investigated in several different ways: (1) In situ, at sites where deposition levels are monitored, short and long term changes can be assessed; (2) on-site manipulations where effects of known additions (e.g. RASMUSSEN & al. 1995, WRIGHT & RASMUSSEN 1998), or removal of pollutants e.g. using a roof can be followed, (e.g. GUNDERSON & al. 1998a); (3) via simulation where artificial mist is applied direct to tree canopies allowing excess liquid to wash down to the soil/roots. Simulation experiments can be undertaken in well defined conditions, e.g. glasshouses, open-top chambers (OTCs) or in the field, an environment fashioned by the weather and soil physical and chemical properties. We have used simulation experiments to study effects of acid mist on spruce since 1986. Initially experiments involved potted spruce seedlings treated in OTCs but they have since included clones and mature grafts, using both artificial composts and natural soils (SHEPPARD 1994, SHEPPARD & al. 1994, 1995a). These experiments, undertaken in controlled conditions, indicated that acid mist containing the 4 major pollutant ions (H^+ , NH_4^+ , SO_4^{2-} , NO_3^-), applied at realistic concentrations, approximating to the annual N and S dose measured in the southern Scottish uplands, (CROSSLEY pers comm) could significantly influence the vitality of Sitka spruce.

Evidence from these OTC studies with red spruce (*Picea rubens* Sarg) (JACOBSON & al. 1990, 1992) and Norway spruce (*P. abies* (L.) Karst) (SHEPPARD & al. 1998a, CAPE & al. 1991) indicated that SO_4^{2-} and H^+ were the main toxic ions, particularly with respect to observed reductions in frost hardiness. Significant effects, either positive or negative, on growth were rarely detected although visible damage was often observed. Foliar S concentrations were doubled following treatment at high frequency with an equimolar solution of $NH_4NO_3 + H_2SO_4$ (1.6 mol m^{-3}). Mature grafts were similarly affected with respect to the level of visible damage (LEITH & al. 1995) but changes in frost hardiness and nutrient concentrations were relatively smaller, implying that bigger trees could tolerate a larger dose (SHEPPARD & al. 1994). The use of natural soils, albeit in an artificial en-

vironment, indicated the potential for soil chemistry to influence the responses of seedlings to acid mist (SHEPPARD & al. 1995a). The growth of seedlings in base rich soil (pH 5.6) was not significantly affected by treatment with acid mist containing equimolar proportions of N and S, whereas on acid mineral and organic soils (pH 4.0) growth was stimulated.

Subsequently, field studies have been undertaken in an attempt to understand to what extent the environment can modify the response of spruce to acidified additions of N and S and in addition, which results seen in controlled environments can be extrapolated to the field. Treatment of a single clone of physiologically mature Sitka spruce (*Picea sitchensis* Bong. Carr.) with N and S at pH 2.5 caused a rapid and persistent reduction in relative stem area increment over the 3 years of treatment (CROSSLEY & al. 1997). No visible damage was observed, however, and sustained significant changes in foliar nutrition and frost hardiness were not found (SHEPPARD al. 1998b). Treatment of 2-year-old seedlings growing nearby failed to cause any effects on growth, frost hardiness or foliar nutrition (SHEPPARD al. 1998c). However, the implications of these earlier studies for determining if and how anthropogenic N and S are likely to affect productivity of Sitka spruce plantations in Britain were compromised by the use of a single genotype and base-rich soil, when Sitka is commonly planted on acid organic soils. A second field experiment, incorporating the original acid N + S treatments together with individual S and N treatments without acidity, was therefore undertaken on a more typical Sitka spruce site. Pre-treatment growth was assessed, replication was increased and spray infrastructure was minimized in an effort to restrict the influence of experimental artefacts (SHEPPARD & al. 1998b). This paper reports some initial findings from the first 2 years of treatment which are contrasted with those from the previous field study.

The specific objectives of this field study were to determine:

1. relative effects of N, S and acidity on the growth, frost hardiness and nutrition of plantation Sitka spruce growing on an acid soil;
2. conditions influencing the growth responses of Sitka spruce to N and S with or without acidity.

Materials and Methods

Site

The experiment covers 1.5 ha within a privately owned and managed forest in south-central Scotland at Deepsyke: 290 m asl, latitude 55°46'N, longitude 3°18'W. Prior to planting with a mixture of provenances (identities unknown) in 1986, the site, on a basin peat, was drained by double moleboard ploughing to create a series of 1 m wide ditches and 3 m wide mounds. The trees (mean height April 1995, 3.8 m), were planted at approximately 2 m spacing at either side of the mound into the raised 'sandwich' created by the inversion of the organic horizon from the ditch

on top of the mound. An initial survey of the site based on 3 pits dug along a south-east to north-west transect indicated increasing acidity and peat depth towards the NW of the site. The site is very acid. The pH of the upper peat horizons ranged from 2.5 to 3.0 (Ca Cl₂) with the mineral horizon around pH 3. Aluminium contents were also quite high for peat, ~100 µeq/kg soil (mineral) and 30 µeq/kg (organic). When treatment commenced basal branches had begun to interlock between the trees although, for the most part, the ground vegetation in the middle of the mound is not shaded and a healthy community of moorland species such as *Carex* spp. and *Deschampsia flexuosa*, *Rhytidiadelphus squarrosus*, *Polytrichum commune*, together with *Molinia caerulea* and *Agrostis capillaris* dominate as a thick mat. The decaying organic litter fraction extends up to 25 cm deep in places with up to 0.5 m depth of peat.

Treatments

The experiment comprises 6 treatments (applied approximately twice weekly, each application supplying 2 mm precipitation equivalent), (Table 1). Acid mist at pH 2.5 made from NH₄NO₃ and H₂SO₄ provides an accumulated annual dose of 50 kg S and 48 kg N ha⁻¹ (2 × 2 mm wk⁻¹) for direct comparison with previous experiments on a mineral soil (CROSSLEY & al. 1997). A double dose of the pH 2.5 treatment (4 × 2 mm wk⁻¹) was included to estimate the relationship between dose and tree response. The control treatment provides acid mist at pH 5 (2 × 2 mm wk⁻¹). To test for the effect of

Table 1

Treatments applied in rain water*, annually to 10-year-old Sitka spruce growing on a drained, acid peat at Deepsyke forest (S. Scotland).

Treatment	Description	Ion concentration mol m ⁻³	Dose** kg ha ⁻¹		
			S	N	H
No treatment	spray gantry in place, no spray		—	—	—
N	NH ₄ NO ₃	1.6	—	48	—
S	Na ₂ SO ₄	1.6	50	—	—
Control	NH ₄ NO ₃ + H ₂ SO ₄	0.1	<1	<1	<1
NS Acid * 1	"	1.6 (3.2)*	50	48	1
NS Acid * 2	"	1.6 (3.2)	100	96	3

* The mean pH of the rain water is about 4.5. The pH of the acidified solution is approximately pH 2.5.

(3.2)*. is the H ion concentration

** Actual inputs to the treatments exceed those given above because of background wet and dry deposition = ~ 6.7 kg S ha⁻¹ a⁻¹ and 8 kg N ha⁻¹ a⁻¹. N is deposited fairly evenly between wet and dry deposition (52% and 48% respectively). By contrast 1.7 times more S is deposited as wet rather than dry deposition. The use of rain water to disseminate the treatments will enhance the inputs by a further 10 or 20% of the wet deposited background input (FOWLER & al. 1997).

additional water in the mist treatments, a treatment with the spray booms in place but not dispersing spray was included. Effects of N and S additions individually, in the absence of acidity, were evaluated by applying N as NH_4NO_3 and S as Na_2SO_4 (Fig. 1). Each treatment was applied to 4 replicate blocks each containing 10 trees to increase the likelihood of detecting effects.

Because of the large variation in tree height and form, over 4000 trees were measured prior to identifying groups of 10 trees (plots) which had single unbroken leaders, were not excessively small and were at least 4 lines of trees removed from other plots. Once the site had been mapped and 24 plots defined, the site was divided geographically into 4 quadrants each containing 6 plots which were then assigned randomly to the 6 treatments. The layout of the plots is shown in Figure 1.

In 1995 (March) a pond was dug at the site (capacity 125,000 dm^3) and lined with polyethylene sheet to collect direct rainfall. Black woven sheeting was used to cover the pond to reduce evaporation and deter algal growth. Water from the pond was pumped through a 10 μm filter (to remove particulate matter) into a 6000 dm^3 header tank. This fed water into 5 (500 dm^3) treatment tanks in a cabin. The treatment solutions were made up immediately prior to spraying by adding aliquots of the concentrated treatment solution to the tanks of rainwater. Actual N, S and H deposition in rain are monitored monthly at a site 2 km to the north-east, together with meteorological data every 20 minutes. Annual atmospheric N, S and H (wet + dry deposition) inputs to the adjacent moorland were approximately 8.1, 6.7 and 1.0 $\text{kg ha}^{-1} \text{a}^{-1}$ respectively (FOWLER & al. 1996).

Over the winter of 1995 scaffolding to support the sprayers was assembled. This consisted of a framework of galvanized steel poles (13 m $\times$ 5 m) supporting 24 full cone sprayer units. Trees were sprayed from both sides at a discharge pressure of 1.5 bar. This resulted in droplets in the order 100–250 μm diameter, equivalent to a fine drizzle or 'Scotch mist'. In this experiment, the hardware in the forest was kept to a minimum and spray drift problems were reduced by a border of at least 4 guard rows of trees not included in the experiment. In 1996 spraying commenced with bud burst in May (31 May), and the final spray was applied at the end of January 1997 after frost testing. In 1997 spraying started a week or so before all buds had flushed (30 April). The final doses were achieved by the middle of November (19 November). The pattern and frequency of spraying was determined by local conditions, being mainly determined by windspeed. Spraying was not undertaken when it was hot and sunny or raining. The double dose in which the mist is applied twice as frequently was occasionally sprayed twice on one day, usually in the evening while the whole site tended to be sprayed about 8.00 am when it was calm.

Measurements

Girth

Because the trees were young, and in order to provide comparable data to those from the previous field experiment, regular girth measurements were taken near the stem base, between whorls 8 and 9 from the leader. Bark roughness was removed by abrasion and 100 mm wide plastic film wrapped 3 times round the trunk (CROSSLEY & al. 1997). In 1995, the pre-treatment season, 5 measurements were taken using a diameter tape. In 1996, measurements were made every 2 weeks between April and

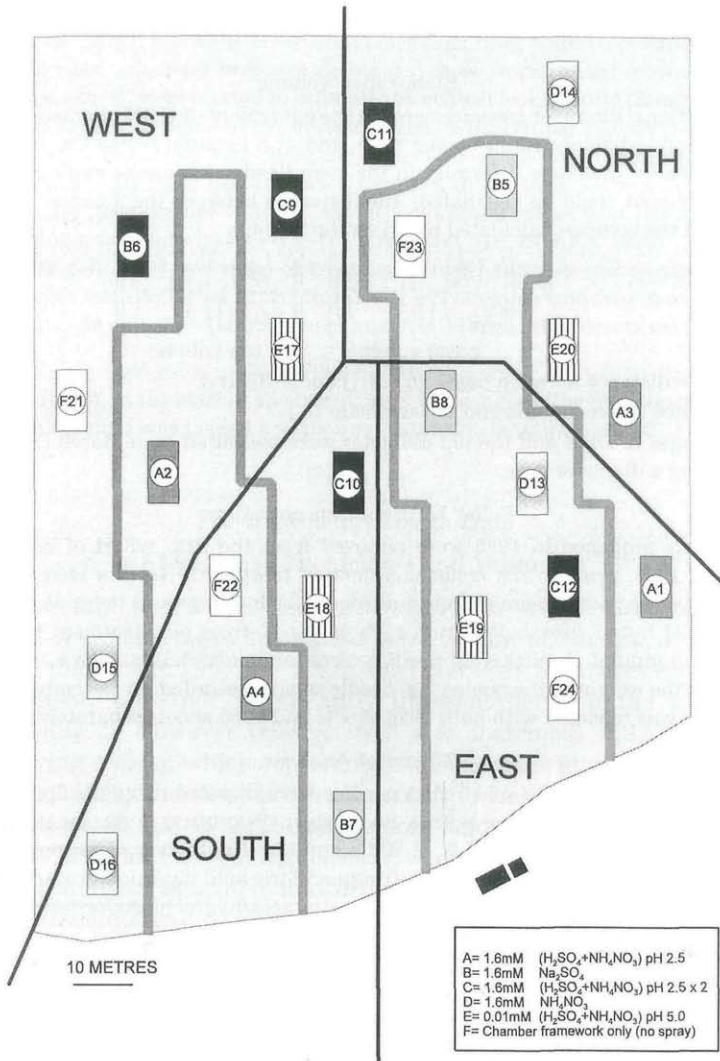


Fig. 1. Layout of the six experimental treatments (A–F) used to examine the effects of N, S and acid inputs on the vitality of Sitka spruce. The site is divided into 4 blocks (see solid black lines) designated south, west, north and east. The hatched continuous lines represent brashed walkways leading into the experimental area and out again. Each treatment chamber is represented once per block as 10 trees arranged in 2 parallel lines of 5 trees, surrounded by a scaffolding framework supporting the spray facility. Treatment E represents the control, a dilute salt solution at the pH of the rain water (~ 4.8), treatment F with no spray will be used to evaluate the impact of additional rainwater. Treatments B and D enable effects of S and N in the absence of acidity to be assessed and A and C provide acidified (pH 2.5) N and S at a single (*) and double dose (*2).

October. Subsequently, from March 1997, Vernier dendrometer bands were fitted, and read weekly.

Stem-taper, Volume

Additional diameter measurements at the equivalent of DBH (diameter at breast height), approximately 1.3 m up the stem, and at a position below the 1995 leader and any leader breakage deformity in the stem (leader loss) were made so that volume increment could be calculated. The distances between the 3 bands were measured and the volumes calculated based on the formula

$$V = \left\{ \left(\frac{\pi h_1}{3(r_1 - r_2)} \right) * (r_1^3 - r_2^3) \right\} + \left\{ \left(\frac{\pi h_2}{3(r_2 - r_3)} \right) * (r_2^3 - r_3^3) \right\}$$

basal volume top volume

where h_1 = distance between basal girth (r_1) and DBH (r_2)

h_2 = distance between DBH and top diameter (r_3)

Changes in DBH and the top diameter were measured each March (1996, 1997, 1998) using a diameter tape.

Foliar Nutrient Concentrations

Shoots produced in 1995 were removed from the fifth whorl of each tree in February 1996, prior to the commencement of treatment. Needles were separated from the twig by immersion in liquid nitrogen. Both foliage and twigs were dried at 70 °C for 24 h and subsamples from each of the 10 trees per treatment block were pooled and ground. A further 10 needles were randomly chosen from each tree, re-dried and the weight of the pooled 100 needle sample recorded. In December 1996 the procedure was repeated with both 1996 shoots and 1995 shoots separately.

Chemical Analysis

Oven dried, ground (<0.10 mm) needles were digested using a sulphuric acid/hydrogen peroxide procedure with a Se catalyst (PARKINSON & ALLEN 1975, ALLEN 1989) for measurements of total N, P, K, Ca and Mg. Total S was measured following digestion of oven dried needles in a nitric/perchloric acid digestion procedure (MAFF 1986). Inorganic S was measured in an acetic acid/hydrochloric/ortho-phosphoric acid extract.

K, Ca, Mg, S and inorganic S were analysed using sequential Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; JOBIN YVON model 38+). N and P were analysed using an automated colorimetric system (Skalar) based on indophenol blue and molybdenum blue respectively. Procedures were validated against the National Institute of Standards and Technology certified plant reference materials. Organic S was estimated by difference (total S – inorganic S).

Frost Hardiness

In December 1996 following the first year of treatment, current year 1996 shoots (at least 8) were removed from the sixth whorl and washed with deionized water. One shoot per tree was frozen (at temperatures, -29, -35, -38, -41, -44, -49 °C) using a programmable freezing cabinet which cooled at 5 °C h⁻¹, held the preset temperature for 3.0 h and warmed to 2 °C at 10 °C h⁻¹ (FOWLER & al. 1989). Following freezing a one centimetre section was excised and placed in a vial to which a 15 ml aliquot of deionised water was added. The solution conductivity was measured after 1, 24 and

120 h before autoclaving (105 °C for 5 mins), left to stand for 24 h and remeasured (MURRAY & al. 1989). Normalised electrolyte leakage rates were calculated for each shoot section and values for unfrozen shoots and those frozen at the coldest freezing temperature, -49 °C, were plotted to estimate the critical leakage rate (SHEPPARD & al. 1995b). Shoots were designated live or dead based on the critical leakage rate and the proportion of dead shoots was fitted as a function of freezing temperature using probit analysis (SAS 1985) for each block based on the 10 trees. Differences in mean treatment LT_{50} (temperature which killed 50% of shoots) values were determined by ANOVA using treatment $\times$ block (4) as the error term (GENSTAT V 1990).

In 1997, following two years of treatment, frost hardiness was re-assessed. As in 1996 shoots were collected in the first week of December and frozen at -15, -21, -24, -27, -30, -33 and -39 °C. Subsequent analysis followed that described for 1996.

Shoot Extension

At budburst, at the start of treatment, an east facing leading bud approximately 1.5 m off the ground was tagged and its final extension length measured

Results

Pre-treatment Growth Data

Stem Areas, Increments and Volumes

Both before (April 1995) and after the 1995 growing season (April 1996), pre-treatment mean stem areas were similar across the site, with a range less than 6% in 1995 and 5% in 1996 (Table 2). Stem area increments pre-treatment, were not significantly different between the treatment blocks (Table 2). However relative stem area increment (RSAI) over the 1995 growing season, which represents the increment relative to the start value (April 1995), did show pre-treatment differences among plots, with a range between 30 and 45%. The single dose acid mist treatment plot gave the largest RSAI and the double dose treatment plot the smallest (Table 2). Statistical analysis indicated that differences between treatments in the experiment would need to exceed 13% to demonstrate treatment effects at the 95% level. At the end of the 1995 growing season, stem volumes were not significantly different among plots, either for the basal volume, top volume, or the combined volume (Table 2).

Foliar Nutrient Concentrations

N concentrations in one-year-old foliage ranged from 1.35 to 1.42% and indicated no spatial differences. Likewise, S, P, K, Ca and Mg concentrations were similar for the pre-assigned treatment plots (Table 3). Concentrations of inorganic and organic S indicated most of the S was organic (~ 55%).

Effects of one Year's Treatment on Growth

Conditions at the site during 1996 did not permit 100% application of the 'double dose' N + S * 2 acid treatment during the growing season.

Table 2

Differences in 10-year-old Sitka spruce stem growth parameters growing at Deepsyke forest (S. Scotland), prior to treatment (n = 4).
(Superscripts denote treatment rankings for each measurement)

1995-1996 PRE-TREATMENT			ASSIGNED TREATMENTS					ANOVA		
	Time	Unit	No Spray	N	S	Control	NS Acid	2 NS Acid	P Value	LSD
Stem basal area	April 1995	cm ²	0.5 ¹	29.3 ³	28.1 ⁶	28.8 ⁴	30.2 ²	28.7 ⁵	0.744	3.84
	April 1996	cm ²	42.8 ²	41.6 ³	40.4 ⁵	41.3 ⁴	43.4 ¹	39.9 ⁶	0.776	5.6
Stem rel. area inc. (RSAI)	1995	%	40.6 ⁴	40.2 ⁵	44.4 ²	43.6 ³	44.7 ¹	39.3 ⁶	0.048	4.1
Stem basal vol.	April 1997	cm ³	3033 ¹	2943 ³	2911 ⁵	2843 ⁵	2977 ²	2522 ⁶	0.62	652
	April 1996	cm ³	1401 ²	1305 ³	1020 ⁶	1440 ¹	1224 ⁴	11.25	0.452	5.13
Stem total vol.	April 1996	cm ³	4428 ¹	4295 ²	3931 ⁵	4284 ³	4165 ⁴	3646 ⁶	0.640	1054

Table 3

Pre-treatment (February 1996) nutrient concentrations (% dry wt) in rinsed (deionized H₂O) one-year-old foliage of 4m tall Sitka spruce growing at Deepsyke forest (S. Scotland)

1995-1996 PRE-TREATMENT		ASSIGNED TREATMENTS					ANOVA	
No spray	N	S	Control	NS Acid	2 NS Acid	P value	LSD	
N	1.37	1.40	1.37	1.40	1.42	1.35	0.886	0.12
S total	0.12	0.13	0.12	0.12	0.12	0.12	0.187	0.01
S inorganic	0.05	0.06	0.06	0.05	0.06	0.05	0.280	0.01
S organic	0.06	0.07	0.06	0.07	0.06	0.07	0.385	0.01
P	0.16	0.17	0.17	0.17	0.17	0.16	0.451	0.01
K	0.53	0.53	0.48	0.49	0.51	0.52	0.763	0.03
Ca	0.37	0.37	0.32	0.36	0.36	0.34	0.284	0.05
Mg	0.14	0.15	0.15	0.14	0.15	0.14	0.549	0.02

When the plant material was harvested, only 80% of the target dose had been applied, i.e. the treatment received 60% more than the single dose (Table 1).

Stem Areas, Increments and Volume

After one season's treatment absolute stem basal areas ranged from 55 to 60 cm². Stem areas increased by between 36 and 39% over the 1996 start values but there were no treatment effects (Table 4). Following two years of treatment relative stem basal area increments still exhibited no significant effects of N and S additions ($P = 0.694$). Percent RSAI ranged from 124% in the N + S acid * 1 treatment to 140% in the N treatment with a detection level of 18%. Neither volume increments, basal or top, nor the total increment indicated effects of N, S or acidity in the first year of treatment although the relative increase in volume was high, between 52 and 56%, effects of the different treatments. After 2 years of N, S and acid treatments, effects were detected for total relative stem volume increment over the 2 treatment years ($P = 0.07$). Increasing S and N inputs in the acid treatment had a significant positive effect on volume. Relative volume increments ranged from 112% (+N), 116% (control) through to 126% and 131% (pH 2.5 * 1*2) respectively. The +S and no treatment increased by 121 and 119% respectively.

Shoot Extension

Treatment effects were almost detected ($P = 0.056$, Table 4). Shoots receiving the single dose acidified N + S treatment extended more than those receiving N or the control, (dilute spray).

Foliar Nutrient Concentrations and Needle Weight

In addition to treatment effects, different growing conditions from year to year also affected nutrient concentrations. One-year-old foliage sampled in the winter of 1995 from the untreated trees had, generally, higher nutrient concentrations than measured in one-year-old foliage sampled in the winter of 1996. Total and inorganic S and Mg were significantly lower following the 1996 growing season (Table 5). Ratios of the major nutrients with respect to N, however, did not change suggesting a growth dilution effect, i.e. that the foliage mass may have been greater in 1996 than in 1995. Inspection of the 1995 needles revealed they were much smaller in size than the needles grown in 1996.

Treatment Effects: Current Year Needles (1996)

Needle weights were not affected by N, S or acidity (Table 6). Concentrations of N, S and Mg were significantly influenced by treatment

Table 4

Differences in stem growth parameters and shoot extension for Sitka spruce trees after one year's treatment at Deepspyke forest (S. Scotland). Values in each row followed by the same letter are not significantly different ($P > 0.05$) Tukey's t-test

1995-1996 PRE-TREATMENT				ASSIGNED TREATMENTS				ANOVA		
	Time	Unit	No spray	N	S	Control	NS Acid	2NS Acid	P value	LSD
Stem volume	Apr. 97	cm ³	6821	6604	6147	6528	6442	5640	0.76	1758
Basal stem area (BSA)	Apr. 97	cm ²	58.4	56.6	56.0	56.4	59.8	55.2	0.85	8.4
BSA inc.	96-97	cm ²	15.6	15.0	15.6	15.1	16.4	15.3	0.90	2.9
Rel. BSA inc.	96-97	%	36.6	35.6	38.7	36.5	38.1	38.2	0.38	3.4
*Basal volume inc.	96-97	cm ³	1333	1313	1382	1236	1363	1185	0.85	371
**Top volume inc.	96-97	cm ³	1049	995	834	1008	914	819	0.78	415
Total volume inc.	96-97	cm ³	2383	2308	2216	2244	2278	1994	0.90	720
Rel base volume inc.	96-97	%	44.6	43.4	47.9	43.5	46.4	46.1	0.28	4.5
Rel top volume inc.	96-97	%	76.1	73.6	83.5	72.5	79.4	73.7	0.37	11.8
Rel total volume inc.	96-97	%	53.8	52.2	56.5	52.6	55.0	54.1	0.62	5.7
Shoot extension*	96-97	cm	21.1a	18.8b	21.3a	19.7b	22.0a	21.4a	0.056	2.2

* Basal below DBH (at approximately 1.3 m height up stem)

** Top above DBH, up to first sign of leader loss (deformity)

* Values followed by the same letter are not significantly different (95%, Tukey's t-test)

Table 5

Effect of season on nutrient supply: mean nutrient concentrations (% dwt) and needle weight (mg) in one-year-old foliage taken from the 'untreated' treatment for Sitka spruce growing at Deepsyke forest (S. Scotland) on 28 February 1996 and 3 December 1996 (n = 4).

	Pre-treatment 28. 02. 96	After first season's treatment 3. 12. 96	P value	LSD
Wt 100 needles	34.1	33.9	0.91	6.6
N	1.37	1.17	0.16	0.34
S	0.12	0.09	0.03*	0.02
S in	0.054	0.035	0.05*	0.02
S org	0.063	0.057	0.52	0.03
P	0.16	0.15	0.31	0.03
K	0.53	0.53	0.84	0.07
Ca	0.28	0.32	0.69	0.24
Mg	0.13	0.09	0.01*	0.02

(Table 6). The acid double dose N + S treatment significantly increased foliar N concentrations. Foliar total S concentrations were significantly higher in the three S treatments being highest in the double dose acid treatment. Inorganic S was not significantly increased but significant increases were seen in organic S. Mg concentrations were significantly affected by treatment and were lowest in the control and unsprayed treatments.

Treatment Effects: One-year-old Needles (1995)

Needle weights were quite variable and no treatment effects were found (Table 7). S and N inputs affected foliage formed in the year prior to treatment, (1995) with significant increases in N concentrations with double acid N + S, and significant increases in S, particularly organic S from both the single S dose and double S + N acid dose. Mean values based on all treatments indicated current year foliage (1996) had heavier needles (+10%), higher N concentrations (+10%), lower inorganic S (-18%), higher P (+27%), higher K (+30%) and considerably lower Ca (-100%) concentrations than one year old (1995) foliage.

Electrolyte Leakage and Frost Hardiness

Unfrozen Shoots

In 1996, leakage values from unfrozen shoots at the beginning of December ranged from 0.53 to 0.66% d⁻¹ and were not significantly affected by treatment (Table 8). Shoots treated with S alone had the lowest leakage

Table 6

Nutrient concentrations (% dwt) in current (1996) Sitka spruce foliage from trees at Deepseyke forest (S. Scotland), collected in December 1996 after one season's mist treatment. The NH_4NO_3 , NS Acid and 2NS Acid treatments supplied 48 and 76 kg N ha⁻¹ respectively. The (Na_2SO_4) and NS Acid and 2NS Acid treatments supplied 50, 50 and 80 kg S ha⁻¹ respectively. Values in each row followed by the same letter are not significantly different ($P > 0.05$), Tukey's t-test.

	No spray	N	S	Control	NS Acid	2NS Acid	P value	LSD	Mean
Needle wt. (mg/100)	33.9	33.9	34.6	33.2	35.9	33.9	0.96	6.3	34.2
N	1.17 b	1.25 b	1.27 b	1.22 b	1.27 b	1.45 a	0.012*	0.136	1.27
S	0.09 c	0.10 c	0.11 b	0.09 c	0.11 b	0.12 a	<0.001***	0.012	0.10
S inorg.	0.03	0.03	0.04	0.03	0.04	0.05	0.11	0.012	0.04
S org.	0.06 b	0.06 b	0.07 a	0.06 b	0.07 a	0.07 a	0.011**	0.009	0.06
P	0.15	0.14	0.15	0.15	0.14	0.15	0.766	0.02	0.15
K	0.53	0.43	0.49	0.44	0.48	0.51	0.374	0.11	0.48
Ca	0.32	0.29	0.35	0.28	0.35	0.34	0.197	0.07	0.32
Mg	0.10 a	0.11 b	0.11 b	0.10 a	0.11 b	0.11 b	0.037*	0.01	0.11

Table 7

Nutrient concentrations (% dwt) in one-year-old (1995) Sitka spruce foliage from trees at Deepseyke forest (S. Scotland) collected in December 1996 after one season's mist treatment. The NH_4NO_3 , NS Acid and 2NS Acid treatments supplied 48 and 76 kg N ha⁻¹ respectively. The (Na_2SO_4) , NS Acid and 2NS Acid treatments supplied 50, 50 and 80 kg S ha⁻¹ respectively. Values in each row followed by the same letter are not significantly different ($P > 0.05$), Tukey's t-test.

ASSIGNED TREATMENTS								ANOVA		
	No spray	N	S	Control	NS Acid	2 NS Acid	P value	LSD	Mean	
Needle wt. (mg/100)	31.1	29.1	28.0	35.6	33.2	29.6	0.535	10.6	31.1	
N	1.10 b	1.17 ab	1.15 b	1.12 b	1.15 b	1.25 a	0.037*	0.09	1.16	
S	0.09 b	0.10 ab	0.11 a	0.09 b	0.10 ab	0.11 a	0.024*	0.016	0.10	
S inorg.	0.03	0.03	0.04	0.03	0.032	0.037	0.284	0.014	0.03	
S org.	0.06 b	0.07 ab	0.07 ab	0.06 b	0.07 ab	0.08 a	0.047*	0.010	0.07	
P	0.12	0.12	0.13	0.11	0.11	0.12	0.356	0.014	0.12	
K	0.39	0.34	0.40	0.33	0.37	0.39	0.273	0.07	0.37	
Ca	0.63	0.58	0.69	0.62	0.65	0.63	0.198	0.08	0.64	
Mg	0.10	0.13	0.11	0.10	0.11	0.11	0.513	0.028	0.11	

Table 8

Effect of field misting on ion leakage (% d⁻¹) from unfrozen current year Sitka spruce foliage, sampled both in December 1996 and December 1997.

	1996 % d ⁻¹	1997 % d ⁻¹
No spray	0.66	0.63a
N	0.66	0.69ab
S	0.53	0.59a
Control	0.60	0.64a
NS Acid	0.61	0.80b
2 NS Acid	0.63	0.81b
P value	0.11	0.006
LSD	0.10	0.12

rates. In 1997 leakage rates were similar to those measured in 1996 but ranged higher, from 0.59 to 0.81 % d⁻¹ (Table 8). Treatment effects were highly significant ($P = 0.006$), with a large increase in leakage rates for shoots treated with acidic S + N mist.

Frozen Shoots

1996

Most of the freezing temperatures used in this assessment exceeded the hardiness level of most of the shoots. However, LT₅₀s were calculable by linear interpolation for 3 of the 4 blocks. The critical leakage rate was 1.2% d⁻¹, derived by comparing the range of leakage rates for unfrozen shoots and those frozen at -49 °C. LT₅₀s ranged from -27.0 °C (acid treatments) to -32.0 °C (Na₂SO₄) (Table 9). The acid treatments S + N exhibited the lowest level of frost hardiness; contrast analysis of variance, comparing treatments receiving acid mist and those receiving non-acid mist, showed the effect to be statistically significant ($P = 0.036$). Likewise trees receiving N (S + N × 1, × 2 or N) were significantly less hardy ($P = 0.014$).

1997

A critical leakage rate of 1.62% d⁻¹ discriminated between live and dead shoots. LT₅₀s (°C) for all treatment plots were similar, with means for each treatment between -30.9 and -33.1 °C, with generally small confidence limits. The freezing temperatures used produced a good gradation of damage, permitting improved estimation of the LT₅₀ compared with 1996 (data not shown) based on the 95% fiducial limits (Table 9). These limits indicate that LT₅₀s can be estimated with a range of 3 to 6 °C about the mean. Analysis of variance based on treatment means indicated statistically significant treatment effects, with control shoots and those from

Table 9

Lethal temperatures, ($^{\circ}\text{C}$) causing 50% shoot death (LT_{50}) in current year Sitka spruce foliage experiencing different misting treatments based on critical leakage rates (kd^{-1}) of 1.2 and 1.6% d^{-1} in 1996 and 1997 respectively. 95% confidence limits indicate the fiducial limits of the LT_{50} value. (n/a missing)

1996			Treatment mean	1997		Treatment mean
	LT_{50}	$^{\circ}\text{C}$ Block		LT_{50}	Confidence limits	
No treat- ment	S	-33.7	-31.0ab	-31.6	-29.5 to -34.3	-30.9b
	W	n/a	± 1.4	-30.3	-28.9 to -31.9	
	N	-29.2		-31.3	-29.4 to -33.8	
	E	-30.0		-30.5	-28.9 to -31.3	
N	S	-29.2	-27.9ab	-30.7	-29.3 to -32.3	-31.1b
	W	-23.9	± 0.7	-30.8	-28.9 to -33.2	
	N	-30.5		-30.5	-28.4 to -33.0	
	E	-23.9		-32.5	-30.7 to -34.9	
S	S	-33.4	-32.1a	-31.0	-29.6 to -32.9	-31.3b
	W	-31.0	± 0.7	-31.1	-29.5 to -33.1	
	N	-31.9		-32.3	-30.0 to -35.5	
	E	-31.0		-30.8	-28.9 to -33.0	
Control	S	-33.7	-29.2ab	-32.5	-30.7 to -34.9	-32.8a
	W	n/a	± 1.2	-31.0	-29.6 to -32.9	
	N	-29.2		-34.1	-31.8 to -37.7	
	E	-28.0		-33.5	-31.5 to -36.3	
NS Acid * 1	S	-26.3	-26.7ab	-31.7	-29.6 to -34.3	-31.3b
	W	n/a	± 0.2	-30.5	-28.5 to -32.8	
	N	-26.6		-32.3	-26.6 to -76.1	
	E	-27.1		-30.8	-29.6 to -33.1	
NS Acid * 2	S	-24.1	-27.4ab	-33.5	-31.5 to -36.3	-33.1a
	W	n/a	± 2.3	-32.9	-31.0 to -35.4	
	N	-31.7		-32.6	-30.6 to -35.6	
	E	-26.4		-33.5	-31.5 to -36.3	
P Value			0.12			0.004
LSD			4.2			1.2

acid S + N * 2 treatments being significantly more frost hardy than the rest although differences were on the small side 2 to 3 $^{\circ}\text{C}$ (Table 9).

Discussion

This experiment was undertaken to examine the potential for combinations of N, S and acidity, applied as mist, to influence tree vitality in a genetically variable stand comprising more than one provenance, growing on an acid soil typical of sites planted with Sitka spruce in northern Britain.

Experimental Protocol

The fore-runner to this experiment (CROSSLEY & al. 1997, SHEPPARD & al. 1998b) was undertaken with a single, N efficient clone (SHEPPARD & al. 1985) which typically invested a high proportion of assimilate in stemwood production (CANNELL & al. 1983). The clone, grown in a base rich soil, showed systematic differences in height growth across the site in response to the increasing nutrient availability. Blocks of four trees were enclosed within temporary walls during spraying which restricted spray drift and minimized interference with light and turbulence. The experimental evidence was based on 80 trees with crown bases ranging from 0.10 m to 0.50 m above ground level.

In this experiment numbers of trees per block were increased from 4 to 10 and blocks per treatment from 2 to 4. Restriction of drift was achieved by using non-experimental trees as a screen. This also eliminated the need to brash the trees and the potential for interference with light and turbulence regimes. The pre-treatment season RSAI data gave an indication of potential environmental effects. The positive side of increasing the numbers of experimental trees was the increased sensitivity for detecting statistically significant treatment effects. RSAI treatment differences were detectable ($P < 0.05$) at between 12 and 15%. The negative side of handling such large numbers of trees is the large time and financial commitment to chemical analysis (see also WINNER 1994). The scale of the experiment meant physiological measurements, such as water relations and photosynthesis, were not feasible. Biochemical determinations were restricted to one or two individual trees, of intermediate basal diameter, per chamber. This detailed information will be used to facilitate interpretation of the growth measurements which were undertaken on all 240 trees.

Interaction Between N/S/acidity Treatments and Growth

In this experiment neither one year, nor the combined effect of 2 years treatment with N/S/acidity have significantly influenced RSAI, and there was no pre-treatment bias in the selection of plots. This is in marked contrast to the result from the afore-mentioned field experiment, on a base-rich mineral soil (CROSSLEY & al. 1997) where treatment with S + N acid * 1 caused significant, ~20% reductions in RSAI over unsprayed control trees. By contrast, relative stem volume increments (%) have, after the second treatment year, shown a significant positive response to the addition of N + S acid * 1, * 2. Growth rates for this and the previous experiment were similar with annual stem height increments of about 1 m, and both sets of trees were coning and had interlocking basal branches. The major differences between the experiments were at the level of genetic variability, and the soil type. Clones which favour stemwood production as the main assimilate sink may show a different level of response from other genotypes

to acid mist (see OREN 1996). Alternatively the different responses may be wholly mediated via changes in soil chemistry, i.e. the response of a base rich mineral soil compared to those of an acid, predominantly organic soil.

Growth Responses to N

In this experiment two years of N application, at approximately 2 kg N per week between May and November, failed to influence relative stemwood area or volume increments. Lack of response to N in field grown Sitka spruce was also reported by THOMAS & MILLER 1992, under similar conditions. They supplied eleven-year-old trees with 50 kg N at 10 kg N ha⁻¹ month⁻¹ by supplementing natural rainfall with NH₄NO₃. Foliar N status was enhanced (+22%), in contrast to this experiment, but DBH was not significantly altered. Evaluation of N response in Sitka spruce suggests a positive N response is restricted to trees where foliar N concentrations fall well below 1.4% (TAYLOR 1987). Similarly, mature Norway spruce (> 20 years old) growing in Sweden failed to increase stemwood area in response to N when foliar N concentrations exceeded a threshold 1.5% N (SIKSTRÖM & al. 1998). Mean foliar N concentrations in this experiment, measured in current year needles from the lower part of the crown, at around 1.3%, suggest therefore we are unlikely, at the present time, to see a significant growth response to N alone. However, we shouldn't dismiss the concluding remarks of MCINTOSH 1983 who wrote 'fertilizer responses on shallow peats are the least predictable, because such soils are inherently variable'. We must also acknowledge that in forest stands, and pot experiments too, growth responses to fertilizer inputs are often delayed because of timing of the fertilizer application in relation to the ontogenic development status of the plant (OREN 1996). MCINTOSH 1981 found no increase in Sitka spruce height growth in response to N fertilization where N was applied after June (once shoot extension had ceased). Thus the absence of a growth response after one year may simply reflect the timing of the nutrient additions. So far, in this experiment, no response has been seen even after 2 years of semi-continuous N application. The response to N will also depend on the availability of other major nutrients, particularly P and K (MCINTOSH 1983). Results from an N, P, K bioassay (DIGHTON & HARRISON 1990) conducted in May 1996, provide no evidence that prior to treatment the P and K demands of the trees were not being met. Once the trees close canopy however, nutrient demand will be met to a large extent from internal cycling and efficient cycling of the nutrients in the newly developing litter layer (MILLER & MILLER 1987). N inputs, and associated acidification may influence future growth trends via effects on the demand for, and availability of, other major nutrients – e.g. P (CARREIRA & al. 1997), P and K (SOGN & ABRAHAMSEN 1998).

Potential for Soil Chemistry to Dominate Tree Response to N, S and H Inputs

In a comprehensive review of fertilization and growth experiments undertaken in Sweden over the last 30 years or more, BINKLEY & HOGBERG 1997 concluded that on average N fertilization either encourages growth or (less often) fails to increase growth. They cite many studies where soil acidity has increased, concomitant with reduced base cation, Ca + Mg : Al ratios. Surprisingly, perhaps, their evidence indicates that trends such as increasing acidity, in already quite acid soils (pH < 4) have not been associated with significant reductions in yield in the short term.

In this experiment, we have limited information concerning the effects of the N, S and H inputs on the soil. After one year of the S + N acid * 1, * 2 mist treatments the concentration of base cations, (Ca and Mg) leached from one washing of a 10 cm soil core indicated increased (+36%) concentrations of base cations despite the soil pH being lowered. Similar increases in base cations and growth, were seen in a pot experiment, where Sitka spruce seedlings grown in tubs of an acid organic soil, pH 4.1 (CaCl₂) responded more positively to acid treatment than seedlings grown on a base rich mineral soil (pH 5.9) (SHEPPARD & al. 1995). Improved growth, in response to soil acidification from N and S additions, has also been seen in *Calluna* (CRESSER pers. comm.) and likewise attributed to a temporary increase in the availability of base cations via anion displacement. These changes in soil chemistry in British peaty soils are thus very similar to those described for Swedish soils in response to acidified N and S inputs (BINKLEY & HOGBERG 1997). However because the reservoir of exchangeable bases is naturally low in UK soils (WHITE & al. 1995) and, in view of the concomitant increase in soluble Al, it is unlikely this improvement in growth in response to acidified N+S inputs will persist. In the Netherlands large inputs of (NH₄)₂SO₄ (120 kg N ha⁻¹ yr⁻¹ + 40 kg N ha⁻¹ yr⁻¹ background deposition) initially (after one year) stimulated growth of a 40-year-old *Pinus sylvestris* stand but thereafter growth declined, tentatively linked to an increase in soil Al concentrations (EERDEN & al. 1997). In Norway a long term experiment evaluating NH₄NO₃ inputs in the presence of acidified rain (H₂SO₄) to pH 3 recorded a significant reduction in stem diameter growth of Scots pine in response to acidification and a non significant positive response to N at the end of a 5 year treatment period (SOGN & ABRAHAMSEN 1998). In that experiment control plants had low N concentrations and Ca:Al ratios appeared to reduce with time. These observations serve to highlight the value of long term experiments to understand how forests cope with acidifying S and N inputs and the importance of soil chemistry.

Treatment Effects on Foliage (Nutrition and Ion Leakage)

Foliar nutrient data measured after one year's treatment provided no evidence of nett cation loss by foliar leaching. Changes in soil solution base

status were not seen as enhanced foliar Ca concentrations although Mg concentrations were higher in both the N + S acid treatments and those supplying soluble anions, i.e. the NH_4NO_3 and Na_2SO_4 treatments. Significant changes in foliar nutrient status were also seen for S and N. Organic S concentrations were significantly increased in relation to the supplied dose of S whereas N was only higher (+19%) in the double dose treatment (N + S acid * 2). Although significant, these changes represent quite small increases, with debatable physiological importance and may simply reflect the high level of replication and thus enhanced sensitivity of the experimental design. These results confirm that the doses were sufficient to influence tree nutrient status, either via foliar exchange, or for base cations, via changes in soil chemistry.

In the second treatment year current year foliage did show evidence of effects of treatment with acid. Shoots receiving N + S at pH 2.5 had significantly higher leakage rates than shoots treated with S or N in the absence of acidity. The response, however, was not dose dependent. Higher rates of ion leakage have typified the response of spruce shoots treated with acid N + S in OTC's (SHEPPARD & al. 1994) but have been less consistently observed in the field (SHEPPARD & al. 1998c). The mechanism causing acid treated shoots to leak ions at a higher rate than control shoots is still to be elucidated. Recent data from experiments by DEHAYES (DEHAYES pers. comm.) working with red spruce, suggest membrane integrity is compromised because the acidity is causing membrane bound Ca^{2+} to be leached. Increased ion leakage rates are entirely consistent with a reduction in membrane integrity such as might arise when Ca^{2+} ions are replaced by smaller H^+ ions in their bridging positions (cf roots MOORE 1974), although this type of exchange would have been expected to show a response dose, i.e. increased exposure frequency.

Effects of N/S/acidity Treatment on Frost Hardiness

Measurements of frost hardiness have been included in this study as an indicator of physiological status. Midwinter hardiness levels reflect the level of cryoprotectants, carbon made available to combat freeze induced stress. Reductions in frost hardiness are indicative of changes in C assimilation, or partitioning or both and as such provide a sensitive indicator of changes in the physiological functioning of the tree which are unlikely to be otherwise obvious. After one year of treatment significant effects on frost hardiness were almost detectable ($P = 0.12$) and, as seen in OTC studies, treatment with the combination of N, S and acidity reduced frost hardiness. In contrast to previous experiments with red spruce (CAPE & al. 1991), the N alone treatment also made the shoots more frost sensitive. However after 2 treatment seasons and when the test freezing temperatures were better matched to the hardiness of the shoots, thus increasing

the sensitivity of the test, the effects of N, S and H additions were not clear cut. This data set provided a very precise estimation of LT_{50S} , as judged by the confidence intervals and the level of reproducibility detected significant differences of $<2^{\circ}\text{C}$. However the importance of such small differences – smaller than the test temperature intervals is arguable: our interpretation of this data set is that they provide no evidence of N, S or acidity inducing physiologically significant perturbations to the trees at this stage of treatment.

The enhanced ion leakage rates seen in the second year of treatment were not matched by reductions in frost hardiness in the acid S + N treatments. The lack of correlation between ion leakage rates in the absence of freezing and from frozen shoots has also been a feature of OTC studies (SHEPPARD & al. 1994). Then, it was concluded that enhanced rates of ion leakage from unfrozen shoots were not a useful indicator of pollution effects on frost hardiness. However, the recent data of DEHAYES & al. 1997 suggest we should take a second look at these observations in the light of newly emerging mechanistic implications.

DEHAYES & al. 1997 showed that membrane bound Ca plays a pivotal role in maintaining cell viability against frost damage. More recently DEHAYES has shown that spruce growing at high altitude sites may be at risk from frost injury because of the acid component in the cloud/rain depleting concentrations of membrane bound Ca. High altitude red spruce (*P. rubens* Sarg.) which frequently exhibit low foliar Ca concentrations (MCLAUGHLIN & al. 1991, 1993) showed a marked improvement (up to 10°C) in their levels of mid-winter hardiness when cloud water (pH 3.5–3.7) was excluded (VANN & al. 1995). In our OTC experiments and in this field experiment foliar Ca^{2+} concentrations have been adequate to high and the reduction in ion leakage rates by acid mist although significant did not represent a large proportional ($<20\%$) change in leakage rates. Likewise membrane integrity is only one of several component mechanisms contributing to frost hardiness status (LEVITT 1980). Thus the absence of correlation between ion leakage rate and frost hardiness may be a reflection on the mechanisms of cryoprotection operative at the time of sampling. The usefulness of measurements of ion leakage rates from unfrozen shoots as a bioindicator for acid mist damage potential would appear to merit further attention.

Conclusions

Early results from this well replicated field exposure experiment, based on a large sample of trees (40 per treatment), suggest we still have much to learn about the potential for acid mist to influence trees and the timescale over which effects develop. The results of the frost study were only consistent with the accepted trend for acid mist effects, which

emerged from OTC studies, in the first treatment year. In the second year of treatment less than 2.5 °C separated the treatments and responses to N, S and acidity were inconsistent with earlier results. We shall need to continue with these studies to increase our understanding of how N, S and acidity in acid mist influence frost hardiness (carbon acquisition and allocation) in the field, and the likely role of foliar mediated effects. The absence of a growth response to N reflects the N supply at the site which at present appears to be sufficient to satisfy the growth potential. The apparent benefit from the addition of N + S with acidity appears to reflect increasing concentrations of base cations in the soil solution arising from the addition of mobile anions. So far no deleterious effects of the rising Al concentrations have been detected but if these persist, we would predict eventual reductions in growth. In the remaining years of treatment we shall be concentrating on indicators of N excess i.e. increased N leaching, accumulation of inorganic N and a reduced C:N ratio in the litter/humus, quantifying changes in soil chemistry and working up a N budget for the site so we are better equipped to predict the effects of increasing N deposition to Sitka spruce forest ecosystems.

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