The nature of high altitude precipitation and its effect on Scots pine (Pinus sylvestris L.) and Sitka spruce (Picea sitchensis (Bong.) (Carr.)).

by

Callum George McKenzie Henderson

Ph.D.
University of Edinburgh
1990

CONTENTS

Chapter 4 **Leaching of ions from Scots pine and Sitka spruce in controlled experiments**

Chapter 5 **The exchange of elements between cloudwater and the needles of Scots pine and Sitka spruce in the field**

7.4 Results

Chapter 8 **The release of ions in snowmelt from fresh snow and from snowpack on spruce shoots**

8.2 Method

8.3 Results

ABSTRACT

The nature of high altitude precipitation and its effect on Scots pine (Pinus sylvestris L.) and Sitka spruce (Picea sitchensis Bong.)(Carr.)) was investigated. Scots pine and Sitka spruce are important British forestry species and the effect of acidic cloud on these had been inadequately investigated.

At a field site in the Scottish Southern Uplands, impacted cloudwater was either collected from, or measured with a microelectrode in situ on, artificial polypropylene shoots which closely mimicked live shoots. Temporal variation of pH within cloud deposition events was strong and the potential for damage by acid clouds was seen to be high.

Impacted cloudwater was also collected from, or measured in situ on, live shoots and the interchange of ions between shoots and cloudwater investigated. Leaching of basic cations was considerable but not sufficient to neutralize cloudwater acidity. Dry deposition on live shoots appeared to increase the acidity of impacted cloudwater on shoots.

The change in pH of evaporating droplets was measured in situ with the microelectrode. Droplet acidity increased dramatically as a result of evaporation. The potential for damage by snow was investigated by collecting and analysing snowmelt. Ground snowpack showed an initial flush of ions (including H^+) on melting, followed by a steep decline to very low ion concentrations. Meltwater from live shoots did not show this flush/decline but did exhibit higher acidity levels which were also related to the pathway of the snowmelt through the snowpack.

A series of four glasshouse experiments were undertaken in which a system for applying simulated acid cloud (SAC) was developed. Six to eight-year-old Scots pine and Sitka spruce were subjected to SAC at pH 5.6, 3.0, 2.5 and 2.0. Considerable leaching of Ca, Mg, K, NO_3^--N and PO_4^{3-}-P occurred and showed an increasing response to increasing acidity of SAC.

Both extension growth and needle area declined with increasing acidity but the decline was statistically insignificant. Visible injury on Scots pine needles was limited to those subjected to SAC at pH 2.0. Sitka spruce damage occurred to a very small extent at pH 2.5, but was again largely restricted to pH 2.0. Artificial abrasion of needles did not increase susceptibility to acid damage. Acid damage generally progressed from tip to base and at the junction of the brown and green tissue a darker brown band formed. Tissue underlying brown areas was subject to cell collapse and underneath the dark brown band the extracellular spaces were filled with an unknown material and the cells had not collapsed. The structure of the epicuticular wax was not affected by SAC acidity and even over necrotic areas the crystalline wax remained intact. The functional integrity of the cuticle, investigated by measuring cuticular conductance and contact angles, was unaffected by SAC.

Controlled experiments on the changing acidity of evaporating droplets took place with three-year-old Scots pine and Sitka spruce in a large windtunnel, again with the use of the microelectrode. Droplet evaporation again increased acidity dramatically and there was also a close relationship between the increase of acidity of the droplet and that predicted by a direct relationship to the amount of water evaporated.

Scots pine and Sitka spruce thus appear to be resistant to direct effects of acid cloud at pH 3.0 which is the lowest generally encountered in the Scottish Southern Uplands. However acidity enhancement through evaporation, extreme pH values at the start or finish of cloud events and high acidity flushes at the start of snowmelt may all expose trees to pH levels approaching those causing direct damage. In addition, the nutrient budgeting of trees may be adversely affected and lead to long term reduction in growth.

ACKNOWLEDGEMENTS

My thanks are extended to my supervisors. Prof. M.H. Unsworth of the Institute of Terrestrial Ecology (ITE) supervised the early part of investigation at ITE and Dr. A. Crossley supervised the latter part. In particular Dr. Crossley gave frequent help and instruction with practical details of experimentation, and tuition and help in scanning electron microscopy (SEM) and photographic development. My thanks also to Deirdre Wilson who assisted Dr. Crossley. Prof. P.G. Jarvis, my supervisor at the University of Edinburgh, provided consistent direction and constant stimulation throughout the thesis and I would like to thank him for both that and his patience during the writing up period.

I am especially grateful to Marjorie Ferguson, who in one of her rasher moments offered to type this thesis. Her many hours of labour are much appreciated. I would also like to thank Dr. D. Fowler and Dr. J.N. Cape for advice given during the work, and Dr. J. Dick for moral support and a steady flow of helpful suggestions. Prof. I. Rorison and his helpers at the U.C.P.E., Sheffield spent a considerable amount of time explaining and illustrating the use of hydroponics and Dr. J. Grace and Dr. P. Van Gardigen are both to be thanked for advice and help with the use of resources at Kings Buildings, University of Edinburgh. My thanks are given to Mr. A. Gray for instruction and considerable help in the analysis of samples. I would also like to thank Dr. C. Jeffree and his technicians for their supervision and help with the frozen-hydrated freeze-fractured SEM.

I would also like to thank Mrs. S. Adair, Miss L. Scoular and Mrs. L. Dickson for their help in the library, Mr. F. Harvey, Mr. J. McCormack and Mr. R. Ottley for their help in the glasshouses, Mr. R. Smith for statistical advice, Mrs. R. Addinall and Mr. T. Duffy the computer support staff, and Dr. A. Sandford for technical advice. My thanks to my wife Izzy for her support. Thank you Father.

INTRODUCTION

During the mid seventies, concern grew in West Germany that Silver fir (Abies alba) was undergoing a serious growth decline which had first appeared in the early seventies (Prinz et al. 1987, Roberts 1987). This "Fir-drying" or "Tannensterben" became particularly severe after the dry year of 1976 (Fröhlich 1984, Prinz et al. 1987). Symptoms included chlorosis and yellowing of needles (particularly the upper side of older needles), casting of older needles, a premature reduction in height increment, sub-top dying, the prevalence of wet wood, a reduction in the fine root system and mycorrhizal rootlets, increased adventitious bud development and eventually death (Fröhlich 1984, Krause et al. 1986, Prinz et al. 1987, Roberts 1987).

Around the autumn of 1980 other species, particularly Norway spruce (Picea abies), also appeared to be affected with damage which increased dramatically after the dry summers of 1982 and 1983 (Fröhlich 1984, Ulrich 1985, Binns 1987, Bucher 1987, Prinz et al. 1987, Roberts 1987, Prinz and Krause 1989). Symptoms were similar to those of Silver fir although reduction of height was not so apparent. The severe yellowing of Norway spruce became an increasingly used diagnostic tool because of the ability of Norway spruce to retain yellowing needles longer than Silver fir (Prinz et al. 1987). These symptoms were different from the familiar browning of needles attributable to SO_2 damage and was thus termed "novel forest decline" or "Waldsterben" (Prinz et al. 1987, Roberts 1987).

As a response to this rapid decline an inventory of forest tree health was taken in West Germany in 1982 followed by systematic sampling from 1983 onwards. The German surveys were joined in 1984 by similar surveys in Switzerland, Austria, Eastern France and Northern Italy (Bucher 1987). The German surveys showed that whereas 8% of coniferous trees were damaged in 1982, the percentage rose to 34% in 1983, 50% in 1984 and 52% in 1985 with Silver fir particularly badly affected at over 87% (Roberts and Blank 1985, Binns 1987, Blank and Roberts 1987). Some of this increase may have been attributable to increased awareness of the problem (Ballach and Brant 1984, Fröhlich 1984), and these figures also included slightly damaged trees which are now generally regarded as only a warning stage of decline. Surveys from other countries showed that a high percentage of forest all over Europe was rapidly declining (Krause et al. 1986, Bucher 1987) as were, apparently, some forests in North America (Johnson and Siccama 1983, Linzon 1983, Tomlinson 1987, Ke and Skelly 1989). More recent surveys in central Europe have shown a slowing down of decline for conifers and in some areas even an improvement, whilst the health of hardwoods continues to deteriorate (Blank and Roberts 1987, Kandler et al. 1987, Kandler 1988, 1989, Prinz and Krause 1989).

In the UK surveys along similar lines to those carried out on the continent were begun in the winter of 1984 by the Forestry Commission and focussed on Norway spruce (Picea abies) as a link with the continent, Sitka spruce (Picea sitchensis) as the primary economic species of Britain, and Scots pine (Pinus sylvestris) as a native and important forestry interest with a wide distribution across Eurasia (Forestry Commission 1985, Binns 1987). The Forestry Commission attitude to the results from 1984 was that the trees showed no sign of the excessive crown thinning or yellowing that was characteristic of the central European decline and the poor condition of some plots was attributed to pests and climatic conditions (Binns 1987). Results from 1985 showed an improved state of health (Binns 1987). However there was still concern over the state of Britain's trees, particularly as the Forestry Commission survey had focussed on trees less than 45 years old when most damage on the Continent was confined to older trees (Linzon 1984, Binns 1987, Prinz and Krause 1989). Subsequent U.K. surveys included trees over 50 years-old and showed much higher damage in the older Sitka and Norway spruce and a general deterioration in appearance of all ages between 1985 and 1986 and an even greater deterioration between 1986 and 1987, such that crown thinning and needle yellowing were present to a similar extent as in West Germany. However no link with air pollution was proposed as no direct cause and effect mechanism was isolated.

Over this period a number of hypotheses were put forward as the possible reason for forest decline, most of which attributed the decline to air pollutants. Although photochemical oxidants have been implicated more recently as an inciting mechanism for stress in Europe (Prinz and Krause 1989) and as the major contributor to damage in localized areas in North America (Miller and McBride 1989), much of the attention has focussed on "acid rain".

The pH of precipitation in equilibrium with CO_2 in the atmosphere is 5.6 and "acid" precipitation is regarded as precipitation with a pH below 5.6 (Fowler et al. 1982, Barret et al. 1983). The main contributors to acidity in acid precipitation are SO_2 and NO_x, generated from fossil fuel burning (Unsworth 1983). It is the increase in these gases that has been the cause of the relatively recent upward trend in precipitation acidity. The gases themselves, or derived aerosols, can also be deposited outwith the agency of hydromeotors (rain, hail, snow, sleet and cloud droplets): a process known as dry deposition which further increases the input of acidic species into the terrestrial environment.

Several reviews outline the growth in awareness of acid precipitation. Of particular note was the recognition of long range pollutant transport by Evelyn and Graunt (see Cowling 1982) and the recording in 1727 by Hales (Cowling op. cit.) that dew and rain "contain salt, sulphur etc. for the air is full of acid". Smith's book "Air and rain: the beginnings of a chemical climatology" published in 1872 is often quoted as the first work on acid rain and possible effects on plants but it was not until well into the next century that strong links between gaseous emissions, acid precipitation and environmental effects began to be demonstrated (Gorham 1958). Despite findings by Mrose (1966) which indicated a mean rain pH of 4.5 in mountains in Germany and cloudwater pH reaching values less than pH 3.0, it was only when results of a Scandinavian network operated from 1948 were published by Oden (1968) showing a progressive decrease in rain pH, that monitoring and research grew rapidly.

Because of short term annual fluctuations, monitoring over a number of years was necessary to reveal trends in precipitation pH. Changes in analytical techniques and work practices call into question some of the results of such long term monitoring programmes (Watt 1984) but they provide enough data to identify general trends. Amongst these programmes are the European Atmospheric Chemistry Network and measurements from Rothamsted (Goulding et al. 1986) that indicate a steady increase in acidity from 1956 and 1930 respectively. Recent trends indicate a levelling off of pH around 1965 (Oden 1976) with the pH of rain in the late sixties and through the seventies in non-remote parts of Europe averaging around 4.2 (Mrose 1966, Oden 1976, Barret et al. 1983). Since the late seventies there has been an increase in pH such that in some instances rainfall acidity in the UK was up to 25% lower in the mid-eighties compared with the late seventies although there were large variations from year to year and location to location (Barret et al. 1987). Results for the UK in 1986 and 1987 show similar values to those of 1985 with a pH low centring around Humberside at less than pH 4.3 and steadily increasing to around 4.7 in Wales, the South West of England, Northern Ireland and the Highlands of Scotland (WSL 1987, DOE 1988). Deposited acidity, derived from concentration of pollutants and volume of precipitation was largest across north central England, Snowdonia, Galloway, parts of the Borders and the south west Scottish

Highlands (WSL 1987, DOE 1988). As the concentration of acidity in precipitation has recently fallen so also has the deposited acidity. This recent decrease in acidity has occurred largely because of a decrease in precursor gases, particularly SO_2, although direct links between SO_2 emissions and acidity of precipitation are notoriously difficult to identify (Oden 1976). SO_2 emissions in the U.K. increased from one million tonnes per year in 1860 to around six million by 1970 with much of that increase taking place between the 2nd World War and the 1973 oil crisis (Fisher 1984, Chester 1987). Over that period (1945-1973), U.K. SO_2 emissions doubled whilst NO_x emissions also doubled to around 1.8 million tonnes. Since then, SO_2 emissions have declined in the UK (and over the rest of Europe) by about 40% to around three and a half million tonnes (Barrett et al. 1987, DOE 1988, 1989a, 1989b) but NO_x emissions have remained at similar levels. Thus HNO_3 forms an increasingly important constituent of acid precipitation (Barrett et al. 1987, WSL 1987).

Despite the recent slight decline in acidity, the trend over the last century has very obviously been an increase and it has been this increase in acidic deposition that has been the focus of hypotheses concerning possible causes of forest decline. Two main categories of damage assessment exist. Firstly, the damage mechanism may either be reversible canopy damage, or irreversible (or at least slow recovery) damage via the soil. Secondly stresses can be divided into:

i) <ins>predisposing</ins> stresses which chronically weaken forests and include soil moisture and nutrient status, climate, air pollutants, genetic factors and competition;

ii) inciting stresses which trigger decline and include insect attack, mechanical damage, frost, drought and air pollutants;

iii) contributing stresses which accelerate decline and primarily involve pathogens.

It is noticeable that air pollutants are involved both as predisposing stresses and as inciting stresses. A brief survey of hypotheses of forest decline reveal the main foci of research.

1. Membrane damage by ozone increases losses of nutrients leached as a result of acidic precipitation, particularly acid clouds, leading to nutrient deficiency (primarily Mg or K) and chlorophyll degradation on exposure to light. Soil acidification may exacerbate the problem by hindering resupply and droughts or pathogens may also act as inciting or contributing stresses (Krause *et al*. 1986, Prinz *et al*. 1987, Ke and Skelly 1989, Prinz and Krause 1989).

2. Low rates of Ca, Mg and Zn supply from acid topsoils are the main predisposing stresses. Ozone damage (physiological and anatomical) and increased leaching by acid precipitation, particularly acid clouds, are inciting stresses. Health can be restored by fertilization (Zöttl and Hüttl 1986, Zöttl 1987, Zöttl and Hüttl 1989).

3. Acid deposition leads to soil acidification and the release of toxic heavy metals, particularly aluminium. Root growth and nutrient uptake are adversely affected, the tree is weakened, and inciting stresses such as drought, pulses of acid deposition or defoliation trigger decline (Ulrich *et al*. 1980, Hütterman 1985, Ulrich 1985, Matzner and Ulrich 1987).

4. The chronic impact of wet and dry pollutants (particularly acid clouds - Johnson and Siccama 1983) has lowered forest vitality. Inciting stresses such as extreme pollution episodes, drought or pathogens lead to visible but unspecific symptoms on predisposed trees. The main ecological process affected is mineral cycling (Johnson and Siccama 1983, Seufert *et al*. 1989).

5. Alternative causal agents, e.g. pathogens in South Tyrol, Italy (Bucher 1987), ozone damage on Ponderosa pine in Southern California (Miller and McBride 1989) and SO_2 damage in Eastern Europe (Hutchinson 1987, Greszta *et al*. 1989), lead to localized damage and decline.

There are many other variations and syntheses of these hypotheses but it is becoming increasingly clear that:

a) there is not just one forest decline syndrome but a large number of different "diseases" affecting different species in different locations (Blank and Roberts 1987, Rehfuess 1987, Prinz and Krause 1989);

b) the role of nutrient depletion and forest nutrition appears central to the forest decline syndrome (Hutchinson 1987);

c) an increasingly important role is being ascribed to acid clouds. Precipitation inputs attributable to clouds are high (Kerfoot 1968, Lovett 1984, Milne et al. 1988), acidity is often ten times higher than in rain (Prinz pers. comm., Gervat 1985, Schmitt 1988) and forest decline is most severe at high elevation where acid clouds are most frequent (Roberts and Blank 1985, Hutchinson 1987, Bucher 1989, Prinz and Krause 1989); and

d) the present understanding of the way air pollutants affect trees is still very inadequate and much research is still needed (Andersson 1986, Bucher 1987).

This work therefore focusses on examining the following hypotheses:

a) Decreases in growth associated with forest decline are a result of processes other than a short term, direct effect of acid clouds. Subjecting Scots pine and Sitka spruce to simulated acid cloud should not lead to significant decreases in growth.

b) The damage threshold, in terms of discolouration and necrosis, of Scots pine and Sitka spruce is at an extremely low pH level, although not outwith the pH range of extreme acid cloud events.

c) Damage to needles is not limited to discolouration and internal damage and histological perturbations inside discolouring needles are substantial.

d) The intricate structure of the epicuticular wax of conifer needles, particularly the wax rodlets which are easily weathered, should be particularly sensitive to acid clouds and will fuse together or 'melt' after exposure to acid cloud.

e) Associated with the above degradation of wax; wettability and permeability to water of needles will increase.

f) The theory of cation exchange suggests higher acidity in acid clouds would lead to higher leaching of cations from needles. Nutrient losses are substantial and could have a strong effect on the nutrient status of trees exposed to acid clouds.

g) In turn these cations neutralize the acid clouds by cation exchange with H^+, thus increasing the pH of droplets in contact with the needles and reducing the risk of damage.

h) Increased leaching is compensated for by increased uptake of ions through the roots.

i) Inputs of acidity to upland regions are far larger, and extreme pH values far lower, than monitoring of the acidity of rain has shown because of higher acidity levels in clouds. Both temporal and spatial variations lead to considerable short term fluctuations in the acidity level of cloud that trees are exposed to. Increased turbulence caused by the structure of forests leads to large amounts of cloud deposition with its higher potential for damage than rain.

j) Drying droplets on needles increase in acidity through evaporation and expose the needles to extremely low pH values.

k) Trees at high elevations may be subject to short pulses of high acidity associated with snowmelt.

The experiments done to test these hypotheses involve Sitka spruce (Picea sitchensis (Bong.) Carr.) (Britain's most widely planted tree) and Scots pine (Pinus sylvestris L.) (Britain's only native commercial conifer and of particular interest with respect to native pinewoods in the UK and also widely distributed throughout Eurasia). Simulated conditions have been modelled on Scottish upland sites and field work was done locally. The observations and experiments allow direct conclusions to be drawn concerning British forestry and the possible decline of British trees.

CHAPTER 2

The effect of simulated acid cloud on extension growth and needle area of Scots pine and Sitka spruce.

2.1 Introduction

Much of the attention paid to the effects of acid precipitation on crop plants has concentrated on reductions of growth, as economic yield of crops is very often directly related to productivity. For forest trees however, surveys of damage have focussed on discolouration of needles and crown thinning, with less attention paid to growth. This is also a result of the difficulty in measuring effects of acid precipitation on the growth of mature trees.

Decreased productivity is important as a precursor to death, an influence on competitive ability, success and succession, a cause of decreased aesthetic value and a cause of economic losses. Tree growth is thus one of the most important criteria in the study of forest decline and tree vitality (Morrison 1984, Kandler 1989).

Analysis of tree rings in Sweden (Sweden 1971, Jonsson and Sundberg 1972) led to claims that tree growth had declined as a result of acid precipitation, but later field and laboratory experiments have failed to substantiate these claims (Jonsson and Svensson 1982, Morrison 1984). Various tree ring analyses and investigations of basal area throughout the world have led to opposing conclusions. Some authors supported the view that tree growth had declined in line with the increase in acidity of precipitation (Jonsson and Sundberg 1972, Johnson *et al*. 1981, Puckett 1982), whereas others found no consistent evidence of decreased productivity resulting from acid precipitation (Cogbill 1977, Strand 1980, Jonsson and Svenssen 1982). The reason for such conflicting results may well be the result of differences in the growth pattern of different species and also in the same species in different areas (Schmid-Haas 1989).

Results from controlled experiments on trees are also variable. Increased growth with increasing acidity precipitation has been found for *Pinus strobus* (Wood and Bormann 1977),*Pseudotsuga menziesii* (Lee and Weber 1979) and *Pinus banksiana* (MacDonald *et al*. 1986), whereas decreased growth with increasing acidity has been found for *Betula alleghaniensis* (Lee and Weber 1979), various broadleaves (Neufeld *et al*. 1985) and both broadleaves and conifers (Percy 1986). Both no effect (McColl and Johnson 1983, Reich *et al*. 1986) and increased growth at intermediate acidity (Lee and Weber 1979) have also been observed.

Different species thus behave very differently under the same experimental conditions (Neufeld *et al*. 1985, Percy 1986). Experimental findings for one species can, therefore, not be applied to other species without severe reservations. There is a need for work on Scots pine and Sitka spruce because of their important roles in British forests, and the lack of studies of acidic precipitation on the growth of these species.

Many experiments have been conducted on young seedlings (e.g. McColl and Johnson 1983, Neufeld et al. 1985, Percy 1986, Reich et al. 1986). There are great difficulties in setting up controlled experiments with mature trees because of the scale of experimental apparatus involved. However the use of trees past the establishment phase may well produce results which are more applicable to mature trees than results for seedlings.

The amount of nitrogen deposited by acid precipitation may also affect the growth of trees and obscure the effects of acidity. The nitrogen content of Picea abies can be increased by spraying with simulated acid rain containing high levels of nitrate (Roberts et al. 1988) as can that of Pinus strobus (Wood and Bormann 1977). Increased growth with increasing acidity of precipitation in Pinus banksiana has been ascribed to nitrogen fertilization (MacDonald et al. 1986). Under conditions of limited nutrients, seedlings exposed to simulated precipitation of low pH with its higher nitrogen content grew faster, but when nutrient solution was added to the growth medium to compensate for the lower nutrient level in the low acidity treatment, differences in growth disappeared (Raynal et al. 1982).

It is possible that growth may also be affected by acid stimulation of cell enlargement. Cell enlargement is a function of osmotic potential, hydraulic conductivity, wall yield stress and cell wall extensibility (Lockhart 1965). Cell wall extensibility has been linked to acid-induced growth whereby auxins cause proton secretion with resultant loosening of cell wall bonds and cell enlargement (Labavitch 1981). Van Volkenburgh and her co-workers have developed the theory and have found increased cell enlargement with acidic solutions and thus a faster growth rate (Cleland and Rayle 1978, Van Volkenburgh and Cleland 1980, Van Volkenburgh et al. 1985). What is therefore of particular interest is whether acidic precipitation could act as an acidic buffer leading to apoplast acidification, increased cell wall extensibility and thus increased cell enlargement and shoot elongation.

Both dwarfism and increased growth of needle have been reported. Hindawi and Ratsch (1974) claimed that misting of Pinus sylvestris with H_2SO_4 at pH 2.0 or less produced short needles but their experiments gave mixed results. Such dwarfism was not found by Wood and Pennypacker (1976) and increased needle length with increasing acidity was found by Wood and Bormann (1977).

This study therefore set out to investigate two basic growth processes; shoot elongation (which before canopy closure can be closely correlated with needle biomass, total dry matter production and stem volume (Cannell et al. 1976)) and expansion of needle area. The use of Scots pine and Sitka spruce as the experimental species provided much needed information on these two poorly studied yet important species. The composition of the applied solution was based on the composition of local precipitation samples, thus avoiding both under- and over-estimation of any nitrogen fertilization effect. The use of trees that had developed past the seedling stage allowed more reliable application of results in explaining the decline syndrome found in mature trees.

2.2 Methods.

Growth measurements were an integral part of two out of a series of four glasshouse experiments looking at tree response to simulated acid cloud (SAC).

In experiment 1 six-year-old Scots pine were subjected to SAC over a period of twelve weeks during which the trees flushed after being transferred to a warm glasshouse. Thirteen applications were given during the pre-flushing phase and twenty-four applications during the flushing phase giving a total of thirty-seven applications or 118.4 mm of precipitation. The deposition rate was 1.8 mm h^{-1} and applications lasted approximately 106 minutes. Spraying took place on Mondays, Wednesdays and Fridays. Full details of SAC chemistry and spraying techniques are given in Appendix 1.

There were five misting chambers constructed with wood and polythene sheeting and measuring 1.85 m high x 1 m x 1 m. Two chambers were given mist at pH 3.0 both before and during flushing, one was given pH 3.0 before and pH 5.6 during flushing, one pH 5.6 before and pH 3.0 during, and one pH 5.6 both before and during flushing. Full details of glasshouse conditions are given in Appendix 3.

Each chamber contained six trees.
To allocate trees to misting chambers the previous years extension growth was measured. The trees were then placed in order according to that measurement and allocated in rotation to each of the chambers. Thus all chambers contained trees with similar aggregate extension growth. Full details of the tree material used are given in Appendix 2.

To measure extension growth in experiment 1 a small mark was made with correction fluid at the base of the previous year's leader. Extension growth of the present year's leader was measured by subtracting the original length of the previous leader from the total length. Measurements were made using a finely divided (½ mm) metal ruler. When the experiment terminated the needles of the leader were harvested and counted and extension growth per needle pair calculated.

In experiment 4 7-year-old Scots pine and 7-year-old Sitka spruce were subjected to SAC over a period of two months during which time the trees flushed. There were two acidic treatments at pH 3.0 and pH 2.5 and a control treatment at pH 5.6. There were two misting chambers allocated to each of the three treatments. Each chamber contained three Sitka spruce and three Scots pine trees giving a total of twelve trees per treatment. Trees were allocated according to the previous year's extension growth as described above for experiment 1. The experiment took place in a warm glasshouse, details of which are given in Appendix 3. Full details of the tree material are given in Appendix 2.

Spraying again took place on Mondays, Wednesdays and Fridays for 106 minutes at 1.8 mm h^{-1}. A total of 27 episodes (86.4 mm) were given. Full details of SAC chemistry and spraying technique are given in Appendix 1.

The same technique for measuring extension growth was used as for experiment 1, described above. In addition to measurement of leader extension growth, the extension growth of all 1st order laterals was measured. Extension growth for Sitka spruce was not assessed.

Needle area was also measured for Scots pine in experiment 4. Forty needles were harvested from each leader and a total of 40 needles were also harvested from the 1st order laterals. Needle area was measured using a light area meter (LiCor Inc., Lincoln, Nebraska).

2.3 Results.

In experiment 1 variation within treatments was large and extension growth was not significantly different between treatments when calculated using analysis of variance (Figure 2.1a). A very similar pattern was obtained for mean extension growth per needle

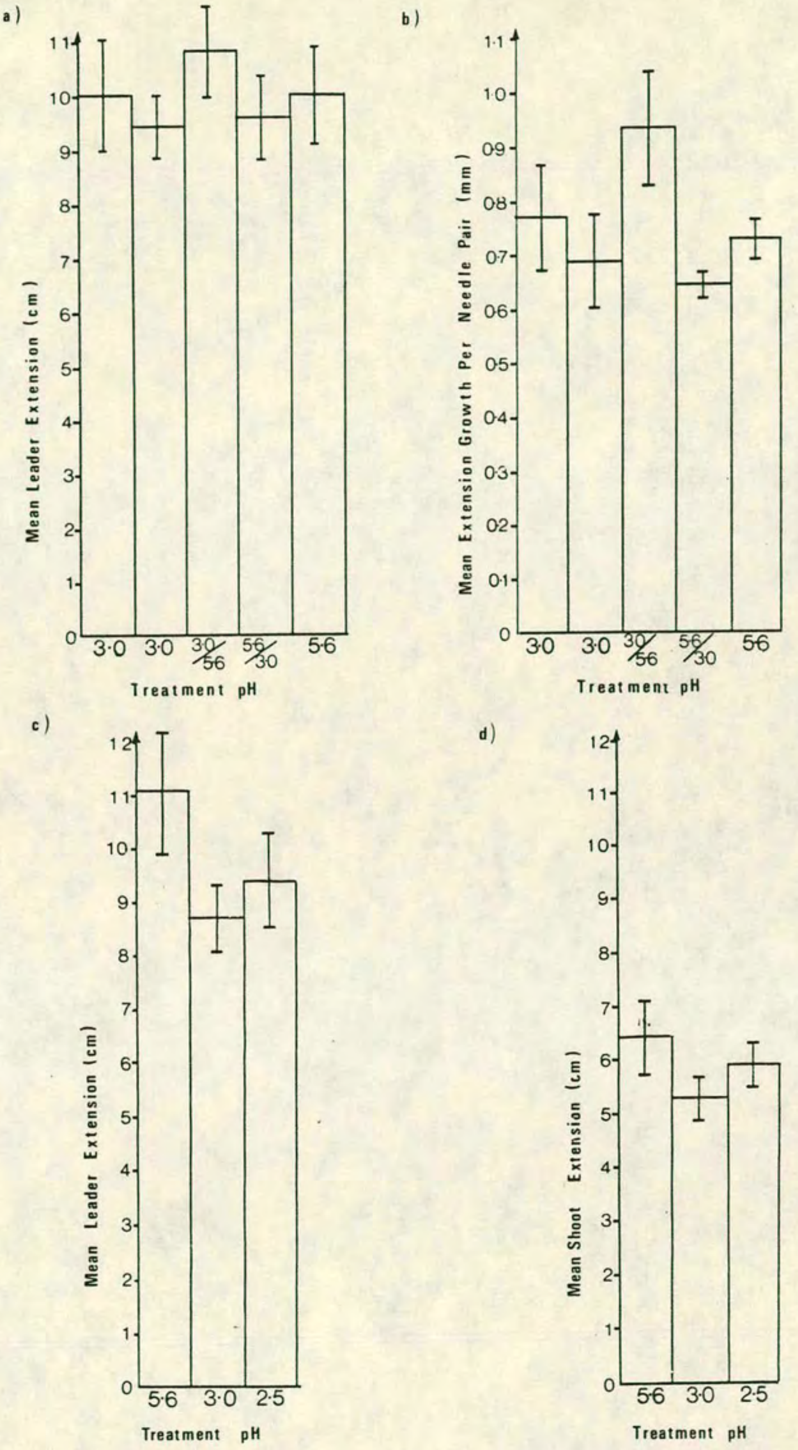


Figure 2.1a Mean extension growth(±S.E.) of Scots pine leaders in experiment 1. Figure 2.1b Mean extension growth per needle pair(±S.E.) of Scots pine leaders in experiment 1. Figure 2.1c Mean extension growth(±S.E.) of Scots pine leaders in experiment 4. Figure 2.1d Mean extension growth of Scots pine 1st order laterals in experiment 4.

pair although differences between treatments were larger (Figure 2.1b). Preliminary analysis of variance produced a non-significant F-ratio for mean extension growth per needle pair. Partitioned analysis of variance between treatments subjected to pH 3.0 during the period of extension growth and those subjected to pH 5.6 during the same period similarly yielded no significant differences. However further partitioning did show that those trees receiving SAC at pH 3.0 prior to extension growth and pH 5.6 during extension had significantly longer extension growth than trees in the rest of the treatments at $P = 0.05$.

In experiment 4 neither extension growth of leading shoots nor of 1st order lateral shoots was significantly affected by acid misting, although extension growth of both was largest for pH = 5.6 (Figure 2.1 c and d). Variation within treatment was again large.

Needle areas also showed large variation within treatment and resultant standard errors (Table 2.1). Results showed a general progressive but insignificant decrease in needle area with increasing acidity. Analysis of variance produced non-significant F-ratios for both leaders and 1st order laterals.

Table 2.1 Mean needle area ($cm^2 \pm$ S.E.) of Scots pine in experiment 4.

Treatment pH	Leading shoots	1st order laterals
5.6	0.881 ± 0.080	0.746 ± 0.081
3.0	0.815 ± 0.071	0.661 ± 0.114
2.5	0.824 ± 0.073	0.614 ± 0.028

2.4 Discussion.

Shoot growth depends on the number of stem units produced and the length per stem unit (Cannell 1979). In seedlings, the stem units, once produced, elongate shortly afterwards within the same season, whereas after the seedling stage of development the elongation process arises from stem units that have overwintered as buds (Cannell *et al*. 1976). Thus, in experiments with older trees lasting a single season, the acidity of precipitation during extension could only affect the length per stem unit and this depends on cell division and expansion.

In normal circumstances these processes are dependent on nutrition, temperature, water stress, photoperiod and premature lignification (Cannell *et al*. 1976, Cannell 1979). Nutrient leaching or altered water uptake by damaged roots caused by acidic precipitation could therefore affect extension growth. In the next chapter I show how both cells and intercellular space can be filled with unidentified deposits in response to acidity and it is possible that increasing acidity may lead to premature lignification or tannin and resin deposition which would be expected to curtail cell elongation.

Alternatively cell wall extensibility may be increased by acid deposition. Hydrogen ions have been found to increase cell extension by "acid growth" but the equipment involves leaves or coleoptiles which were either cut into strips or pieces, or rendered permeable by abrading the cuticle (Rayle and Cleland 1970, Van Volkenburgh and Cleland 1980, Van Volkenburgh et al. 1985). To understand whether acid precipitation could affect cell elongation via cell wall extensibility a better understanding of the flux of incoming hydrogen ions is needed along with precise identification of pH changes beneath the cuticle. The amount of protons in the vacuoles of both barley and Sitka spruce seedlings has been shown to increase in response to precipitation acidity but the seedlings were grown in hydroponic solution and the method of H^+ ion entry by leaf or solution was unclear (Wolfenden and Wellburn 1986, Wellburn and Wolfenden 1987).

The final amount of extension growth will be determined by the interaction between these stimulating and inhibiting effects on cell division and expansion.

One of the major disadvantages of trees or saplings, as opposed to seedlings, was that fewer replicates could be used because of limits on space available in glasshouses and funding available for spraying equipment. Even with seedlings, growth measurement often show large variation within treatments, leading to non-significant differences between treatments (McColl and Johnson 1983, Van Elsacker and Impens 1987, Van Elsacker *et al.* 1988) and in this experiment a large amount of variation in measurement of growth between trees led to large standard errors. It was therefore difficult to determine whether the observed trends were the result of random variation or of real responses that lacked significance because of low replication.

The results do not indicate a significant increase or decrease in needle or shoot growth as a result of increasing acidity. In experiment 1 extension growth of trees subjected to pH 3.0 or pH 5.6 prior to, and during flushing was very similar. Because the number of needle pairs was determined before the experiment began and extension growth occurred, it is the mean extension growth per needle pair that would change if SAC acidity was having an effect on the extension growth of the trees. This does not occur for trees subjected to continuous application of SAC of pH 5.6 or pH 3.0 but is true for SAC treatment 3.0/5.6.

The reason for increased growth in the pH 3.0/5.6 treatment is unclear. It is unlikely to be a result of "acid growth" as cell wall extensibility would be more important during the flushing phase. Neither is it likely to be the result of nitrogen fertilization as the soil was protected from incoming SAC and nitrogen was leached rather than absorbed by the trees.

Experiment 4 confirmed that SAC acidity does not have a significant detrimental effect on the extension growth of Scots pine shoots. Acid treated plants did, however, have shorter extension growth than control plants although this was not progressive with increasing acidity of treatment. Because the final effect of precipitation acidity is a trade-off between stimulating and inhibiting effects, it is often the case that intermediate pH treatments show a relative increase in growth before inhibition outweighs stimulation and leads to a decrease in growth in low pH treatments (Lee and Weber 1979, Raynal et al. 1982, MacDonald et al. 1986, Temple 1988, Ashmore et al. 1989). This stimulation of growth is often considered the result of fertilization, particularly by nitrogen, in the added precipitation (Abrahamsen et al. 1976, Shier 1986, 1987, Ashenden and Bell 1988). This is borne out both by increased nitrogen content of needles (Wood and Bormann 1977, Roberts et al. 1989b) and by the absence of a stimulation of growth after supplemental nutrients are given to all treatments (Raynal et al. 1982). It is, however, possible that altered allocation of carbon assimilates from roots to shoots may also be important (Neufeld et al. 1985, MacDonald et al. 1986, Schier 1987, Van Elsacker and Impens 1987, Temple 1988, Bennett et al. 1989),particularly as some stimulation of growth of above ground parts may take place in response to precipitation acidified only with H_2SO_4 (Lee and Weber 1979).

The insignificant decrease in needle area of the leading shoots followed a similar pattern to leader extension growth. It appears that acidity has the same effect on both needles and shoots. The observed progressive decrease in needle area of 1st order laterals with increase in acidity was not statistically significant. If, however, this decrease did reflect a real reduction in needle area it would represent a considerable reduction in photosynthetic area. This in turn could lead to reduced growth in subsequent years.

The results are in general agreement with other experiments on growth which show that conifers are rarely significantly adversely affected by precipitation acidity in short term growth experiments until the pH is reduced to about pH 2.0 (Table 2.2). Although these other experiments have largely been conducted on seedlings, which have different growth patterns, they do show a lack of growth reduction by acid precipitation at the commonly occurring pH levels of precipitation in the U.K. A lack of effects in short-term experiments was also the general conclusion of the National Acid Precipitation Assessment Programme in the U.S.A. (Lefohn and Krupa 1988). It should be noted, however, that different experimental conditions, species, and plant ages preclude blanket generalisations and individual situations continue to be in need of study.

There are few published experiments which have assessed needle area of young trees (as opposed to seedlings), and those dealing with seedlings report a large variation in response. Needle length of Pinus strobus seedlings increased with increasing acidity (Wood and

Table 2.2 Acidity levels of precipitation and above ground growth effects on conifers.

Author	Species	Age	pH at which above ground growth declines	Lowest pH at which above ground growth does not significantly decrease
Abrahamsen *et al* 1976	*Pinus ponderosa*	Mature		3.0
	Picea abies	Mature		3.0?
	Pinus sylvestris	Mature		3.0
Wood & Bormann 1977	*Pinus strobus*	Seedlings		2.3
Matziros & Nakos 1978	*Pinus halipensis*	Seedlings	3.1	3.5
Lee & Weber 1979	*Pseudotsuga menziesii*	Seedlings		3.0
	Pinus strobus	Seedlings		3.0
McColl & Johnson 1983	*Pinus ponderosa*	Seedlings		2.0
	Pseudotsuga menziesii	Seedlings		2.0
MacDonald *et al*. 1986	*Pinus banksiana*	Seedlings	2.0	2.5
Abouguendia & Baschak 1987	*Pinus banksiana*	2 yr old		2.6
	Picea glauca	2 yr old		2.6
Schier 1987	*Pinus rigida*	8 wk old		3.0
	Pinus echinata	8 wk old		3.0
Van Elsacker & Impens 1987	*Pices abies*	3 yr old		3.5
Ashenden & Bell 1988	*Picea sitchensis*	Seedlings		2.5
Temple 1988	*Sequoia gigantum*	2 yr old	2.0	2.7
	Pinus jeffreyi	2 yr old	2.0	2.7
Laurence *et al*. 1989	*Picea rubens*	Seedlings		3.1
Turner *et al* 1989	*Pseudotsuga menziesii*	1 yr old		2.1
	Pinus ponderosa	1 yr old		2.1
	Tsuga heterophylla	1 yr old		2.1
	Thuja plicata	1 yr old		2.1

Bormann 1977) whereas acid misting of *Pinus sylvestris* seedlings resulted in the production of short needles (Hindawi and Ratsch 1974). Van Elsacker and Impens (1987) obtained similar insignificant decreases in the dry weight of 3-year-old *Picea abies* needles with increasing acidity as in this experiment with needle area, and Laurence *et al*. (1989) obtained no effect of acidity on the needle dry weight of *Picea rubens* seedlings.

These results clearly indicate that the results for each species must be treated separately. Future experiments, with large enough budgets, should concentrate on long term acid applications to non-seedling individuals, have large replication and growth response measurements should be separated out and include a measurement of needle response.

CHAPTER 3

Simulated Acid Cloud and Needle Damage

3.1 Introduction: The Cuticle

The cuticle, which covers the aerial parts of all higher plants (Juniper and Jeffree 1983), is the interface between the atmosphere and the leaf. It is, therefore, the properties of the cuticle that determine the exposure of leaf cells to acid precipitation in contact with the leaf surface. The passage of substances, including pollutants, through the cuticle is controlled by the conductance of the cuticle to that particular substance and the wettability of the cuticle (Berg 1987). The properties of the cuticle are therefore pertinent to this study of the interaction between acidic precipitation and trees.

Plant cuticles consist of several layers (Figure 3.1). Epicuticular wax usually, but not always, covers the surface of the cuticle. The wax consists of long chain aliphatic compounds, typically saturated, unbranched hydrocarbons and closely related compounds with a single functional group as well as wax esters (Holloway 1971, Berg 1987). The quantity and composition of plant waxes varies between species (Percy and Baker 1987). The epicuticular wax overlies a lamellate region (sometimes called the cuticle proper (Chamel 1986), which is composed of cutin and wax.

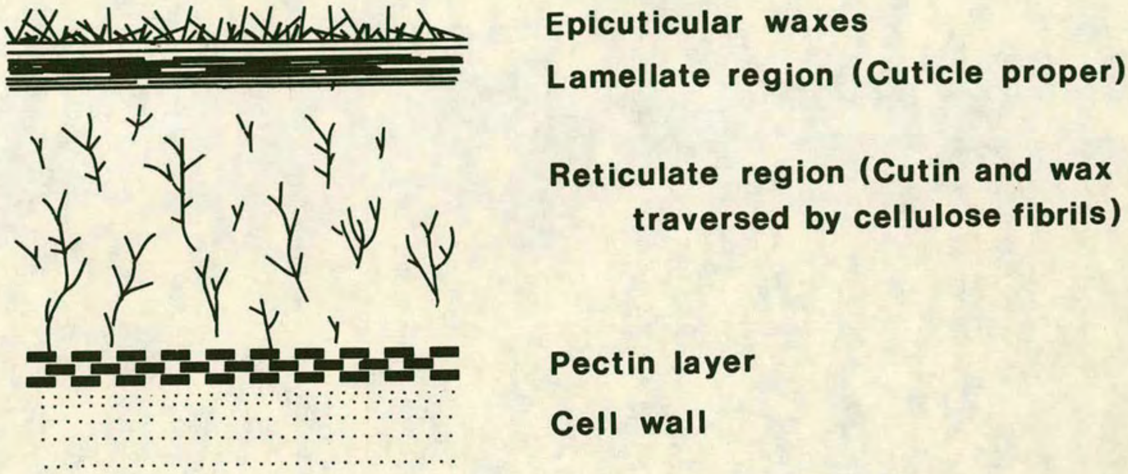


Figure 3.1 Diagrammatic representation of a cuticle

It is the cutin that gives the cuticle its mechanical strength and resistance to degradation (Berg 1987). The bulk of the cuticle is often the layer below, which is a reticulate region of cutin and wax transversed by cellulose fibrils. Below this lies a layer of pectin next to the epidermal cell walls. The cuticles of different species vary greatly in the relative thickness of these layers, and in their wax composition and structure (Wattendorf and Holloway 1980).

The variation in cuticular waxes and structure is influential in the wide variety of plant responses to acidic precipitation. However, it is clear that it is not cuticle thickness that primarily determines cuticular conductance to aqueous solutions but the amount, arrangement and type of waxes (Grace in press).

Some plants are characterised by a waxy bloom, where the epicuticular wax has a crystalline structure which reflects and scatters incoming light. The bluish tint on needles of Sitka spruce is caused by the presence of wax tubules approximately 1 µm long and 150 nm outside diameter (Jeffree et al. 1971). They are especially concentrated in the stomatal ante-chambers and form porous plugs which are instrumental in reducing transpiration (Jeffree et al. 1971, Yoshie and Sakai 1985).

Unlike the waxy plugs of Sitka spruce, the stomatal ante-chambers of Scots pine are lined rather than filled with wax tubules (Figure 3.11c). This waxy occlusion of coniferous stomatal ante-chambers occurs frequently but not universally (Hanover and Reicosky 1971). Yoshie and Sakai (1985) classified the crystalline epicuticular wax on <u>Pinus</u> species into three types.

Type I: Crystalline epicuticular waxes in the stomatal ante-chambers and stomatal bands.

Type II: Crystalline epicuticular waxes only in the stomatal ante-chambers and sparsely in the stomatal rows.

Type III: Crystalline epicuticular waxes only in small amounts in the stomatal ante-chambers.

Scots pine wax is classified as Type II, although Figure 3.2 shows that the crystalline wax structure in the stomatal bands is not particularly scarce. These stomatal bands of wax are seen under low magnification as white glaucous lines about 50-100 μm across both the adaxial and abaxial surfaces of the needles. Wide bands around 100 μm across are usually associated with double rows of stomata. The wax tubules are about 1-1.5 μm long and 60-150 nm in diameter, the diameter being difficult to assess accurately because of the gold coating on SEM specimens (Cape and Fowler 1981, Huttenen and Laine 1983, Crossley and Fowler 1986). The rest of the cuticle is covered with an amorphous layer of wax which appears darker as a result of specular rather than diffuse reflection of light (Crossley and Fowler 1986). Baker (1982) suggested that the tubules are associated with secondary alcohols, ketones and β-diketones, although other waxes are also present. In young needles the tubules are in pristine condition, but as the needles weather the tubules begin to fuse and eventually take on the same appearance as the surrounding amorphous wax (Figures 3.2 b-d).

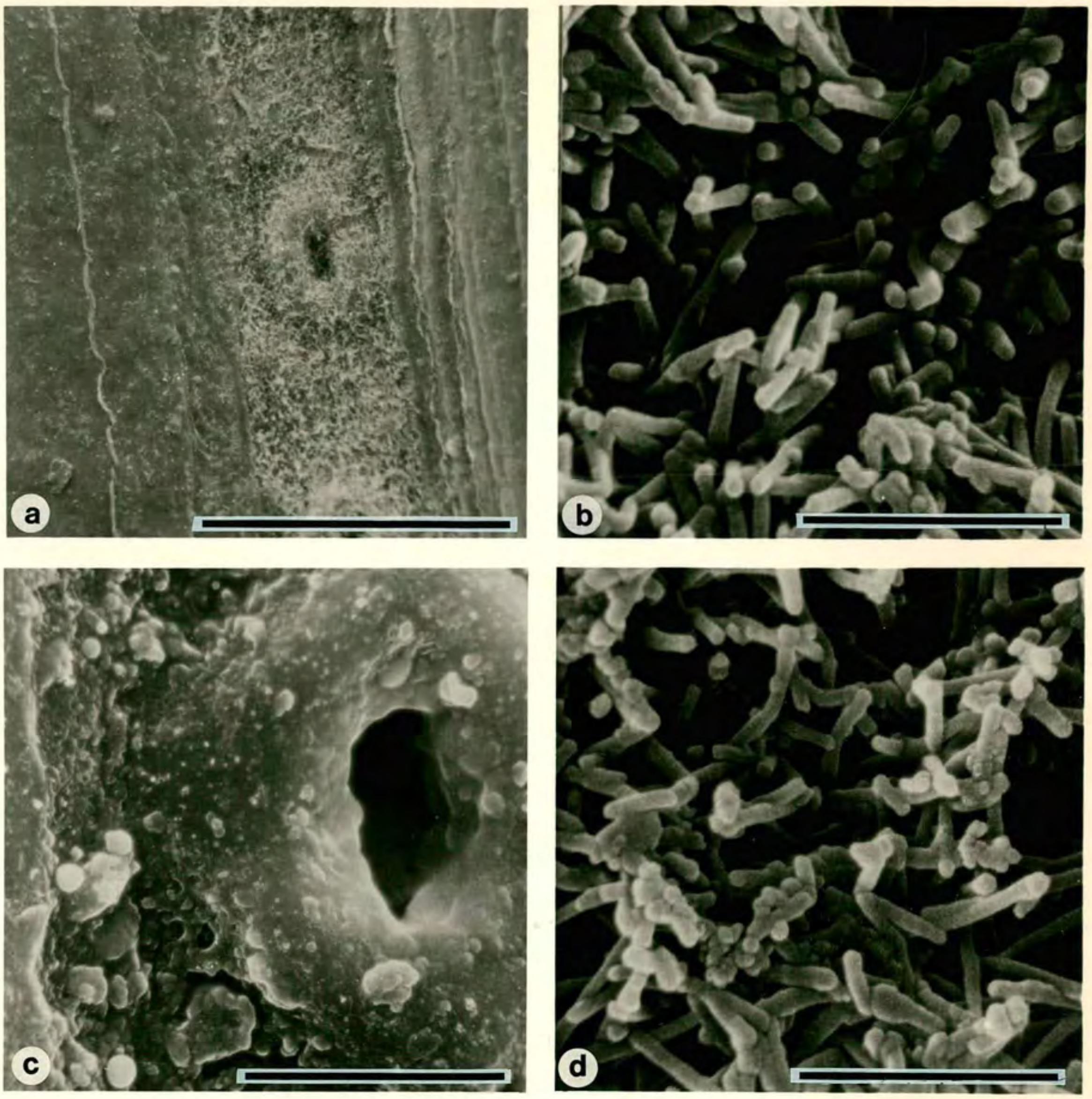
Figure 3.2. Scanning electron micrographs of the weathering of Scots pine epicuticular wax.

Figure 3.2a. Stomatal band with crystalline wax. Bar = 100 µm.

Figure 3.2b. Crystalline wax in pristine condition. Bar = 4 µm.

Figure 3.2c. Crystalline wax has "melted" and fused. Bar = 20 µm.

Figure 3.2d. Tubules of crystalline wax beginning to fuse. Bar = 4 µm.


a
b
c
d

3.2 Introduction: Needle Damage

Cuticles may be injured by damage which does not show up as necrosis or lesions. Cuticle degradation is strongly dependent on environmental conditions (Martin and Juniper 1970, Cape 1983, 1986, Crossley and Fowler 1986), but it has been suggested that air pollutants may also be important in cuticular weathering. Gaseous pollutants and particulate matter are thought to contribute to the erosion of cuticles (Percy and Riding 1978, Cape and Fowler 1981, Huttunnen and Laine 1983, Crossley and Fowler 1986, Grill et al. 1987), but authors are divided as to whether acidic precipitation leads to erosion of plant cuticles (Evans 1986, Percy 1987, Unsworth and Crossley 1987a, Adams et al. 1990, D. Turner pers. comm.). Responses of different species and even clones appear to vary (Percy 1987) and in particular there is a lack of unbiased objective analysis of cuticular condition.

The research in this chapter was therefore carried out to investigate if the epicuticular wax structure of conifers, in particular that of Scots pine, is sensitive to acid clouds, resulting in the fusion or "melting" of the wax tubules.

A further method of assessing the health and effectiveness of needle cuticles is to investigate associated cuticular functioning. Changes in both cuticular conductance and contact angles of droplets resting on the needle surface have been cited as an indication of air pollution effects (Cape and Fowler 1981, Cape

1983, Huttunen and Laine 1983, Wright and Lucas 1987, Cape 1988b, Cape et al. 1988a, Mansfield et al. 1988). There is however very little information on the effect of acid precipitation compared with that on gaseous and particulate pollutants. Changes in cuticular conductance as a result of acidity may be important in the water relations of plants and particularly in the case of conifers, with regard to winter browning. Cuticular degradation leading to decreasing contact angles increases the wettability of the needle surface, thus increasing the area over which adverse effects including leaching can occur. The research therefore sought to find out whether the permeability of water through Scots pine and Sitka spruce cuticles, and the wettability of Scots pine cuticles was increased after exposure to simulated acid cloud (SAC).

However, as well as microscopic or functional damage, acid precipitation can cause lesions and necrosis in plant tissue. Response thresholds, predisposing factors and possibly defence mechanisms combine to produce differences between species and individuals in the degree and extent of injury. In general, conifers are less sensitive to damage than broadleaves (Haines et al. 1980, Scherbatskoy and Klein 1983, Evans 1984, Jacobson 1984, Neufeld et al. 1985). However, variation in experimental technique is large and there is little information on the response of conifers other than seedlings, to acid precipitation that simulates the droplet size and ion concentrations of local cloud events.

Necrotic tissue, by definition, is non-functioning and thus reduces the photosynthetic capability of the plant. In addition, necrotic needles may be shed, tree vitality reduced, competitive ability decreased and ultimately death may occur. Necrosis may also be the end result of cell malfunctioning and thus the extreme result of otherwise non visible effects. It is, therefore, important (both as an indication of stress, and of its own effects) that necrosis caused by acid precipitation is well understood. This series of experiments sets out to identify the threshold of damage for Scots pine and Sitka spruce.

Because glasshouse conditions do not produce the same conditions as the field, some needles were artificially abraded to simulate wind abrasion in exposed sites to ascertain whether such abrasion predisposes conifers to visible damage. The study was not limited to observation of surface colour and lesions as there is a dearth of information on the internal state of needle tissue underlying conifer cuticles subjected to acid precipitation. The research aimed to identify the histological perturbations that occurred as a result of SAC, assess their relationship to external discolouration and uncover any possible defence mechanisms.

3.3 Method

3.3.1 General framework

Needle damage was assessed in four controlled glasshouse experiments. These experiments were:

Experiment 1

Six-year-old Scots pine were subjected to SAC over a period of twelve weeks from December to March during which period the trees flushed after being transferred to a warm glasshouse. Full details of glasshouse conditions are given in Appendix 3. Thirteen applications of SAC were given during the preflushing phase and 24 applications during the flushing phase giving a total of 37 applications equivalent to 118.4 mm of precipitation. The deposition rate was 1.8 mm h^{-1} and applications lasted approximately 106 minutes. Spraying took place on Mondays, Wednesdays and Fridays. Full details of SAC chemistry and spraying technique are given in Appendix 1.

Five misting chambers were constructed with wood and polythene sheeting and measured 1.85 m high x 1 m x 1 m. Two chambers were misted with SAC at pH 3.0 both before and during flushing, one was misted at pH 3.0 before and pH 5.6 during flushing, one at pH 5.6 before and pH 3.0 during, and one at pH 5.6 both before and during flushing. Visible damage was assessed between spraying and needle surface degradation examined by scanning electron microscopy (SEM).

Experiment 2

Eighteen seven-year-old Scots pine trees were allocated at random to three misting chambers constructed as for experiment

1. Each chamber was subjected to SAC of different pH, these being pH 5.6, pH 3.0 and pH 2.0. These trees were sprayed daily on weekdays for three weeks during July and August with a total of 15 applications (48 mm). Spraying technique and deposition rates were as for experiment 1. Visible damage and needle surface degredation were assessed as for experiment 1 and internal disruption examined with freeze-fractured frozen-hydrated SEM using facilities at the Botany Department, University of Edinburgh. Cuticle integrity and function were also assessed by the measurement of contact angles and cuticular conductance.

Experiment 3

144, three-year-old Sitka spruce were transferred from pots to hydroponic solution. They were allocated randomly to two treatments, each with nine replicates of eight saplings. The treatments were SAC at pH 5.6 and pH 3.0. Spraying took place on Mondays, Wednesdays and Fridays at noon with a total of 37 applications (118.4 mm) over a thirteen-week period from December to February. The experimental detail of the hydroponic equipment is given in Chapter 6. Spraying duration was again 106 minutes and details of SAC chemistry and spraying technique, tree material and glasshouse conditions are given in Appendices 1, 2 and 3. Visible damage was assessed and cuticular conductance measured.

Experiment 4

Seven-year-old Scots pine and Sitka spruce were subjected to SAC at pH 5.6, pH 3.0 and pH 2.5. Eighteen trees of each species were allocated to three treatments, each with two replicate blocks. Each block, therefore, had three Sitka spruce and three Scots pine and each treatment had two blocks. The three treatments were SAC of pH 5.6, pH 3.0, and pH 2.5 and a total of 27 episodes (86.4 mm) were given over a nine-week period from March to May. Spraying took place on Mondays, Wednesdays and Fridays at noon at the same rate as for the other experiments. Visible damage, cuticular conductance and contact angles were all measured. Details of SAC chemistry and spraying technique, tree material and glasshouse conditions are given in Appendices 1, 2 and 3 respectively.

Visible damage was also assessed on abraded needles. Needles were abraded to simulate damage at exposed field sites. Crossley (1988) has shown that needles exposed to severe climatic stress such as high wind speeds and particulate deposition undergo more severe cuticular wax structure degradation than similarly aged more sheltered needles. Three levels of damage were simulated.

i) Wax structures were smoothed by cotton wool. Two pea sized balls of cotton wool were attached to inner sides of a pair of forceps. Needles were smoothed by drawing each needle through the cotton wool balls three times. A. Crossley (pers. comm.) has shown that this is effective in smoothing the wax tubule structure of conifer needles. This was confirmed visually by the loss of the glaucous appearance of the stomatal bands indicating that the light-reflecting wax tubules had been degraded.

ii) Cuticles were disrupted by grade 100 emery paper. Two small pieces of emery paper were attached to the inner side of forceps and the needles given two light strokes resulting in cuticular disruption and damage on both adaxial and abaxial surfaces.

iii) Needles were given an "open wound". Two small cuts were made in needles using a razor blade resulting in a wound which penetrated $\frac{1}{4}$ to $\frac{1}{2}$ of the way through the needle.

Four shoots on each tree were abraded. Each level of damage was inflicted on nine needle pairs on each Scots pine shoot and fifteen single needles on each Sitka spruce shoot. Each level of damage occupied a different zone (base, lower middle, upper middle and upper) on each of the four shoots to avoid bias resulting from the position of the needles on the shoots. Small blocks of untouched needles separated the different levels of abrasion on each shoot. In addition a longer section of untouched needles was included in each shoot as a control. Needles were assessed for damage at the end of the spraying episodes.

3.3.2 Visible damage assessment

All trees were inspected for chlorosis, browning and tip necrosis between SAC episodes in all four experiments.

In experiment 2 a sample of current-year needles was taken from the pH 2.0 treatment after ten episodes. Needles were chosen randomly by taking several needles a random number of fasciles apart on randomly chosen shoots. This was repeated for several shoots for each of the six trees so that a total of 30 needles were taken. Using a razor blade needles were divided into necrotic (brown) and live tissue and the area of the two types of tissue measured with a leaf area meter (LiCor Inc., Lincoln, Nebraska). Needles with necrotic tips were counted. This process was repeated at the end of the spraying episodes.

3.3.3 Scanning electron microscopy

Needles from experiments 1 and 2 were examined by scanning electron micrscopy (SEM). In experiment 1, ten needles each of current and current + 1 shoots from both treatments (pH 5.6 and pH 3.0) were taken at random.

In experiment 2 a total of 105 needles were examined. Ten needles each of current and current + 1 shoots were taken from each treatment (pH 5.6, 3.0 and 2.0) for examination of the abaxial surface. Another ten current year needles were chosen from shoots in treatment pH 2.0 for examination of necrotic areas. Five needles each from current and current + 1 shoots for each treatment were taken for examination of the adaxial surface, with an extra five current year needles from treatment pH 2.0 for examination of necrotic areas.

These needles were removed with a pair of precision forceps and secured by the base and tips on prepared ridges of card covered with double-sided sellotape at the base and tip. The central untouched sections of the needles were fixed to SEM stubs using double-sided sellotape. The ends of the needles projecting beyond the stubs were trimmed and the stubs engraved with an identification code. Specimens were then dried over P_2O_5 in desiccators before being sputter-coated with gold in an argon atmosphere (53 Pa, 1.5 kV 25 mA) using a water-cooled target and specimen stage.

All specimens were examined using an SEM (Stereoscan IIA, Cambridge Instruments Ltd., Cambridge, UK.) operated at 10 kV, 0° tilt and 120-150 µA beam current. Two photographs of each needle were taken, one at low magnification (c. X 550) and one at high magnification (c. X 2,200). The photographic system on the SEM produced one negative film and one positive (slide) film simultaneously. The high magnification shot was centred on the central stoma in the low magnification picture and was a random representation of wax structure for the needle. Stubs were identified after photography to avoid bias resulting from fore-knowledge of treatment.

To assess wax structure, an arbitrary scale of zero to five, representing pristine structure to amorphorus structure, was used. The slides of each image recorded were projected onto a screen without fore-knowledge of treatment. Scoring was done independently by myself and another observer with considerable experience (Dr A. Crossley). Scoring records showed consistent agreement between the two observers (Appendix 5). Scoring was not undertaken for experiment 1.

3.3.4 Freeze-fractured, frozen-hydrated SEM

Sections of needles were placed onto slots cut in the top surface of copper specimen stubs and fixed using the cryo adhesive Tissue Tek 11 OCT compound (Lab-Tek Products, Division Miles Lab. Inc., Naperville, Ill., USA). Within one minute the specimens were then cryofixed by immersion in nitrogen slush at -210 °C under dry argon gas in a cryo-prep system freezing chamber (SP2000, Emscope Ltd., Ashford, Kent, UK.). After transfer, under vacuum, to the work chamber cold stage, the specimens were fractured with a pre-cooled pointed probe. Fractured specimens were then immediately sputter coated with gold in an argon atmosphere and then transferred to and examined in an SEM (S250, Cambridge Instruments Ltd., Cambridge, UK) working at an accelerating voltage of 6 kv. Both live and necrotic tissue was viewed and photographed and needles with small brown spots were also examined, with the intention of investigating the initial stages of damage.

3.3.5 Cuticular conductance

Hygen (1951, Hygen and Midgaard 1954) developed a technique of periodically weighing excised shoots, needles and leaves and graphing water loss against time. He proposed that the reduction in weight could be divided into a relatively short initial phase when stomata were open and water loss was rapid, a transition phase as stomata closed, and a slow phase when water loss was solely through the cuticle. The method has since been used sporadically but recently has received more frequent use both in investigations of plant response to weathering by normal environmental processes (wind, abrasive rubbing), Grace (1990), and also by air pollution (Fowler et al. 1981, Wright and Lucas 1987, Ziegler 1987, Cape 1988, Mansfield et al. 1988). Grace (1990) assessed the reliability of the technique. Although it is difficult to prove stomatal closure is complete, the transition from phase one to phase three is usually clear and strong enough to suggest full closure of the stomata. As relative water content decreases, evaporation may not occur from immediately under the cuticle and the diffusion gradient may undergo small changes. However, Grace (op. cit., pers. comm.) concludes that the method is worth pursuing and useful in investigating differential water loss between plants with differences in cuticular weathering.

Preliminary experiments by myself and Grace had shown that a longer period than generally allowed by Hygen was necessary to produce a reliable estimate of cuticular conductance. In experiment 2, ten Scots pine needle pairs were taken from each treatment (including ten from both brown necrotic needles and green needles at pH 2.0). In experiment 3, fifteen Sitka spruce needles and in experiment 4, fifteen Scots pine needle pairs, were taken from each treatment. These were placed carefully in a polythene bag and sealed. Using a sensitive pair of light precision forceps they were removed and placed individually into small vessels, one third full of water, and left overnight in a cool shaded place to hydrate. In the morning they were gently dried on filter paper and the base of the needles sealed with silicone rubber (555-588, R.S. Components, Belgium). Needles were then weighed to the nearest 0.01 mg on an electric microbalance (Model 4700, Cahn, Cerritos, Ca., USA) and transferred to a controlled environment chamber. Sitka spruce needles were placed across two strings and Scots pine needle pairs suspended vertically on one string. Conditions in the controlled environment chamber were: irradiation, 400 W m^{-2}; temperature, 15-16 °C; vapour pressure deficit, 1.24 ± 0.03 kPa; and airflow, 0.1 m s^{-1}. Needles were then weighed morning and evening for around 5 days.

The surface areas of needles were measured using a film of small glass balls (Thompson and Leyton, 1971). Needles were balanced on fine wire and coated with an extremely thin layer of adhesive Scotch spray mount (3m, Bracknell, UK). Ballotini glass balls (diameter of 0.08-0.115 mm) (Grade 14, Jencons (Scientific) Ltd, Leighton Buzzard, UK) were then poured over the needle surface forming a monolayer of glass balls stuck to the needle surface. Needles were weighed before and after this treatment so that the gain in weight was directly proportional to the area of the needle. Weight gain was calibrated by coating accurately measured pieces of insulated wire of various lengths with identical balls. The following equation was obtained and used:

$$\text{Area (cm}^2\text{)} = 55.9962 \times \text{weight of balls (mg)}$$

Conductance was calculated from

$$Gc = \frac{W}{t} \times \frac{P}{D}$$

where:

W/t = rate of water loss (mol m^{-2} s^{-1})
D = vapour pressure deficit (Pa)
P = atmospheric pressure (Pa)

3.3.6 Contact angles.

Contact angles of droplets on needles were measured in experiments 2 and 4. Ten current year needles from each Scots pine tree were taken in experiment 2 and ten current and ten current + 1 needles were taken from each tree in experiment 4. Live and necrotic needles of the pH 2.0 treatment were treated separately and ten replicates of each taken per tree. The needles were held under a stereomicroscope with forceps and a 1 mm^3 droplet of glass distilled water was placed in the abaxial surface using a 10 mm^3 teflon coated pipette (Scientific Glass Engineering Ltd., Australia). Contact angles were viewed tangentially through the microscope (x50) which was fitted with a protractor graticule to measure the angle.

3.4 Results

3.4.1 Visible injury

Experiment 1.

After a total of 37 mist events there was no visible damage on any Scots pine needles subjected to simulated acid cloud (SAC) at pH 5.6 or pH 3.0.

Experiment 2.

After fifteen applications neither the control needles (pH 5.6) nor the needles treated at pH 3.0 showed any visible signs of injury.

However, small brown lesions started to appear on needles subjected to SAC at pH 2.0 after six misting episodes (eight days) (Fig. 3.3a). They were more commonly found on the abaxial surface rather than the adaxial, but very quickly spread through the needle to the opposite surface. Lesions were, therefore, rarely limited to one side of the needle but were larger on the abaxial side. After ten episodes, (fourteen days), the lesions had grown and merged and 14.4% of the first year needle surface was brown and necrotic. Damage tended to progress from needle tip to base and 88% of affected needles had necrotic tips. Often the needle tip and adjoining area were completely brown with smaller spots occurring further down the needle. By the end of this treatment (fifteen episodes) almost all needles exhibited some brown areas (Fig. 3.3b). Smaller lesions had generally merged with the advancing edge of damage moving back down the needle tip. The brown, damaged area accounted for 31% of the needle surface area. Damage continued to spread for several days after spraying ceased until a clear demarcation appeared between brown, damaged tissue and green, undamaged tissue. This demarcation was represented by a darker brown band separating the two zones (Fig. 3.4a).

Damage on second year needles took longer to appear, affected fewer needles and smaller areas. Figure 3.4b shows the contrast between first year needles, of which very few escaped injury, and the second year needles, with much less injury.

Figure 3.3a Scots pine needle with acid lesions.

Figure 3.3b Scots pine needles showing extensive damage.

Figure 3.4a Scots pine needle showing live tissue, acid damaged tissue and transition zone.

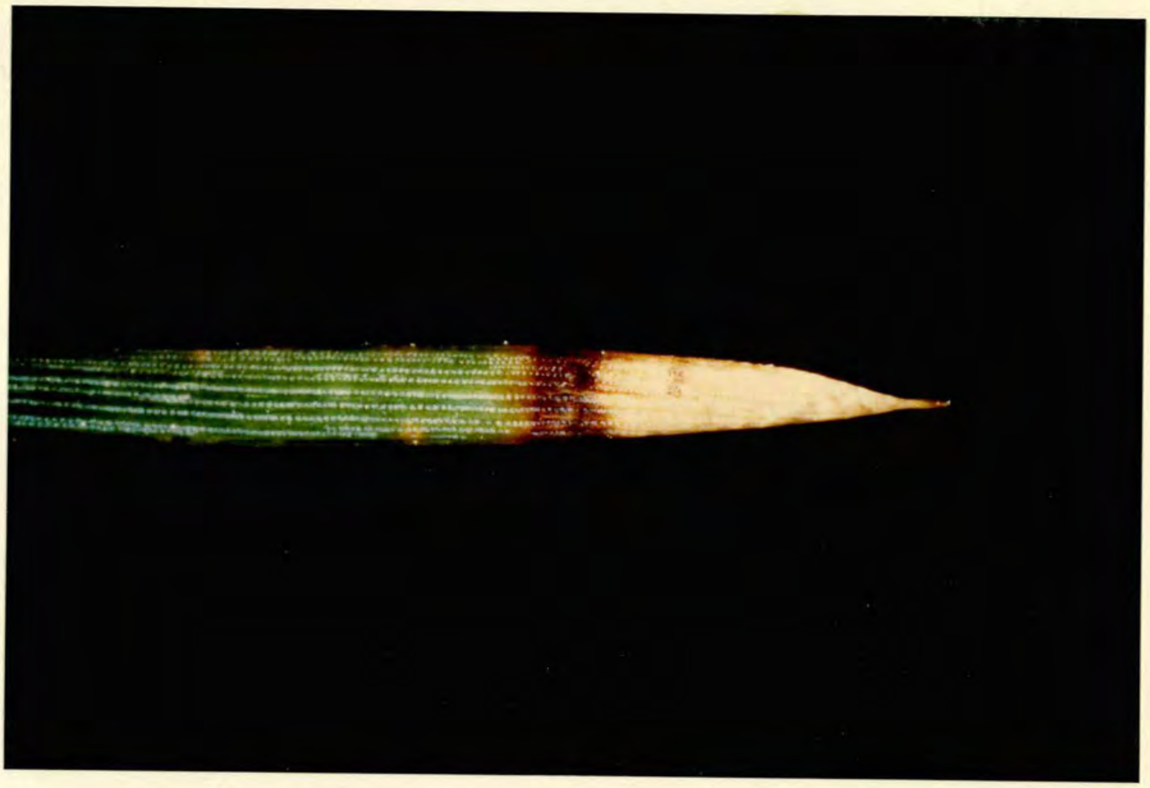


Figure 3.4b Current year needles show extensive damage whereas current + 1 needles still have green tips.

Experiment 3.

Because of infestation by mites during the experiment, it was not possible to assess whether Sitka spruce seedling needles were completely free from injury. However, before the infestation began, there was no evidence of damage to needles.

Experiment 4.

There was no lesion injury apparent on Scots pine needles in any pH treatment (5.6, 3.0 or 2.5). The cuticle of a small minority of needles subjected to abrasion by emery paper was so disrupted that the needles had dehydrated and died but this occurred to the same degree in all pH treatments. Similarly some needles cut by razors also died, and again this occurred to the same extent in all treatments. There was no brown spotting of needles and no tip necrosis associated with abrasion damage on needles.

On Sitka spruce needles there was no damage at pH 5.6 or 3.0 and damage was extremely limited at pH 2.5 affecting only 0.5% of the needles. The damage consisted of small brown lesions on the needles and some tip necrosis. Damage to previous year's needles was slightly greater than the damage to the present year's needles. There was no increase of acid damage in the pH 2.5 treatment on those needles which had been previously abraded.

3.4.2 Needle surface degredation: SEM

Current year needles were characterised by tubules of crystalline wax covering the areas of stomatal bands and partially filling the stomatal antechambers (as in Figure 3.2). Tubules were more often in pristine condition in experiment 1 than experiment 2 because of the shorter period since formation of new needles. Current + 1 needles exhibited extensive fusion and thickening of the crystalline wax tubules and in many areas little or no evidence of the original structure was left. Fungal hyphae were observed on a small number of needles but there was no increase in these hyphae in the high acidity treatments.

No scoring took place in experiment 1 but fusing and thickening of tubules appeared to take place at the same rate and to the same degree in all treatments. The development of wax structure on needles on shoots which flushed during treatment with SAC (pH 3.0) did not appear to be affected or delayed.

Results from experiment 2 indicated that the wax structure of current year needles on the adaxial surface remained close to pristine condition whereas abaxial surfaces had undergone a small but significant amount of erosion (Table 3.1, Figure 3.5 c.f. 3.6 and 3.8). Current + 1 needles were moderately degraded on the adaxial side and moderately to severely

Figure 3.5	Comparison of Scots pine needle surfaces from control treatment (pH 5.6) and after treatment with simulated acid cloud at pH 2.-0. Adaxial surfaces of current year needles.
Figure 3.5a	Crystalline wax in stomatal bands of control needle. Bar = 100 µm.
Figure 3.5 b&c.	Wax tubules of control needles. Bar = 20 µm.
Figure 3.5d.	Crystalline wax on acid (pH 2.0) damaged area of needle is in pristine condition. Bar = 100 µm.
Figure 3.5 e&f.	Wax tubules from damaged needles show no evidence of melting and fusing. Bar = 20 µm.

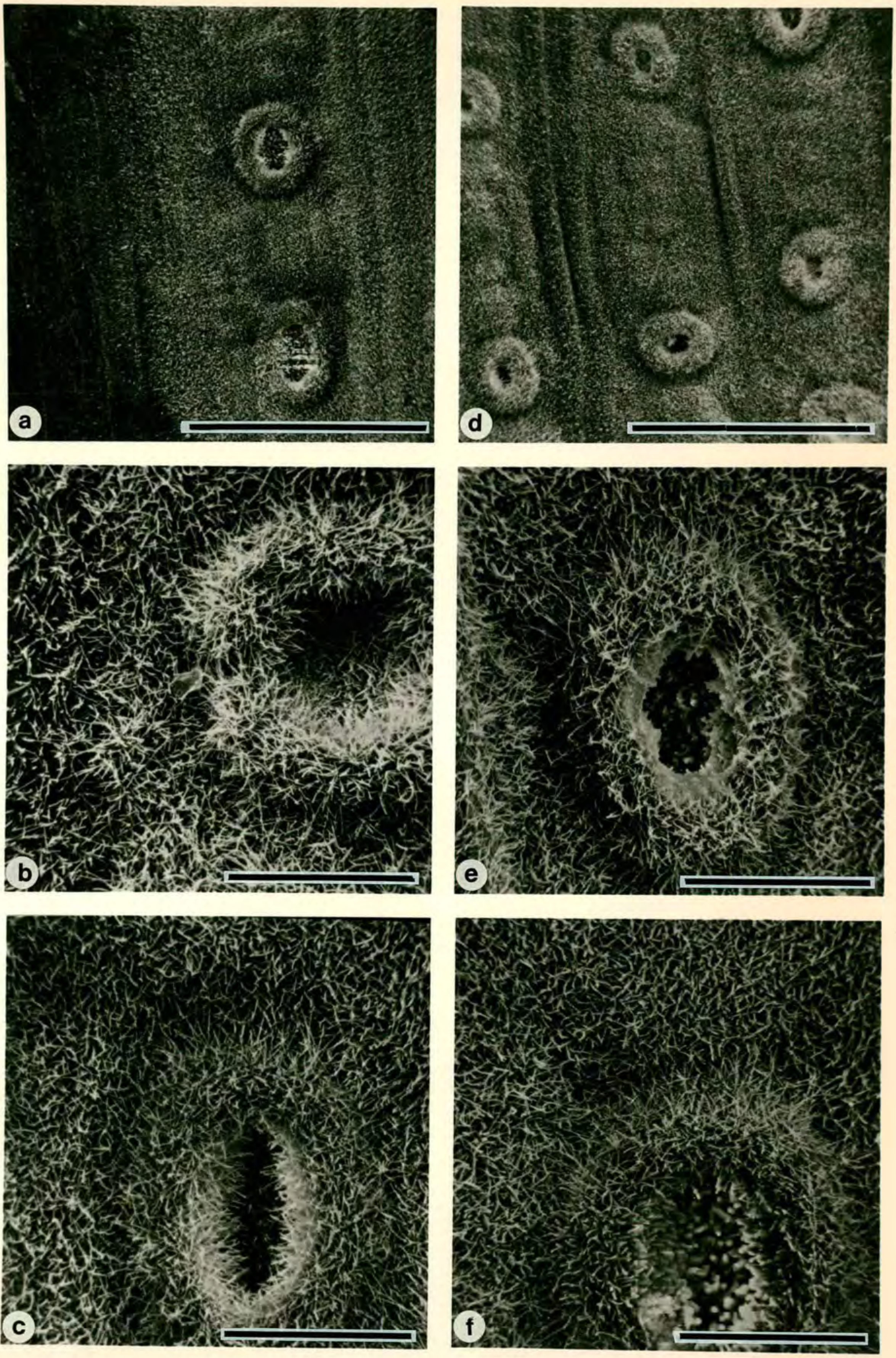
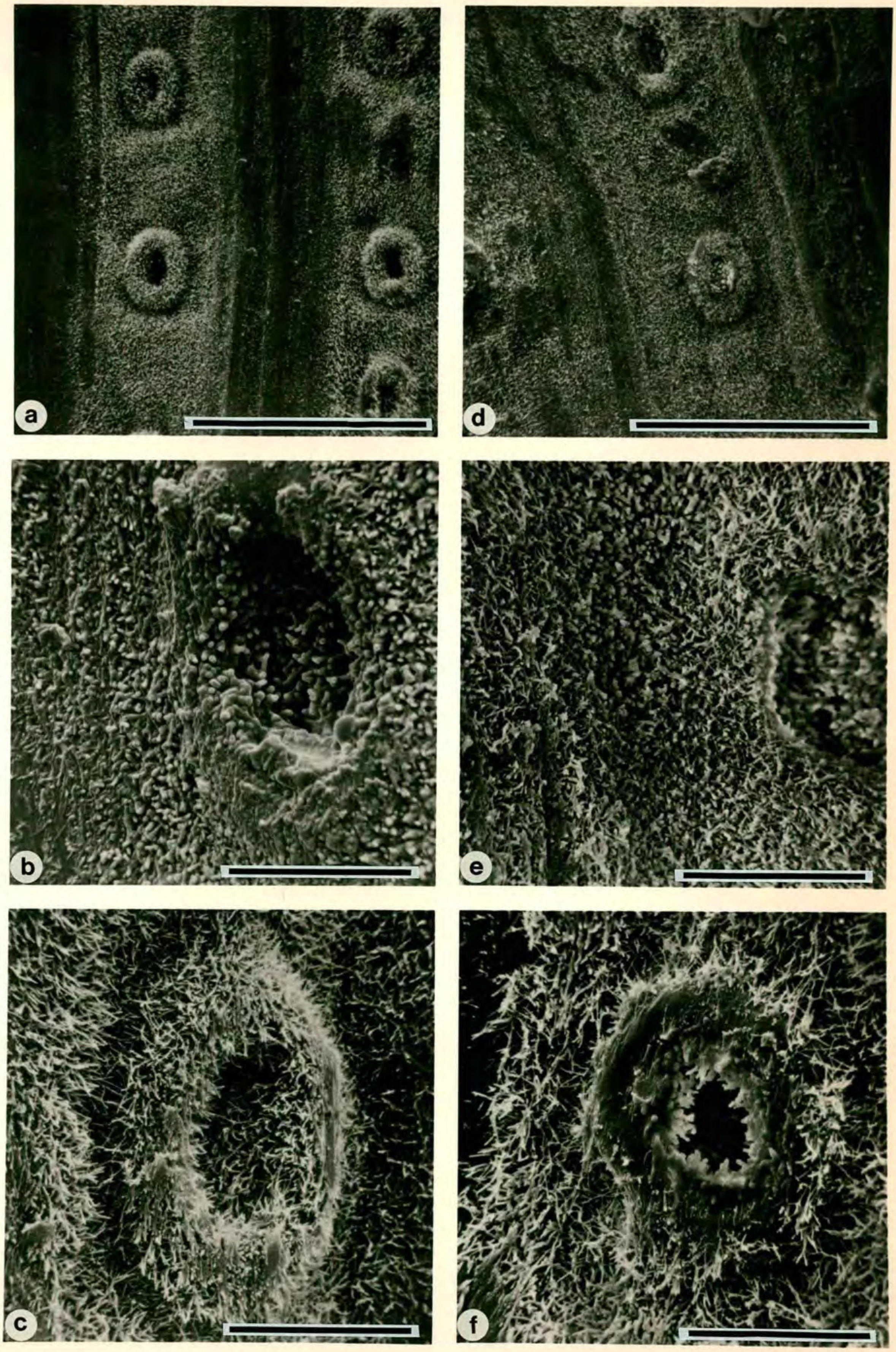

a
d
b
e
c
f

Figure 3.6.	Comparison of control needles and needles subject to simulated acid cloud at pH 2.0. Abaxial surfaces of current year needles.
Figure 3.6a.	Crystalline wax in stomatal bands of control needle. Bar = 100 µm.
Figure 3.6 b&c.	Partially fused tubules of wax of control needle. Bar = 20 µm.
Figure 3.6d.	Crystalline wax in stomatal band of needle subject to SAC at pH 2.0. Bar = 100 µm.
Figure 3.6 e&f.	Wax tubules of acid damaged needles. Bar = 20 µm.


a
d
b
e
c
f

degraded on the abaxial surface (Figure 3.7). No significant increase in erosion or degradation of wax structure occurred as a result of SAC acidity (Table 3.1). Epicuticular wax covering brown necrotic areas of needles was still intact and showed no signs of increased fusing, "melting", "crumbling" or erosion (Figures 3.5 and 3.6). Some artefacts were observed which without careful observation could have been misconstrued as acid damage (Figure 3.8).

Table 3.1 Mean scores (± S.E.) of wax structure of Scots pine needles from experiment 2. Needles with wax in pristine condition are scored at 0, totally degraded wax is scored at 5.

Current	pH 5.6	3.0	2.0	2.0
			(green tissue)	(brown tissue)
Adaxial	0.60±0.16	1.20±0.25	1.60±0.16	0.60±0.22
Abaxial	1.50±0.26	1.80±0.16	2.10±0.16	2.06±0.21
Current +1				
Adaxial	2.80±0.13	2.80±0.25	2.60±0.22	
Abaxial	3.60±0.11	2.89±0.21	3.65±0.11	

3.4.3 Internal damage of needles examined by freeze-fractured, frozen-hydrated SEM.

When frozen needles were struck by the pointed probe live

Figure 3.7. Current + 1 year needles of control and pH 2.0 treatment showing considerable degradation of wax structure. All Bars = 20 µm.

Figure 3.7a. Adaxial surface of control needle.

Figure 3.7b. Adaxial surface of needle subject to pH 3.0.

Figure 3.7c. Adaxial surface of needle subject to pH 2.0.

Figure 3.7d. Abaxial surface of needle subject to pH 2.0.

Figure 3.7e. Abaxial surface of needle subject to pH 3.0.

Figure 3.7f. Abaxial surface of control needle.

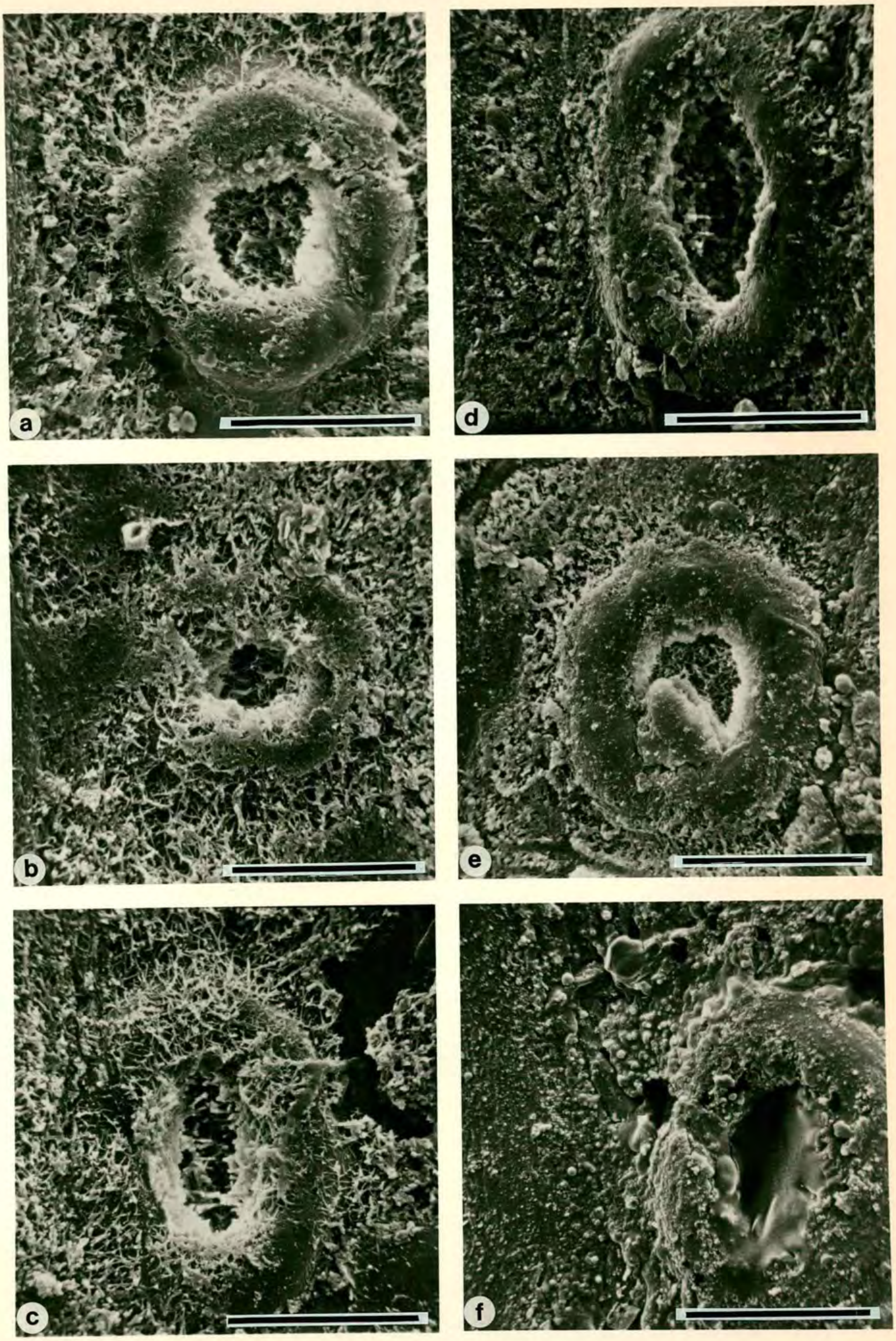

a
d
b
e
c
f

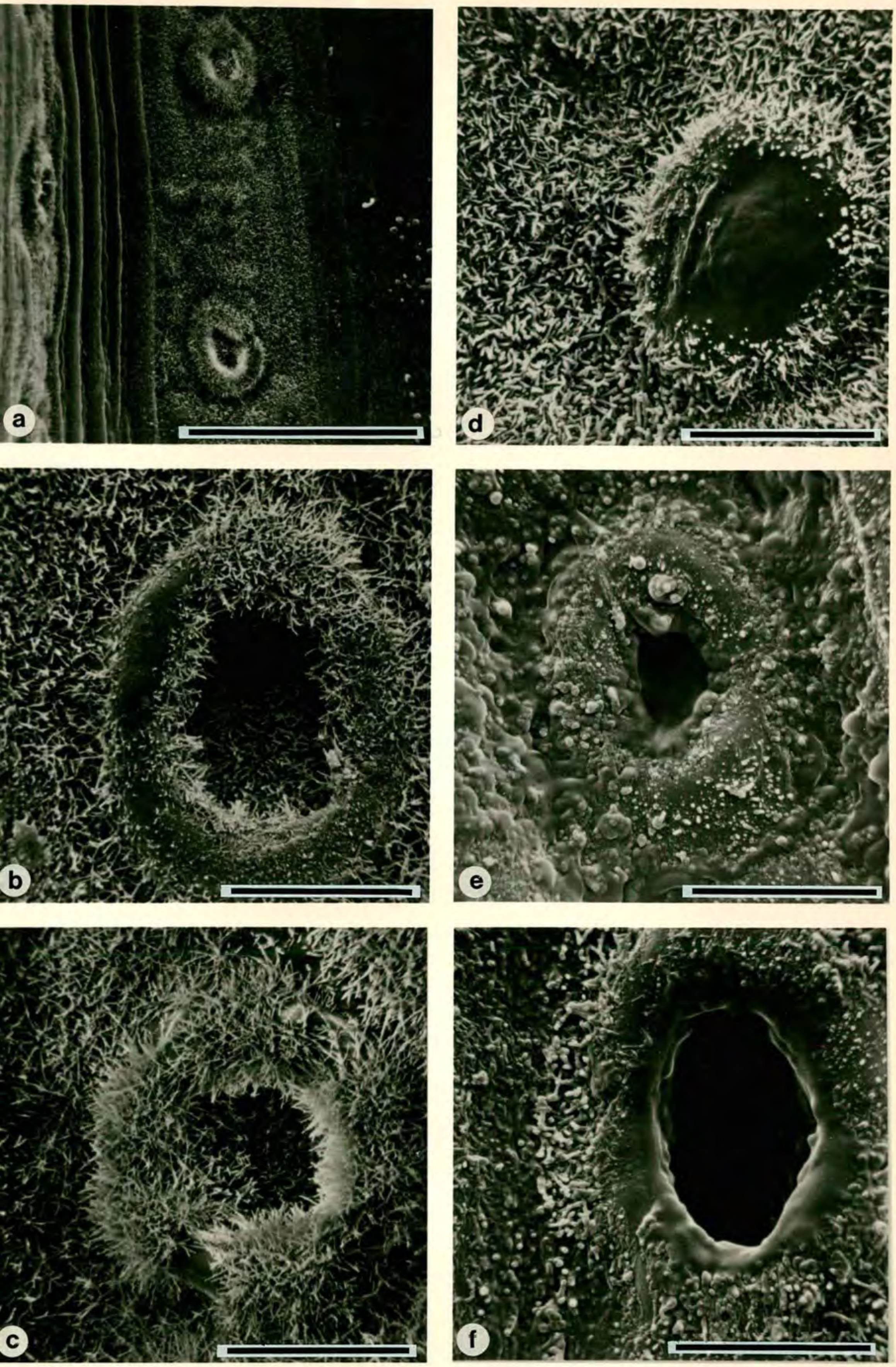
Figure 3.8a-c. Current year needles subjected to SAC at pH 3.0.

Figure 3.8a. Crystalline wax in stomatal band. Adaxial surface. Bar = 100 µm.

Figure 3.8b. Wax tubules of abaxial surface.

Figure 3.8c. Wax tubules of adaxial surface.

Figure 3.8d-f. Artefacts. What appears as extremely "melted" wax around the rim of the stomal antechambers is actually a substance (possibly resin) exuded from inside the needle. The surrounding wax tubules are clearly distinct from the artefact. Bar = 20 µm.


a
d
b
e
c
f

tissue fractured easily but brown tissue was harder to split cleanly and tended to crumble. Control needles were characterised by turgid undeformed cells with easily identified intercellular spaces. Chloroplasts were easily distinguished and numerous (Figure 3.9a+b). Histologically, needle tissue was easy to differentiate into epidermal, palisade mesophyll, spongy mesophyll and vascular tissue (Figure 3.10a+b). Guard-cells and stomatal ante-chambers were clear and distinct (Figure 3.9a). Although the fracturing process made it difficult to assess vascular tissue, it appeared as healthy without signs of crumbling or disruption.

Green tissue subject to SAC even at pH 2.0 showed no differences from control needles. Cells were turgid, with distinct intercellular spaces, the vascular tissue was apparently healthy and stomatal apparatus showed no signs of damage (Figure 3.11a-c).

Tissue underlying brown areas of the needles was subject to complete collapse. Figure 3.12a shows the clear difference between live and necrotic tissue and Figure 3.12b-d displays more closely the change. Examination of tissue below brown lesions showed that damage began with the isolated collapse of epidermal and palisade cells just below the cuticle (Figure 3.13a). Damage may then spread along the underside of the cuticle (Figure 3.13b) or penetrate through to the spongy

Figure 3.9. Scanning electron micrographs of frozen-hydrated freeze fractured Scots pine needle.

Figure 3.9a. Stomatal apparatus of control needle. Bar = 40μm.

Figure 3.9b. Easily identifiable chloroplasts of control needle. Bar = 20 μm.

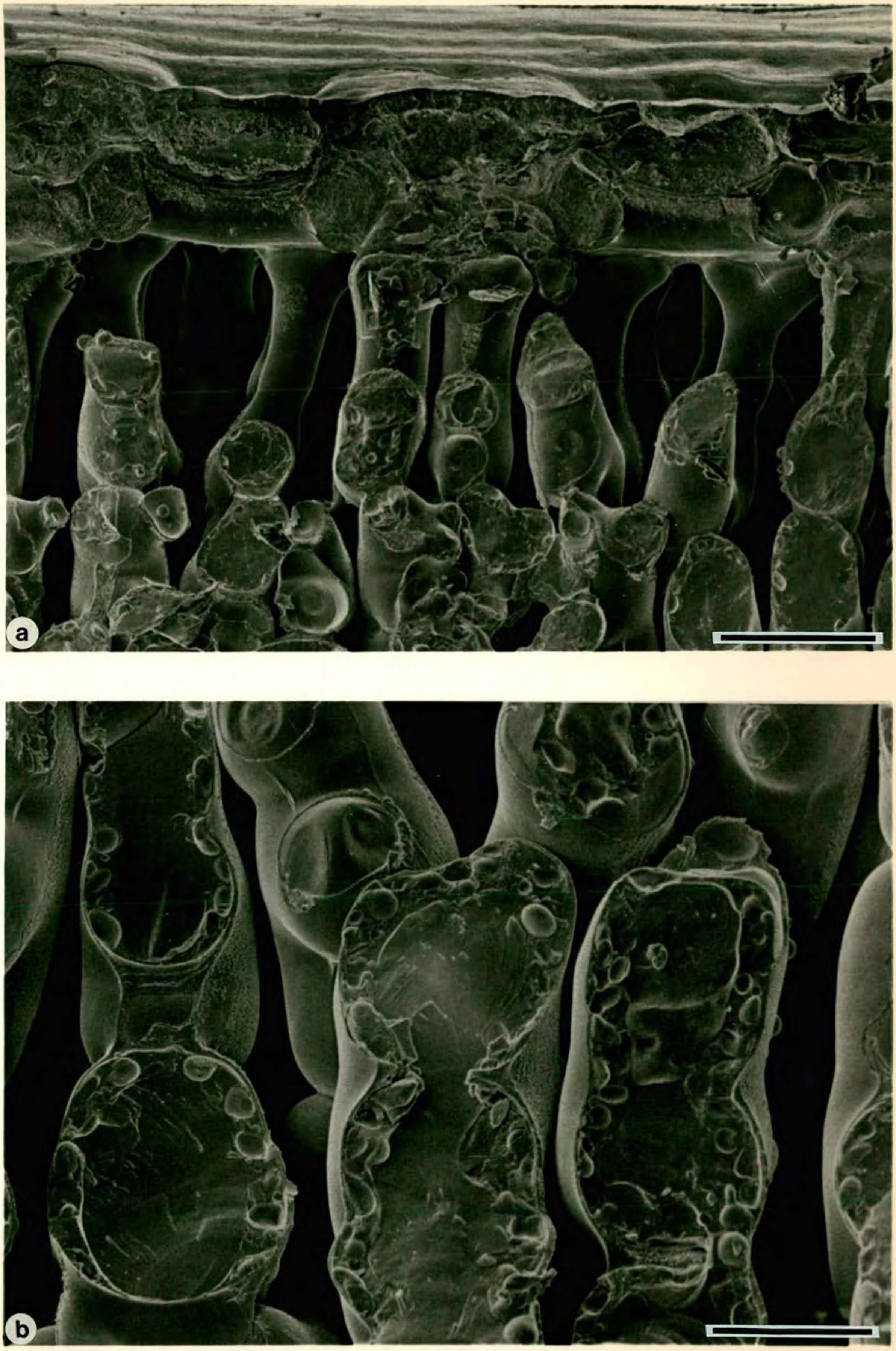

a
b

Figure 3.10a. Cross section of control needle showing epidermal mesophyll and vascular tissue. Bar = 200 µm.

Figure 3.10b. Clear intercellular spaces between cells of control needle. Bar = 40 µm.

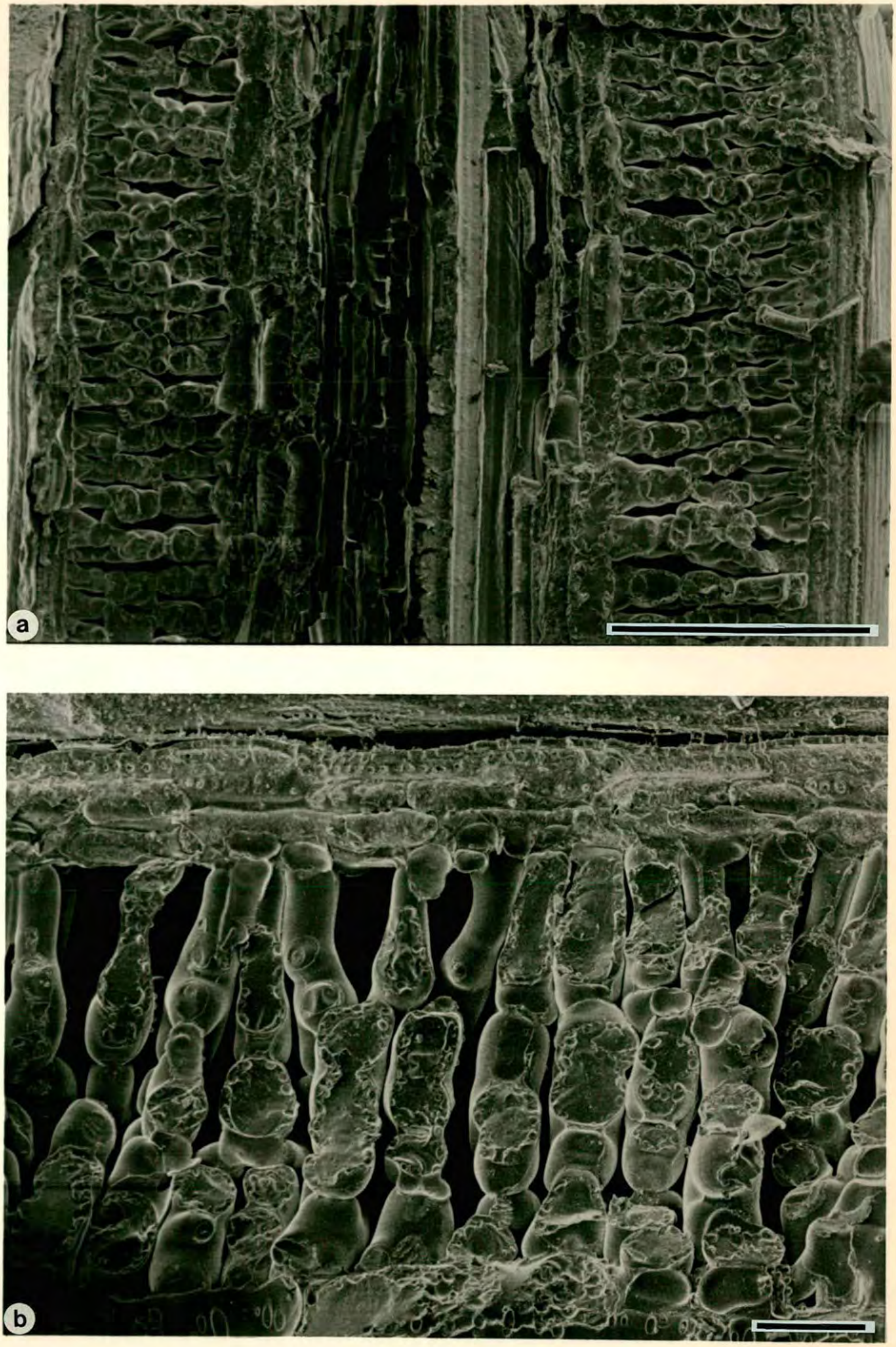

a
b

Figure 3.11 a&b. Cross sections of green tissue from pH 2.0 treatment showing healthy cells and clear intercellular spaces. Bar = 100 µm.

Figure 3.11c. Stomata of green tissue from pH 2.0 treatment with no sign of damage. Bar = 20 µm.

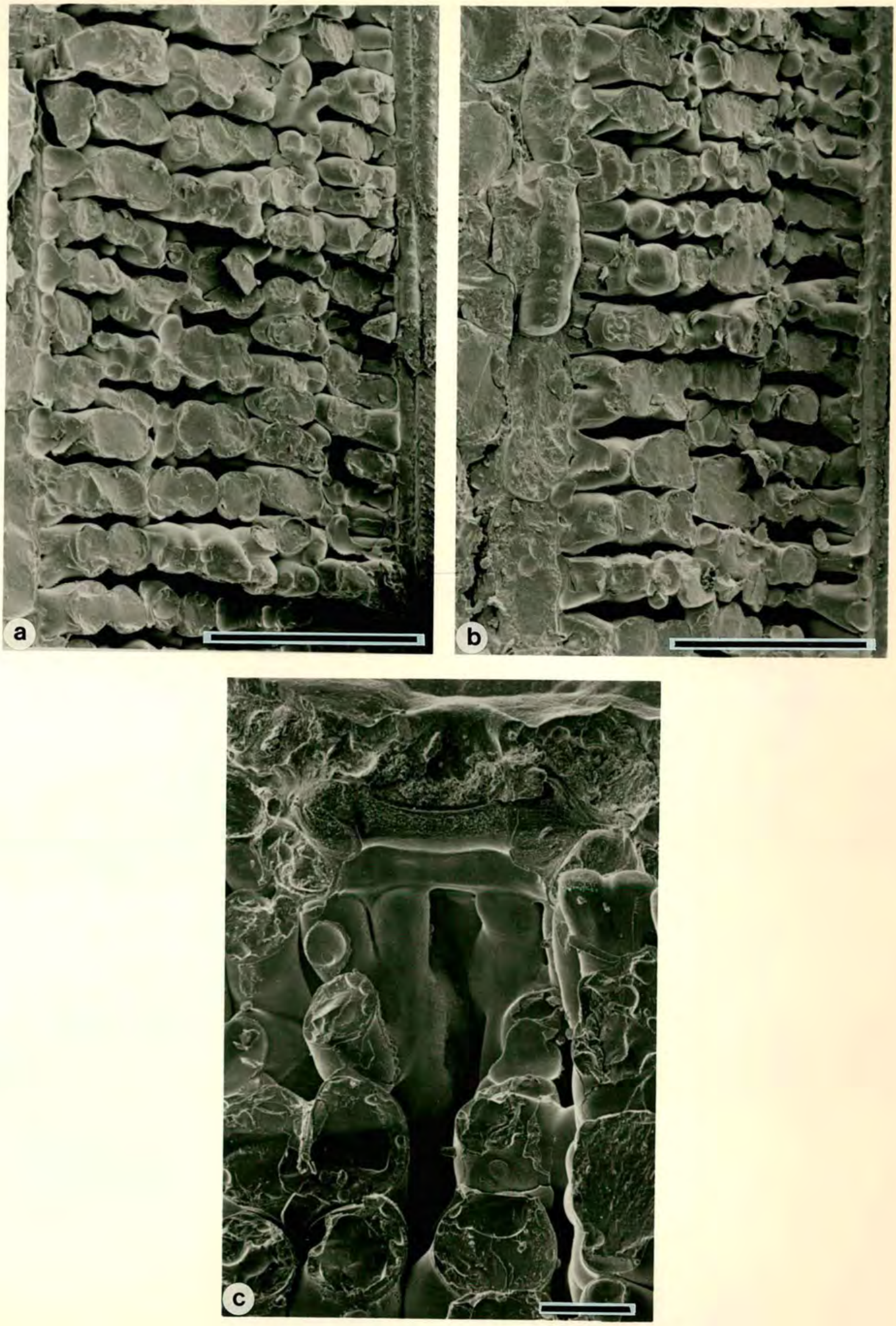

a
b
c

Figure 3.12a. Cross section of damaged needle showing transition from live to necrotic tissue. Bar = 400 μm.

Figure 3.12b. Healthy tissue corresponding to green area of needle. Bar = 200 μm.

Figure 3.12c. Transition zone between green and brown areas of needle corresponding to dark brown band. Bar = 200 μm.

Figure 3.12d. Collapsed cells of necrotic brown area of needle. Bar = 200 μm.

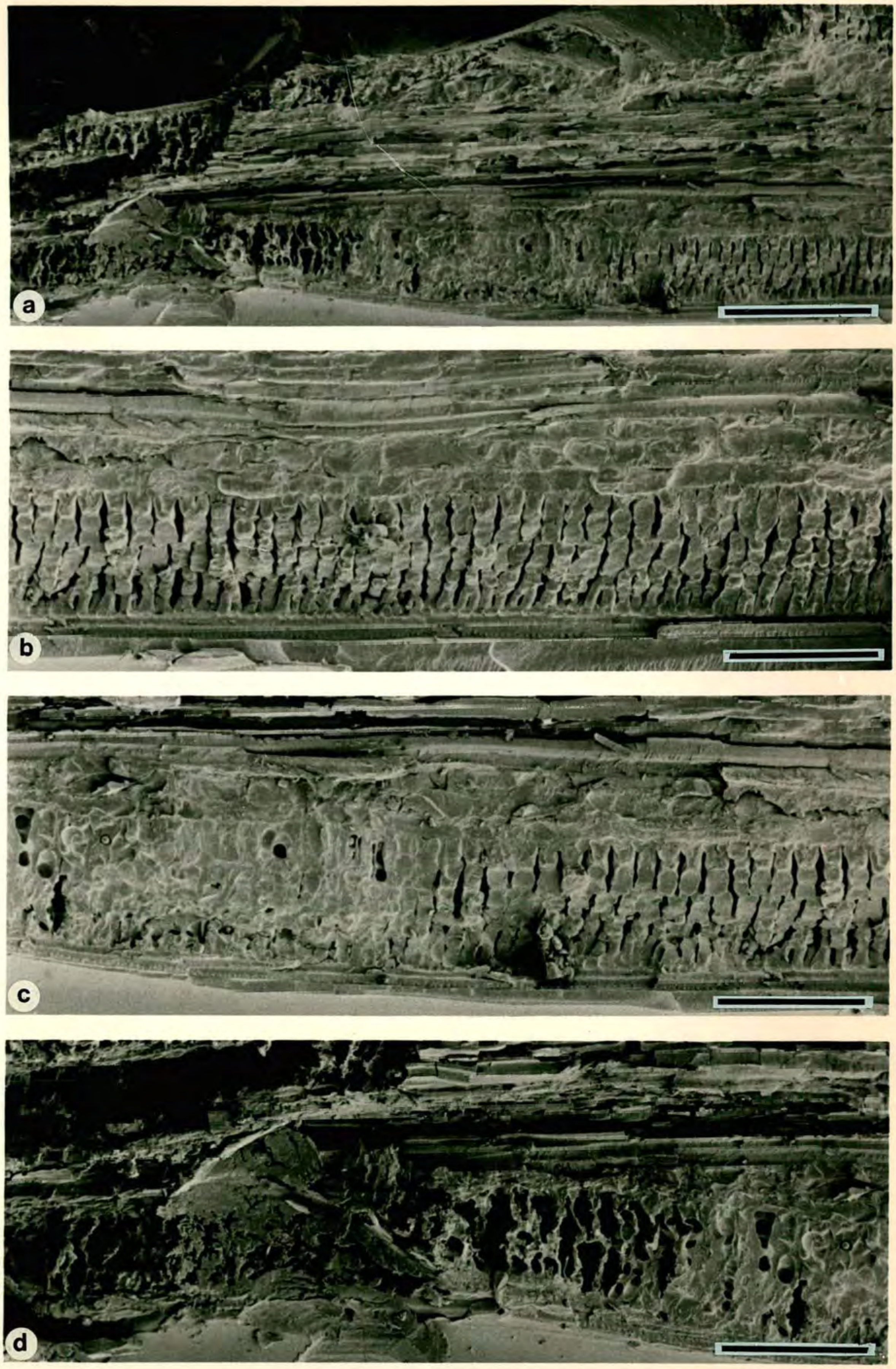
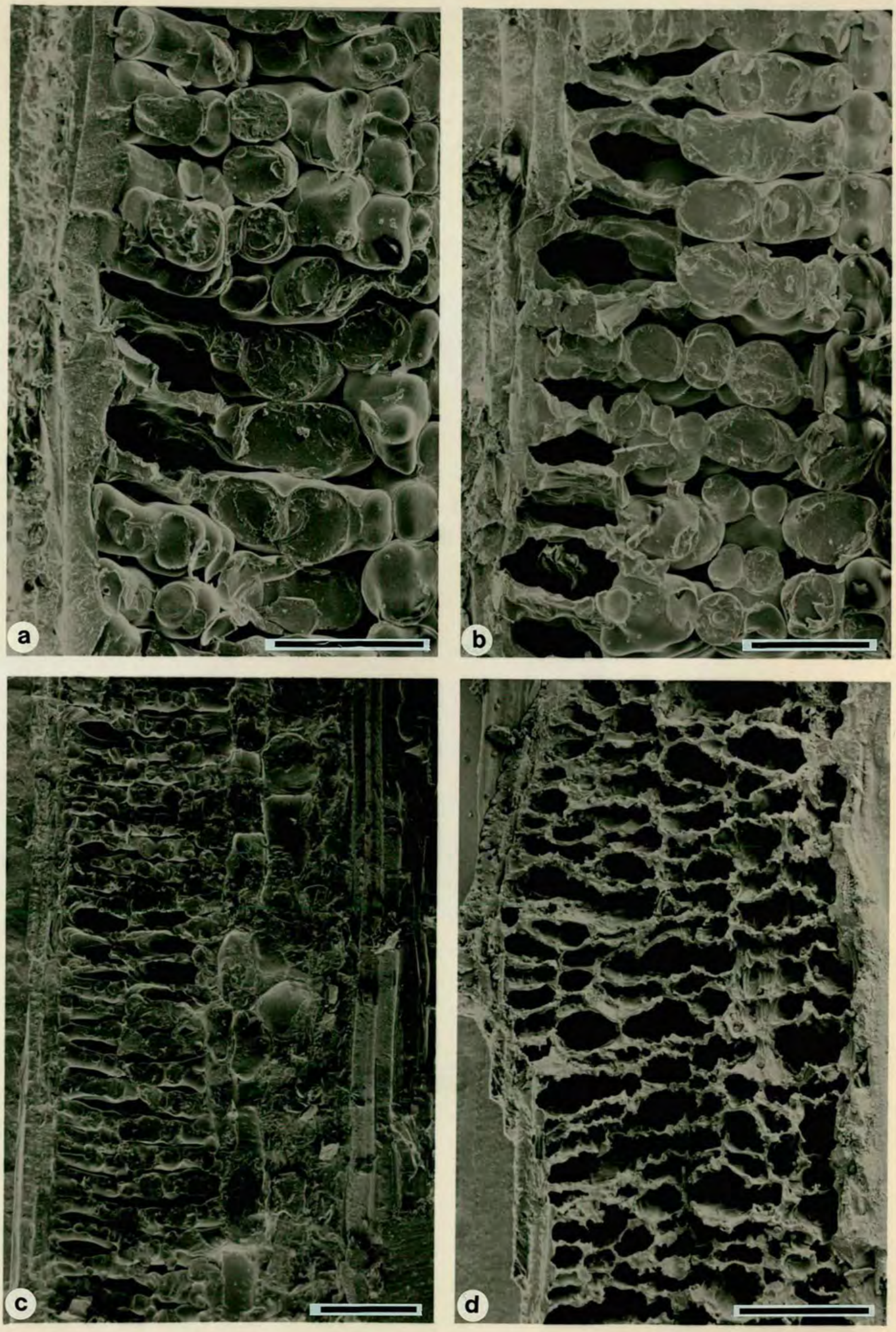

a
b
c
d

Figure 3.13. Progression of necrosis in damaged needle (pH = 2.0).

Figure 3.13a. Several collapsed cells correspond to small brown lesion on needle surface. Bar = 40 μm.

Figure 3.13b&c. Damage spreads along the underside of the cuticle or penetrates through the needle towards the vascular tissue as the lesion grows. Figure 3.13b. Bar = 40 μm. Figure 3.13c. Bar = 100 μm.

Figure 3.13d. By complete browning cell collapse is complete. Bar = 100 μm.


a
b
c
d

mesophyll (Figure 3.13c). Eventually tissue collapse is complete (Figure 3.13d).

As necrosis progressed from tip to base, a barrier began to form as extracellular deposits were laid down until all the extracellular space in this region was filled (Figure 3.14). Cell collapse no longer occurred and chloroplasts were less frequent and often absent. This area of extracellular deposits corresponded to the dark band separating green and brown tissue as seen in Figure 3.4a.

3.4.4 Cuticular conductance.

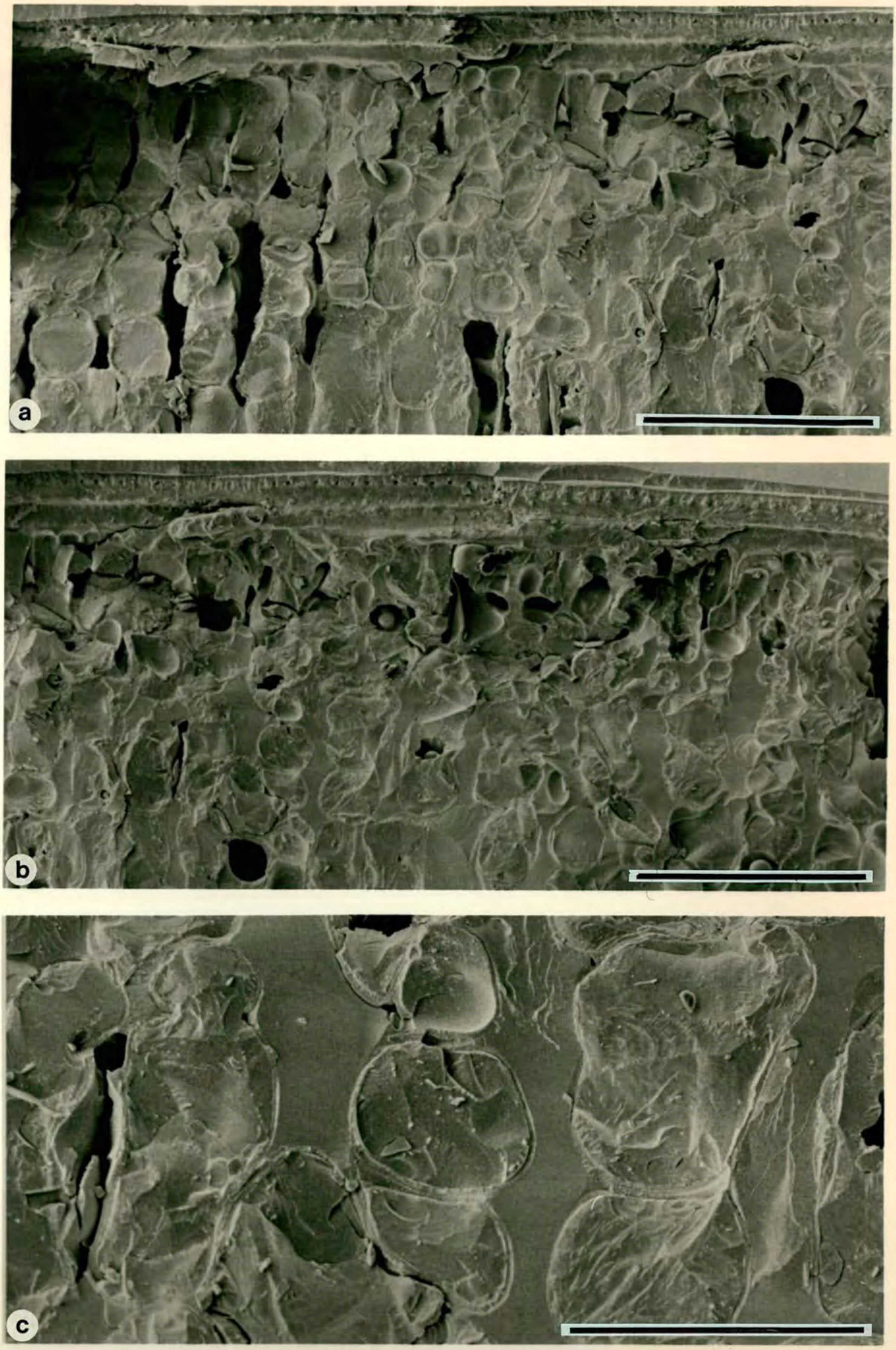
In all experiments conductances fell sharply before levelling out at around 1 mmol m^{-2} s^{-1} (Figures 3.15, 3.16, 3.17). The period prior to levelling out is assumed to include stomatal conductance and cuticular conductance with the period after levelling out being attributable only to cuticular conductance. The transition between the two phases took place at approximately 20 hours from the start of weighing in all experiments.

Initial conductance values vary between experiments probably because of differences in the rate of stomatal closure and also in the time taken to prepare needles for their first weighing. Cuticular conductance shortly after stomatal closure was 1-1.5 mmol m^{-2} s^{-1} and at the end of the

Figure 3.14a. At left hand side of micrograph needles are undamaged but towards the centre and right of the picture intercellular space has been filled up. Bar = 100 µm.

Figure 3.14b. In the centre of the intermediate zone intercellular space is totally absent. Bar = 100 µm.

Figure 3.14c. Close-up of cells shows the absence of chloroplasts. Bar = 40 µm.


a
b
c

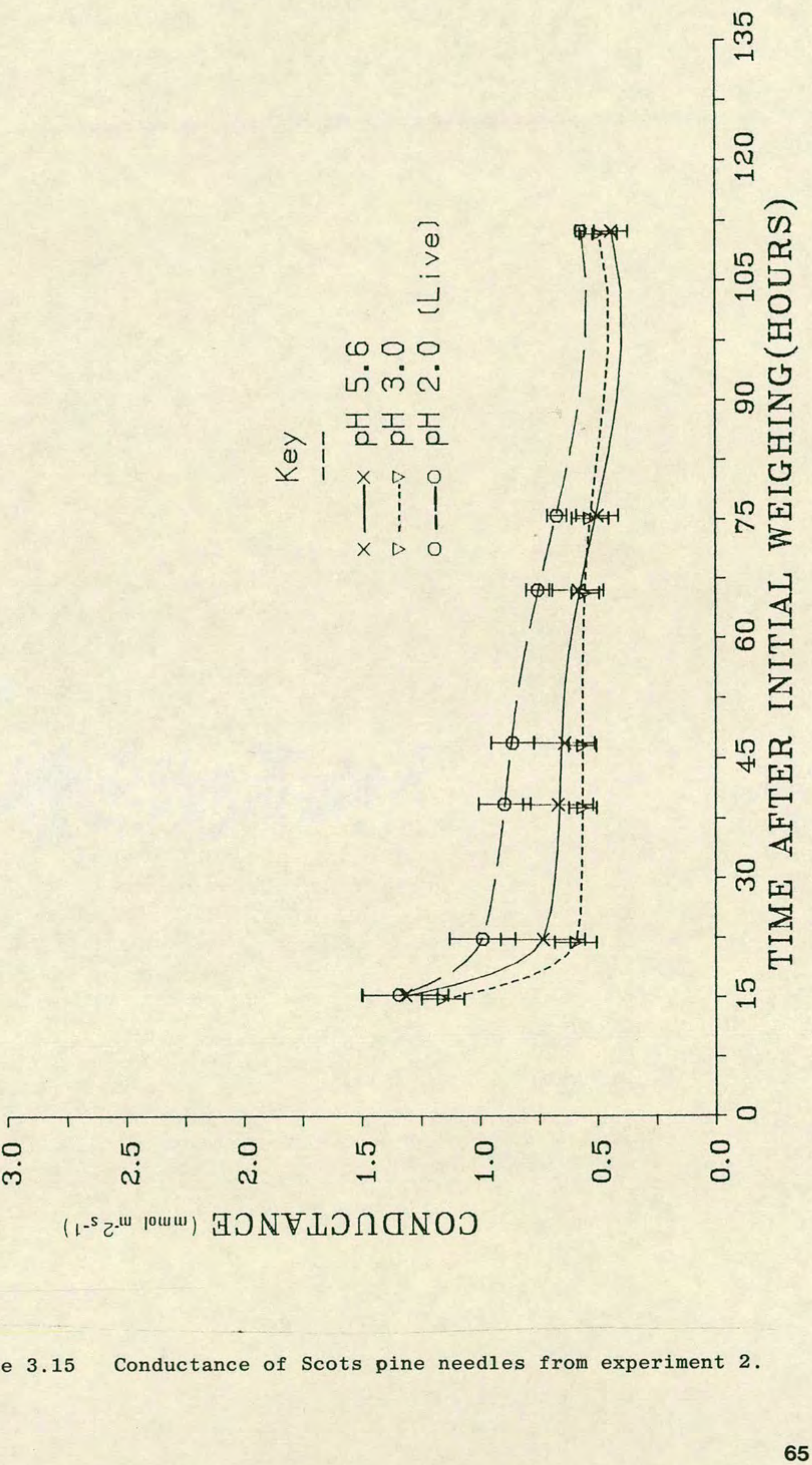


Figure 3.15 Conductance of Scots pine needles from experiment 2.

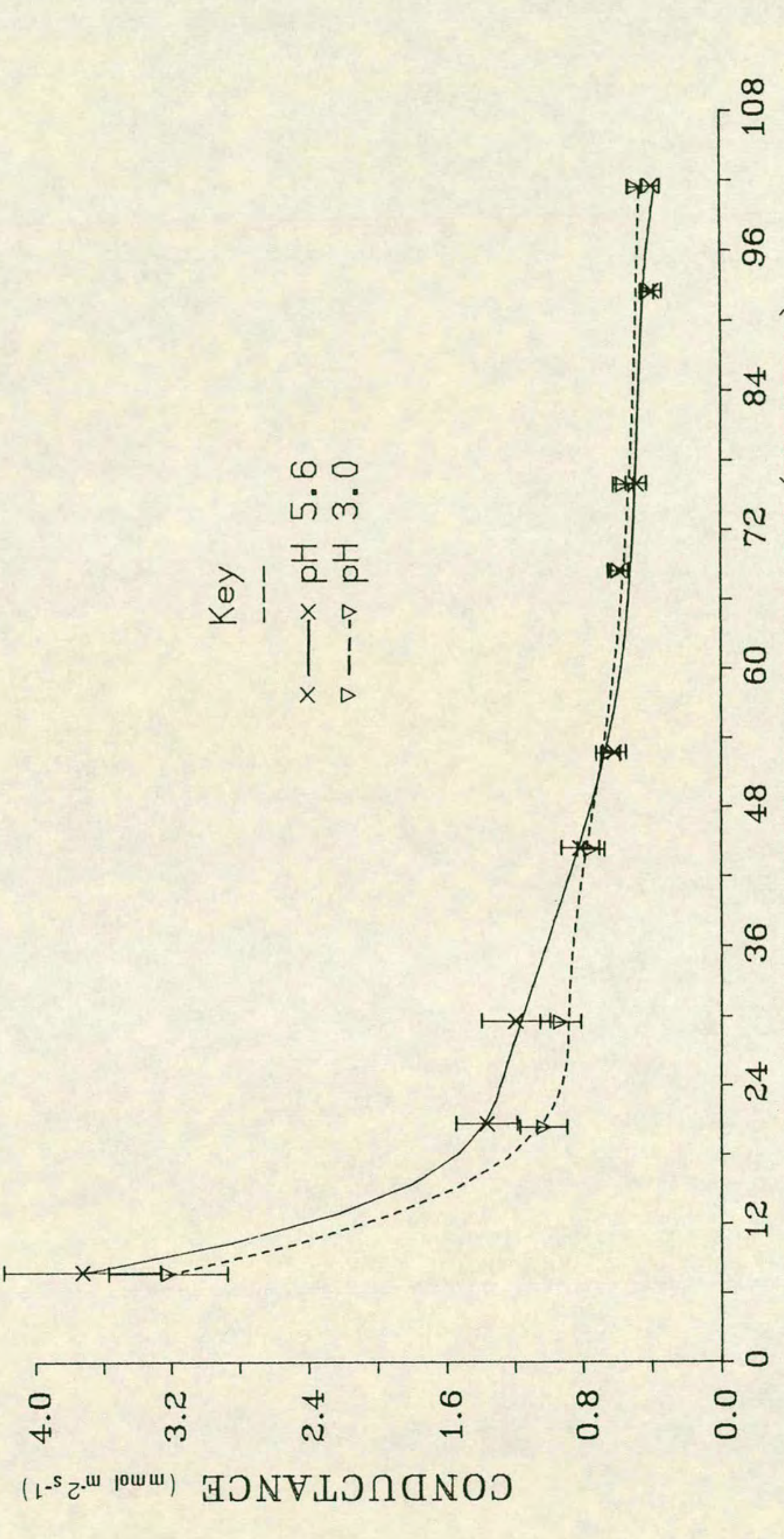


Figure 3.16 Conductance of Scots pine needles from experiment 3.

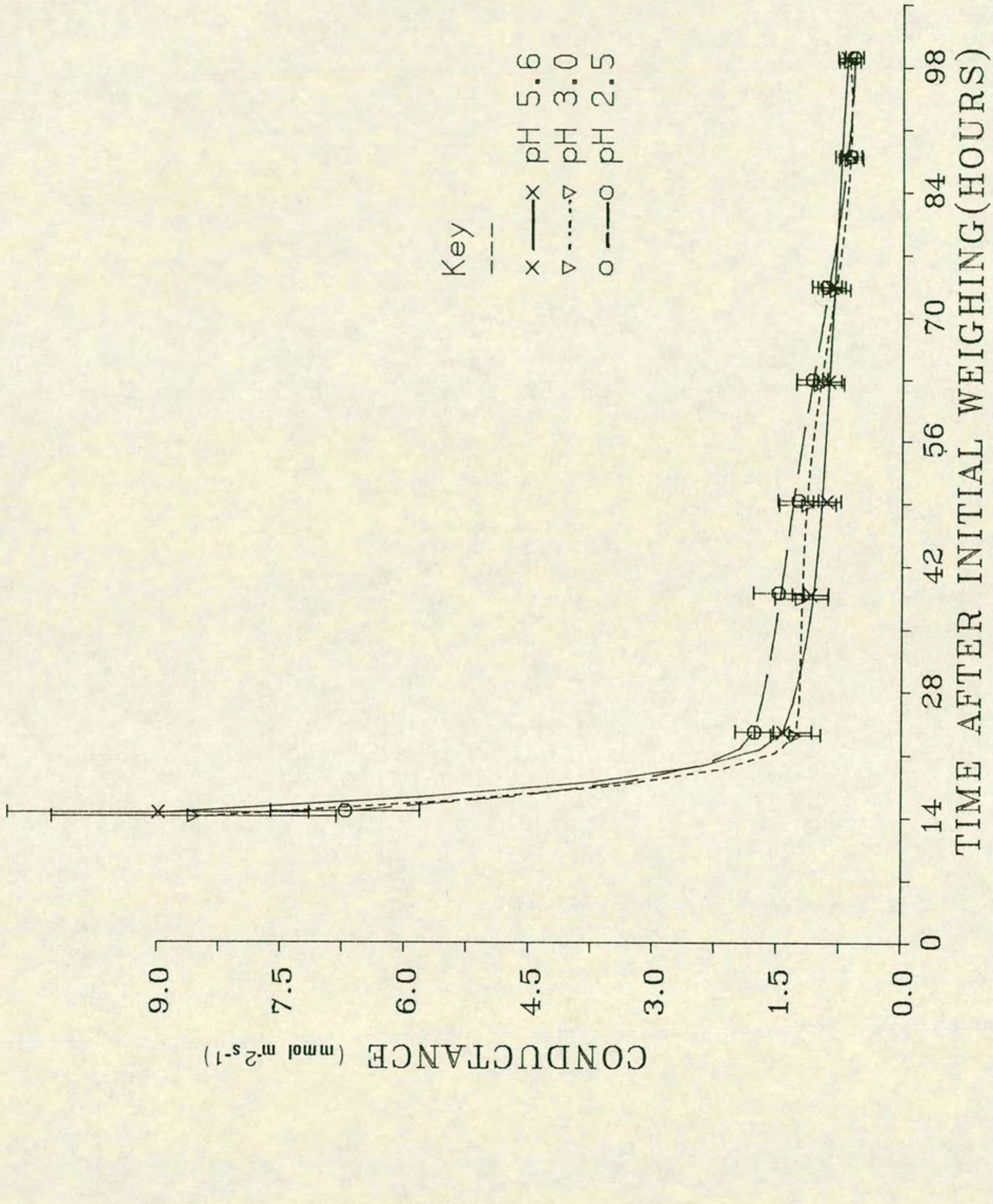


Figure 3.17 Conductance of Scots pine needles from experiment 4.

experimental period was around 0.7 mmol m^{-2} s^{-1} in all experiments. There were no large differences between Sitka spruce and Scots pine cuticular conductances.

In experiments 3 and 4 cuticular conductance values were similar for all treatments. In experiment 2, non-visually damaged needles from treatment pH 2.0 showed initial "cuticular" conductance values (20 hours after the start of the experiment) 34% higher than control needles. After 110 hours differences in conductance values had been reduced to give similar values.

3.4.5 Contact angles.

The first set of results (Experiment 2) indicated the possibility of a decreasing contact angle with increasing acidity (Table 3.1). Contact angles of needles subjected to SAC at pH 3.0 were significantly smaller than for control trees. Differences between needles within treatments were generally small, except for the control needles which showed large variation. In particular, the initial values were noticeably higher than subsequent measurements. There was no further reduction for trees subjected to SAC at pH 2.0 where live tissue was concerned. Necrotic tissue showed much smaller contact angles but this resulted from wrinkling of the needles caused by collapse of internal cells and consequent shrinkage. Where wrinkling was absent, contact angle values were similar to live tissue.

Table 3.1. Contact angles of Scots pine needles from experiment 2.

Treatment	Mean	Standard Error
5.6	96.075a	1.895
3.0	88.350b	1.044
2.0(live)	88.641b	1.405
2.0(necrotic)	72.306c	2.902

Different letters imply significant differences at P = 0.01.

Results from experiment 4 (Figure 3.18) showed a clear reduction in contact angles for the older needles. On current year needles contact angles were approximately 94° whereas current + 1 year needles showed a reduction to around 86° to 88°. There was no effect of acidity on contact angles at any level of treatment. Variation between trees was small compared with experiment 2.

3.5 DISCUSSION

On the basis of visual damage symptoms, these experiments indicate that conifers have a high resistance to damage by acid precipitation. In particular, the damage thresholds of Scots pine and Sitka spruce are well below the pH values of rain events in southern Scotland and also below the lower pH values measured in acid cloudwater at high elevations. Acid cloud events may have pH values below 3.0. However when the cloud base with its lower

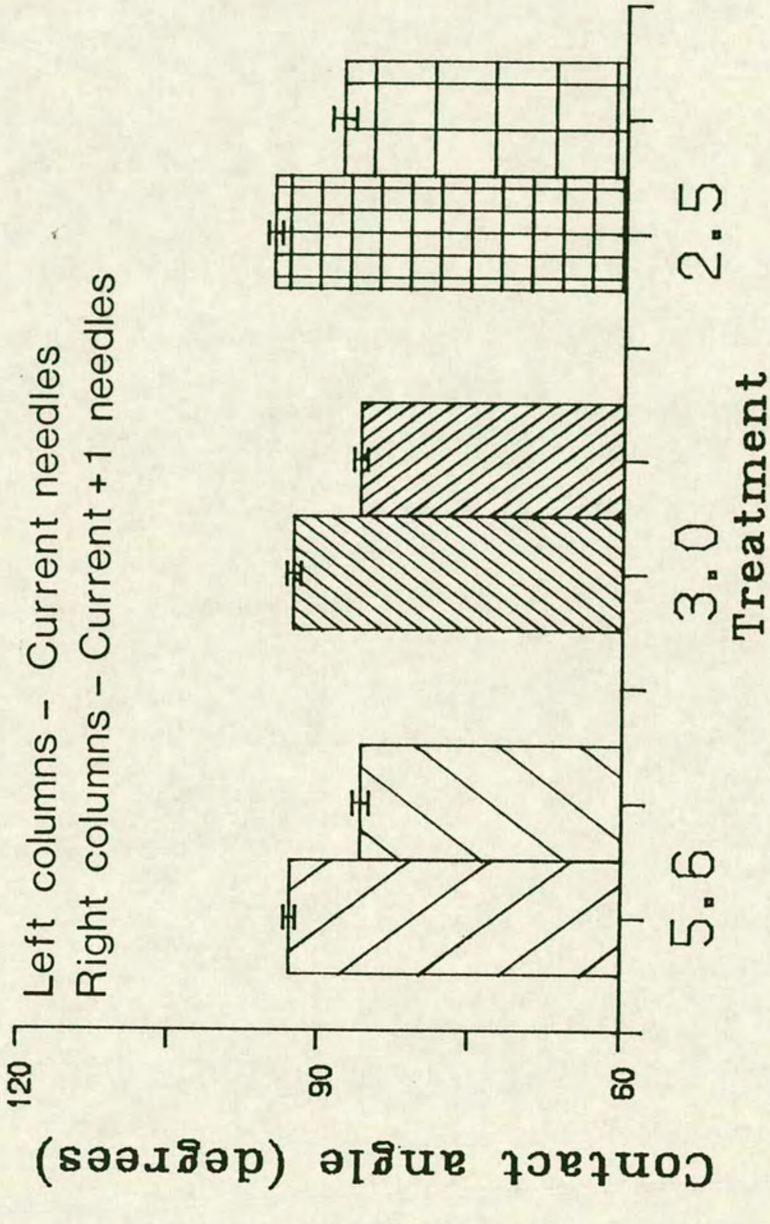


Figure 3.18 Contact angles of Scots pine needles from experiment 4.

liquid water content is in contact with the trees at the onset of an episode the pH is often even lower. It is therefore possible that on isolated occasions threshold levels may be reached for short periods of time. However damage to conifers increases with increasing application time (Jacobsen and Van Leuken 1977, Abouguendia and Baschak 1987), and the delayed onset of damage symptoms until the sixth episode indicates repeated or extended episodes of very low pH cloud would be required to cause visual damage.

These results are supported by evidence from other experiments. Compared with herbaceous plants and broadleaves, conifers are generally resistant to foliar injury. Whereas macroscopic foliar injury thresholds often occur at pH values above 3.0 for herbaceous plants (Evans and Curry 1979, Keever and Jacobsen 1983, Paparozzi and Tukey 1983, Pell and Puente 1986, Percy 1987) and around pH 3.0 for broadleaves (Wood and Bormann 1974, Shriner 1978, Evans and Curry 1979, Percy 1987, Musselmann and Sterrett 1988), acidity often has to be well below pH 3.0 before damage is visible in conifers (Table 3.2). Previous work on Scots pine has used either acid rain or acid mist without background ions. Although such experiments cannot be directly compared with this work using SAC with background ions they indicate a damage threshold around pH 2.5 (Hindawi and Ratsch 1974, Abrahamsen et al. 1976, Skeffington and Roberts 1985b).

Previous work on Sitka spruce using seedlings and simulated acid rain showed considerable variation in damage susceptibility but

KEY TO TABLE 3.2

Method of application

SAD - Simulated Acid Droplet (Background ions included)

SAR - Simulated Acid Rain

SAM - Simulated Acid Mist

HNSR - HCl, HNO_3 and H_2SO_4 rain with no background ions

SNM - H_2SO_4 and HNO_3 mist with no background ions

SM - H_2SO_4 mist with no background ions

AM - Acid mist (detail unknown)

Application is given in episodes per week times number of weeks.

Reference	1	Haines *et al.*	1980
	2	Shriner *et al.*	1975
	3	Wood and Bormann	1977
	4	McColl and Johnson	1983
	5	Percy	1983
	6	Abrahamsen *et al.*	1976
	7	Skeffington and Roberts	1985b
	8	Hindawi and Ratsch	1974
	9	MacDonald *et al.*	1986
	10	Abouguendia and Baschak	1987
	11	Temple	1988
	12	Mengel *et al.*	1988
	13	Seufert	1987
	14	Scherbatskoy and Klein	1983
	15	Percy	1987
	16	Ashenden and Bell	1988
	17	Leith *et al.*	1989
	18	Neufield *et al*	1985

S - Necrotic spots visible but damage very slight.

* - Significant damage,

Table 3.2 Damage of thresholds of conifers. Key to method of application and references follows.

Species	Method & Duration of application	Age of plants	pH																			
			5.0 -7.0	4.1 -5.0	4.0	3.6	3.5	3.4	3.3	3.2	3.1	3.0	2.8	2.7	2.6	2.5	2.3	2.0	1.9	1.5	1.0	Ref
Pinus strobus	SAD 2x1	3 year														–		–		–		1
" "	SAR ?x8	"Seedlings"	–							*				*					*			2
" "	HNSR 1x20	6 week	–		–				–			–					*					3
Pinus ponderosa	SAR 2x12	2 year	–		–							–						*				4
Pinus resinosa	SAR 7x5	5 week	–	–		–									*							5
Pinus sylvestris	SAR Monthly Longterm	Mature									–					*		*				6
" "	SNM 10x10	3 year	–									–										7
" "	SM Various	"Seedlings"	–		–							S			*					*		8
Pinus taeda	SAR ?x8	"Seedlings"	–							*				*					*			2
Pinus banksiana	SAR 7x5	5 week	–	–		–									*							5
" "	SAR 3x11	0-11 week		–	–							*			*			*				9
" "	SAR 1x17	2 year	–	–		–									–							10
Pinus jeffreyi	SAM 3x8	2 year	–	–				–					S				*					11
Picea abies	SNM 10x10	3 year	–									S										7
" "	AM ?x10	5 year	–										*									12
" "	SAR 1x104	8 year			–																	13
Picea glauca	SM 3x1½	2 year	–	–									–									14
" "	SAR 7x5	5 week	–	–		–									*							5
" "	SAR 1x7	2 year	S	S		S									*							11

Table 3.2 (Cont'd)

Species	Method & Duration of application	Age of plants	pH																			
			5.0 -7.0	4.1 -5.0	4.0	3.6	3.5	3.4	3.3	3.2	3.1	3.0	2.8	2.7	2.6	2.5	2.3	2.0	1.9	1.5	1.0	Ref
Picea sitchensis	SAR 3x2 1/3	"Seedlings"																				
		Clone A	–	–				–							–							15
		Clone B	–	–				S							S							
" "	SAR 5x104	1-2 year	–	–			–									*						16
Picea rubens	SAR 7x5	5 week	–	–		–									*							5
" "	SAM 2x22	2 year	–		–		S					*		*		*						17
Pinus rigida	SAR ½x6	"Seedlings"	–		–							–						–				18
Pinus virgiana	SAR 2½x6	"Seedlings"	–		–							–						–				18

serious damage appears also to start occurring around pH 2.5. However, the wide variation in method and duration of application of precipitation and in the age of plants used makes direct comparison within and between species difficult. Damage susceptibility is thought to be affected by the cuticular development of the needle (Abrahamsen *et al*. 1976), soil conditions (MacDonald *et al*. 1986, Roberts *et al*. 1989b, Ashenden and Bell 1988), and also irradiation (Roberts *et al*. op. cit.). Young plants are more susceptible to damage than older plants (Morrison 1984) and this emphasises the importance of using older plants rather than seedlings to achieve realistic results.

Where, however, different species have been present in the same experiment, Scots pine was found to be more resistant to damage than Norway spruce (*Picea abies*) (Skeffington and Roberts 1985b, Roberts *et al*. 1989b) and also more resistant than *Pinus strobus* (Jacobsen and Van Leuken 1977). Care should be taken in such comparisons, as genetic variability within a species may influence its susceptibility to damage (Leith *et al* 1989) and different clones of the same species in the same experiment have shown large differences in their damage response to acid precipitation (Percy 1987).

Although the reasons for differences within species are not clearly established real differences in susceptibility to foliar damage between species may occur as a result of differences in wettability. Very waxy needles with non-wettable surfaces which repel acid droplets may suffer less damage than more easily wetted needles. Abouguendia and Baschak (1987) attributed the low wettability of *Pinus banksiana* compared with *Picea glauca* as the reason for the former's greater resistance to damage. Other authors have also related resistance to damage to low wettability in non-coniferous species (Keever and Jacobsen 1983, Haines *et al*. 1985, Caporn and Hutchinson 1986).

However both Evans and Curry (1979) and Percy (1987) found that the retention of droplets over veins or other areas thinly covered with wax also led to increased susceptibility to damage. It appears that the whole aspect of droplet retention is important rather than merely wettability.

Needle damage in the experiments described here was seen to progress from small brown necrotic spots to large areas of browning, particularly at the needle tips. Such development of damage symptoms is often found in conifers exposed to acid precipitation (Abrahamsen *et al*. 1976, McColl and Johnson 1983, MacDonald *et al*. 1986, Ashenden and Bell 1988, Temple 1988). The reason for necrosis being particularly severe at the tip is likely to be a result of droplets running down needles to the tip where they remain and evaporate. The needle tips are thus exposed to the acid precipitation for longer periods of time and are also subjected to increased concentration of H+ because of evaporation.

In the series of experiments here, droplets were clearly seen to accumulate at needle tips rather than the bases. When droplets did run down to needle bases they tended to continue to run onto the twig rather than remain at the needle base. In the giant sequioa (Sequoiadendron giganteum), where mist droplets tend to run back down the leafy scales to the base, damage is concentrated at the base of the leafy scales rather than the tip (Temple 1988).

Whether foliar damage by acid precipitation leads to reduced productivity is disputed. Although reduced photosynthetic area would appear logically to reduce growth, some authors are sceptical as to whether any growth effects are caused by foliar damage (Morrison 1984). The difficulty in relating reduced productivity to needle damage or loss is that needle damage may lead to reduced growth; or reduced vitality may lead to both needle damage and reduced growth such that cause and effect are not easily identified. Although there appears to be some link between needle loss and a reduction in stem diameter increment (Nöjd 1989, Schmid-Haas 1989), compensating mechanisms may also be at work. For example, Temple (1988) found growth of Sequoiadendron giganteum seedlings was not affected despite severe foliar injury from SAC at pH 2.0 and suggested reallocation of assimilates to foliage for repair and increased meristematic activity were responsible, although the interaction of other effects such as nitrogen fertilization may also be involved. The often observed shift in root/shoot ratios in favour of shoots may reflect a shift in the allocation of assimilates when plants are under stress from acid precipitation (MacDonald et al. 1986, Schier 1987, Bennett et al 1989) but reduced root growth will ultimately lead to reduced shoot growth through an inability to exploit soil ion pools and also increases the danger of windthrow. In the experiments described here 30% foliar damage from SAC at pH 2.0 would require considerable compensating mechanisms to cancel out any growth decline.

The damage seen in the experiments detailed here and in other experiments where needles turn red/brown does not bear a close resemblance to the majority of damage seen in declining forests. Such damage generally includes chlorosis, yellowing, and casting of needles, particularly those of older age classes (Linzon 1984, McLaughlin 1985, Rehfuess 1987, Prinz and Krause 1989, Roberts et al. 1989a). Where browning does occur in the field it appears to be associated with classic SO_2 pollution in eastern Europe (Roberts et al. 1989b), pathogenic infection (Rehfuess 1987) or frost/desiccation injury (Evans 1986, Leith et al. 1989). Thus the needle browning observed here does not indicate that direct damage by acid clouds is responsible for the "novel" forest decline symptoms observed throughout Europe or the recent discolouration and dieback found in Britain.

Despite the rapid progression of needle browning there appears to be some kind of defence mechanism whereby the expanding area of necrotic cells is isolated from the rest of the needle. This involves a band of turgid cells lacking photosynthetic apparatus and with the intercellular space completely filled with an unidentified substance. Because necrosis generally progressed from the needle tip where droplets accumulate, the area of the needle near the base is usually left free from damage. X-ray analysis (not available on the SEM used) would be needed to identify the substance filling the intercellular spaces but it does effectively protect the cells from collapse. These cells themselves appear to lose their chloroplasts but by maintaining their turgidity they, along with the intercellular substance, produce a strong barrier. Damage tends to progress from a cell to its neighbours, forming expanding pockets of damage extending down from the palisade cells to the mesophyll. It appears that once one cell is damaged those around it are more vulnerable and this apparent barrier of intercellular material halts that process.

It may be that this defence mechanism is responsible for the absence of needle abscission observed in the experiments. Although abscission of conifer needles with macroscopic damage may occur (Ashenden and Bell 1988, Mengel et al. 1988, Roberts et al. 1989b), in this series of experiments damaged needles remained on the shoots. Usually, even badly damaged needles maintained a portion of green tissue at the needle base. Although ultimately growth and viability may be affected by the reduction in photosynthetic area, it does not appear that death would directly result from even such severe damage as seen in Figure 3.3b. Two years later damaged trees appeared healthy although no comparison of growth parameters with control trees was undertaken.

The facts that crown thinning is a major identifier of forest decline and that needles were not cast in this series of experiments support the assertion that direct damage by acid cloud is unlikely to be causing widespread forest decline. There is, however, a possibility that at high elevations extended periods of highly acidic cloud may cause localised damage.

In Experiment 1, although epicuticular wax quality was not scored, it did not appear affected by SAC. These needles underwent expansion during the SAC applications so that not only does it appear that Scots pine needles are resistant to the degradation of established epicuticular wax structure but that epicuticular wax production and crystallization are also unaffected. Thus acid precipitation does not appear to endanger Scots pine plants by increasing the potential for desiccation through incomplete maturation or subsequent degradation of the epicuticular waxes. That does not exclude a possible effect on water relations by other means.

It has recently been stated that "there seems good agreement that air pollutants and acid mist influence the rates at which epicuticular wax structure of conifer needles is lost" (Freer-Smith 1988). However, plant cuticles can be isolated by strong acid solutions and remain structurally and functionally intact (Evans 1984, Adams _et al_. 1990). Experimental evidence for cuticle degradation as a result of exposure to pollutants is equivocal.

Field observations of cuticular damage are not specific, whilst objective measurements of controlled applications are not widely available. Percy and Riding (1978), Cape and Fowler (1981), Huttunen and Laine (1983), Crossley and Fowler (1986), Karhu and Huttunen (1986), Grill et al. (1987), and Garrec and Laebens (1988) have all linked accelerated cuticular degradation in the field to high pollution loads. However, cuticle structure is closely dependent on non-pollutant aspects such as climate, water-stress and nutrient status (Jeffree et al. 1976, Juniper and Jeffree 1983). In addition the pollutant "responsible" is often difficult to ascertain, with SO_2, NO_x, O_3 and acid rain sometimes all being present. It is also likely that particles such as pulverised fuel ash (PFA) may play a major role in cuticle degradation, as it is often abundant on damaged cuticles (Crossley and Fowler 1986, Cape 1988c). There does not appear to be any specificity of damage attributable to the different pollutants. However, the majority of controlled studies using pollutant gases indicate erosion or fusion of fibrillar epicuticular wax.

Results from controlled application of acid precipitation, however, appear to show differences in the susceptibility of different species to erosion and also differences in the erosive properties of acids, with sulphuric acid degrading epicuticular wax to a larger extent than nitric acid (Rinallo et al. 1986, Cape 1988c).

Silver fir (Abies alba) is particularly susceptible to fusion of the wax structure (sometimes termed "melting") and erosion or flaking (Rinallo *et al*. 1986, Schmitt *et al*. 1987, Seufert 1987). Cuticular waxes of Norway spruce also degrade on the application of acidic rain at a pH of as high as 3.8-4.0 (Schmitt *et al*. 1987) although where both Norway spruce and Silver fir are present, Silver fir appears to suffer greater degradation of the epicuticular wax structure (Rinallo *et al*. 1986, Schmitt *et al*. 1987). In other experiments Norway spruce has shown no epicuticular degradation on the application of acidic rain (Turunen and Huttunen 1989), but it is generally *Pinus* spp. that show the greatest resistance to changes both in wax chemistry and structure (Horntvedt *et al*. 1980, Skeffington and Roberts 1985a, Percy 1987, Turunen and Huttunen 1989, Turner *et al*. (in press)).

Although the experiments showed no degradation of the physical structure of the cuticular waxes, that did not necessarily mean the functioning of the cuticle was unaffected by the SAC. The maintenance of functional integrity in the cuticle is vital to the vitality and survival of a tree. Incomplete maturation or destruction by abrasion of needle cuticles leading to increased cuticular transpiration has been cited as the reason for winter desiccation of conifers (Baig and Tranquillini 1980, Hadley and Smith 1986, Grace (1990)). A reduction in cuticular resistance as a result of epicuticular wax degradation by SAC could have serious consequences for the water relations of a conifer.

Cuticular conductance values in the experiments described here for Scots pine were similar to those obtained by Cape and Fowler (1981) and Grace (in press). They are extremely low compared with most other plants (Schonerr 1982). Although, in experiment 2, needles from the SAC treatment at pH 2.0 showed initially higher conductance values at the onset of the cuticular conductance phase, the general picture was that the functional integrity of the cuticle was maintained. It is possible that in the above the cause of higher conductance was incomplete stomatal closure, as later conductance values showed similar values to other treatments.

The importance of cuticular conductance measurements lies in their physiological meaningfulness and Cape (1988b) recommends their development as a tool for diagnosing air pollution damage. Although gaseous air pollutants appear to affect cuticular conductance (Cape and Fowler 1981, Mansfield et al. 1987, Wright and Lucas 1987, Seufert et al. 1989), similar work with acid precipitation is scarce. Mengel et al. (1989) found cuticular conductance of Norway spruce was not affected by simulated acid fog when plants had an adequate water supply but, under drought conditions, acid-treated (pH 3.0) plants had a higher cuticular conductance than control plants (pH 5.0). However, unlike this experiment, the needles showed a "disintegration" of the epicuticular waxes. There is reason to expect that epicuticular wax structure and functional integrity of the cuticle are closely linked. The crystalline, soluble cuticular lipids reduce water permeability (Schonerr 1982), and the wax tubules in the stomatal ante-chambers are also excellent antitranspirants (Jeffree et al. 1971). It is therefore not surprising that Mengel et al. (op cit) found that epicuticular wax degradation on Norway spruce was accompanied by change in cuticular conductance, whilst the experiments here showed both structure and function to be intact. It appears that in both structure and function the Scots pine cuticle is more resistant to changes by acid precipitation and specifically acidic cloudwater.

The condition of the wax structure also affects contact angles and wettability. Increased wettability may increase susceptibility to pathogens (Adams et al. 1987, Cape 1988c) and alter leaching by changing ion permeability (either by altering exchange sites chemically or penetration pathways physically). The ease of penetration of pollutant gases and aqueous solution could also be altered (Lendzian 1984) and leaf temperature, frost damage, and sensitivity to ultra violet light may be affected (Martin and Juniper 1970). However, the results from the studies on contact angles clearly indicate that the hydrophobocity of the needle surface is maintained in conjunction with the epicuticular wax structure. Wettability does not therefore increase, thus avoiding the possibility of a positive feedback loop of increasing wettability and susceptibility to damage.

The experiments reported here also clearly indicate that internal damage and epicuticular wax degradation must be separated in the examination of foliar injury by acid precipitation. Substantial cell death occurs without any sign of epicuticular wax degradation, indicating that the epicuticular wax layer does not constitute an effective barrier to severely acidic cloudwater. That is, the cuticle did not need to be damaged or broken down for SAC to reach and destroy the underlying cells. It may well, however, provide considerable resistance and protection as a result of its hydrophobic properties. The fact that damage in many species often occurs where droplets tend to accumulate suggests that the absence of veins, trichomes and hairs and the low wettability of the needle substantially reduce the contact time of SAC and may well be responsible for the low pH threshold of damage. Further work should seek to disclose whether susceptibility to damage is caused entirely by differences in acid penetration of the cuticle or whether differences in internal cell susceptibility are also involved. The lack of damage after artificial abrasion suggests that the resistance to damage is not only a result of a protective cuticular layer but also that resistance remains higher than for herbaceous and broadleaved species even when that layer is smoothed, abraded or broken.

CHAPTER 4

Leaching of ions from Scots pine and Sitka spruce in controlled experiments

4.1 Introduction.

Needles are not inert surfaces, neither do they provide pristine surfaces onto which cloud droplets impact. Therefore, when cloud droplets do impact and subsequently either drip off or evaporate, their composition is changed whilst in contact with the needles. Three main processes occur: solubilization and mobilization of surface deposits, exchange of ions by leaching and absorption, and evaporation. Evaporation will be dealt with in Chapter 7, solubilization and mobilization, and more especially, leaching of ions during controlled experiments will be dealt with in this chapter.

Leaching, in the context of this study, is the removal of substances from plant tissues by an aqueous solution. In this series of experiments it is, more specifically, the removal of substances from needles by simulated acid cloud (SAC) which is investigated. A whole array of inorganic and organic metabolites can be leached from plants (Mecklenberg et al. 1966, Tukey 1970) and this series of experiments concentrates on the leaching of macronutrients, particularly the cations Ca, Mg and K. To assess the impact of acid precipitation on the nutrient status of conifers, the experiments were designed to answer the following questions

i) Does foliar leaching of conifer needles increase with increasing acidity of precipitation?

ii) Does the nutrient ion content of needles change as a result of leaching?

iii) Is foliar leaching sufficient to neutralise the acidity of impacted SAC and thus to raise the pH of impacted SAC on needles?

iv) Are certain ions leached in preference to others and is increased leaching of such ions linked to increased neutralization, i.e., are there any grounds for postulating particular cation H^+ exchange?

The experiments designed to answer these questions consisted of misting young seedlings of Scots pine and Sitka spruce with SAC, collecting and analysing the droplets of SAC which impacted onto the needles and analysing the nutrient content of the needles after the misting episodes were terminated.

4.2 Method.

4.2.1 General framework

Leaching of ions and foliar nutrient content were investigated as an integral part of three of the four controlled experiments undertaken. These three experiments are described below.

Experiment 1:

Six-year-old Scots pine were subjected to SAC in both the preflushing phase (13 applications) and the flushing phase (24 applications) giving a total of 37 applications (equivalent to 118.4 mm) of SAC. There were six trees in each of five misting chambers. Two chambers were given SAC at pH 3.0 before and during flushing, one was given SAC at pH 3.0 before and pH 5.6 during flushing, one pH 5.6 before and pH 3.0 during flushing and one pH 5.6 both before and during flushing. Spraying took place on Mondays, Wednesdays and Fridays at noon. Droplets dripping from needles were collected with a hand held test tube. Direct spray was collected as a control by clamping a test tube in line with spray coming directly from the rotary atomizer.

Experiment 3:

144 three-year-old Sitka spruce were allocated randomly to nine replicates each of two treatments. The treatments were SAC at pH 5.6 and pH 3.0. The spruce were grown during the experiment in hydroponic solution, the details of which are given in Chapter 6. Spraying took place on Mondays, Wednesdays and Fridays at noon with a total of 37 applications (118.4 mm). At the end of the experimental period current year needles were taken from randomly chosen trees from both

experiments and analysed for nutrients as detailed in Appendix 4.

Experiment 4:

In this experiment both needle analysis and droplet collection were undertaken. Eighteen trees of both seven-year-old Scots pine and Sitka spruce were allocated to three treatments each with two replicated blocks. Each treatment, therefore, had two blocks of three Scots pine and three Sitka spruce. Treatments were SAC at pH 5.6, pH 3.0 and pH 2.5. Spraying took place on Mondays, Wednesdays and Fridays at noon and a total of 27 episodes (86.4 mm) were given. Because the collection of droplets by hand held test tubes had proved very time-consuming a vacuum-pipette system was assembled and used. This consisted of a boiling tube with the side arm connected to a vacuum pump. The boiling tube was sealed with a rubber bung after a smaller test tube had been placed inside. Two Pasteur pipettes were connected back to back with a piece of polyethylene tubing and one pipette was inserted through the rubber bung into the smaller test tube. Air was drawn through the two pipettes into the smaller test tube then out through the boiling tube side arm, allowing droplets to be drawn off needles by the outward facing Pasteur pipette and deposited into the internal test tube (Figure 4.1). Droplets were collected from live shoots and from sheets of parafilm placed in the upper branches of the trees.

Figure 4.1 Vacuum/pipette system used for collection of accumulated cloudwater droplets.

Droplets were collected only from current year, non-abraded shoots. Sampling began immediately the spraying stopped and continued for 120 minutes. The effect of evaporation on enhancement of ion concentrations will be dealt with in Chapter 6. In this chapter only differences in leaching of ions will be discussed.

A sample of approximately 2 cm^3 was needed for analysis and this took from 5-15 minutes to collect depending on how abundant droplets were. "Parafilm" did not contaminate samples of deionised water.

Details of SAC chemistry and spraying techniques, tree material, glasshouse conditions, and ion analysis for all experiments are given in Appendices 1, 2, 3 and 4 respectively.

4.3 Results.

4.3.1 Controlled experiments: changes in the composition of simulated acid cloud ions.

Only one set of samples was taken from the Scots pine trees in experiment 1, but samples from the pH 3.0 treatment consistently showed higher concentrations of all ions compared with the control (pH 5.6). The increase was generally around two-fold for all ions analysed (Figure 4.2). The figures show

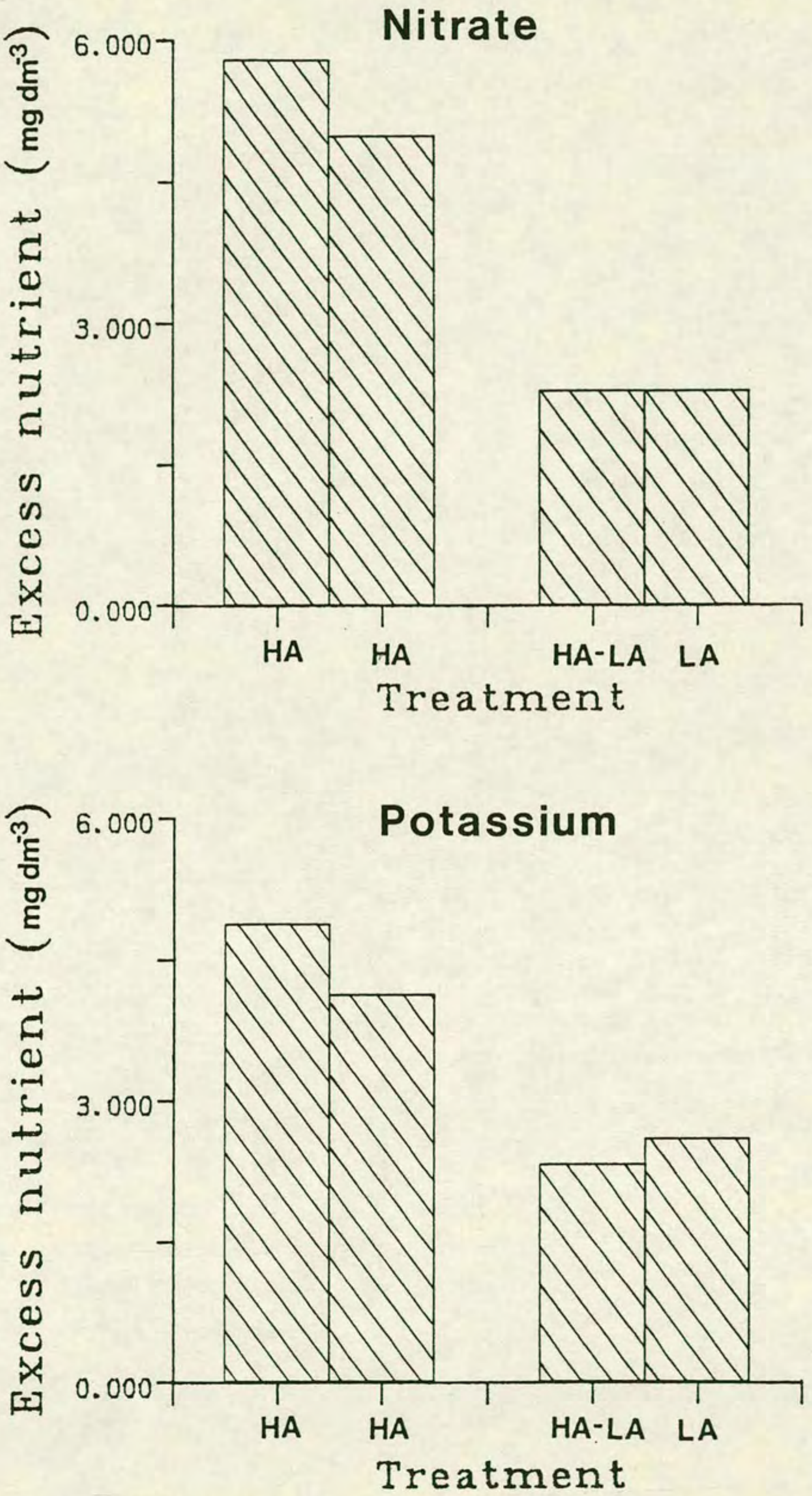


Figure 4.2 Excess (concentration in analysed droplets minus concentration in original spray) nutrients in throughfall of experiment one. Treatments HA were high acidity and HA-LA and LA low acidity when the samples were taken.

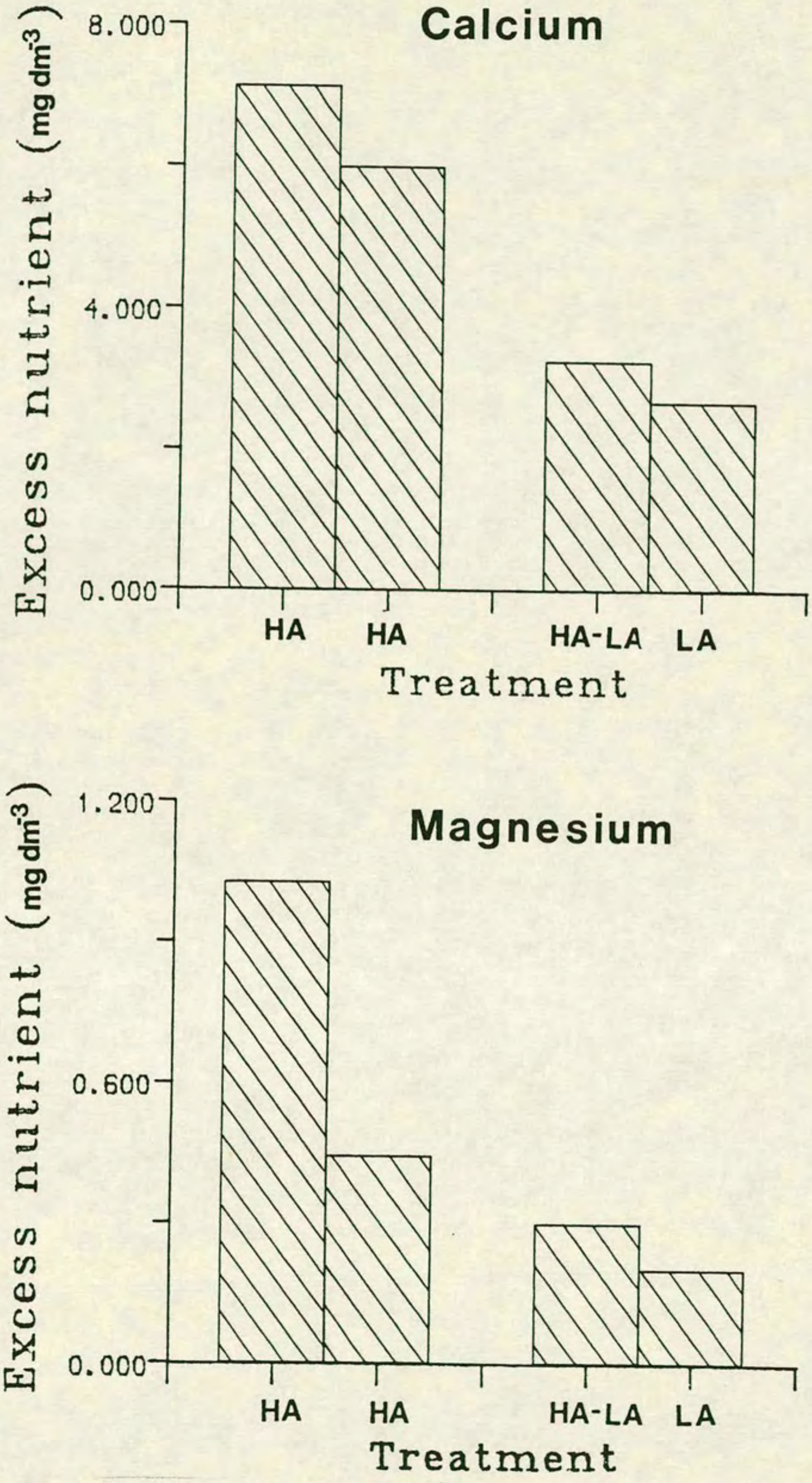


Figure 4.2 continued.

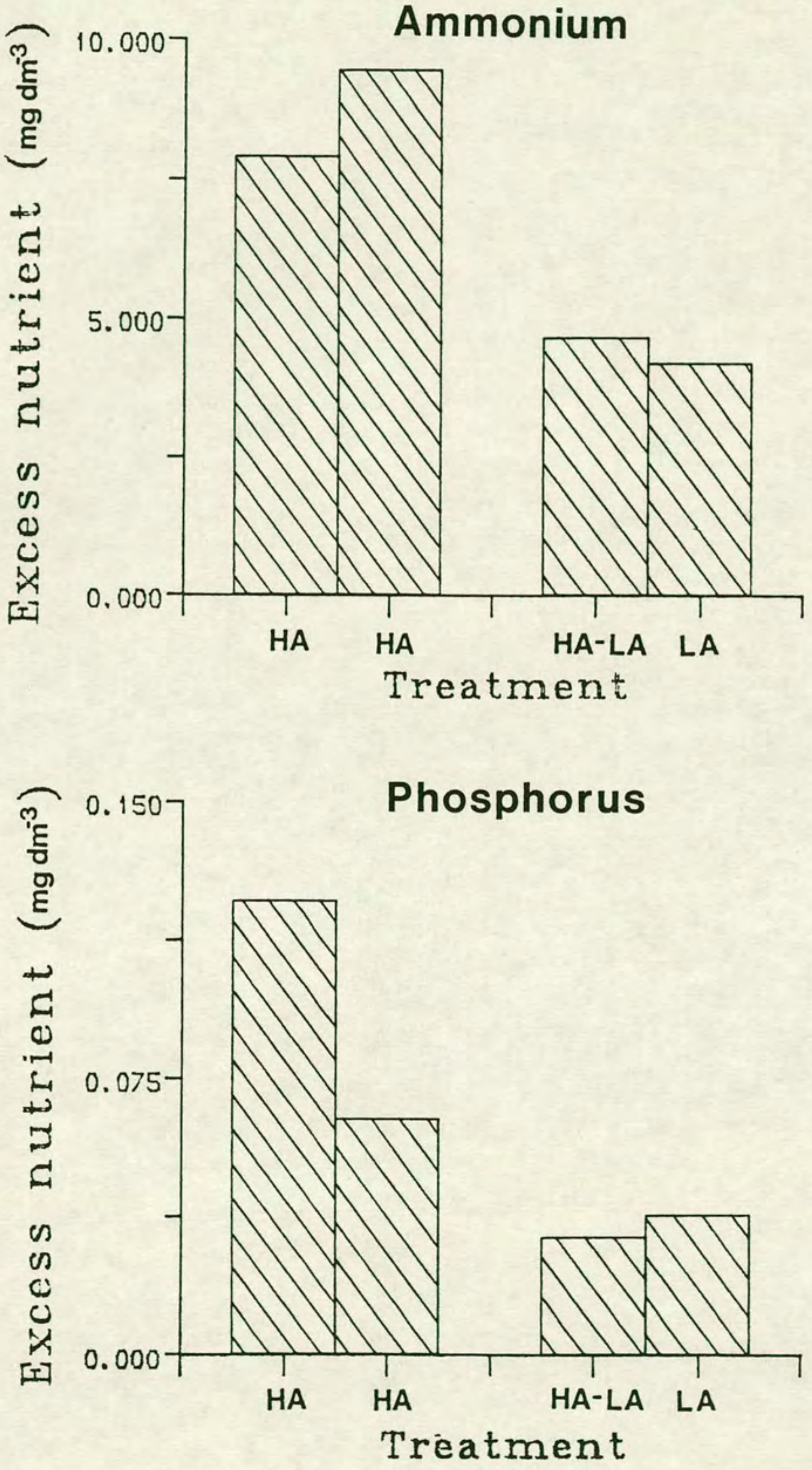


Figure 4.2 continued.

the difference between the analysed droplets and the concentration of ions in the original spray, this difference being described as an excess of ions or nutrients.

However, when multiple samples were taken in experiment 4, the results showed considerable variation between samples from the same species and treatment even when taken at a similar time after spraying.

There was also a less well defined difference between samples from the different treatment pHs. An initial plot of excess nutrients against time for the three treatments with both tree species and "Parafilm" indicates an absence of any strong grouping or separation either by treatment or by species (Figure 4.3 a-e) although several trends can be seen on closer inspection. In particular, samples from "Parafilm" had lower values than values for both tree species indicating an increase in nutrient content of droplets in contact with needles.

Because of the amount of scatter in the data, it is difficult to make direct comparisons of treatment effects on the amounts of excess ions. Further breakdown of the data for magnesium (Figure 4.4 a-c) shows that, as treatment acidity increased, the concentrations of ions in samples from "Parafilm" became increasingly separated from those from the trees. The pH 3.0 treatment also produced more excess magnesium in Scots pine

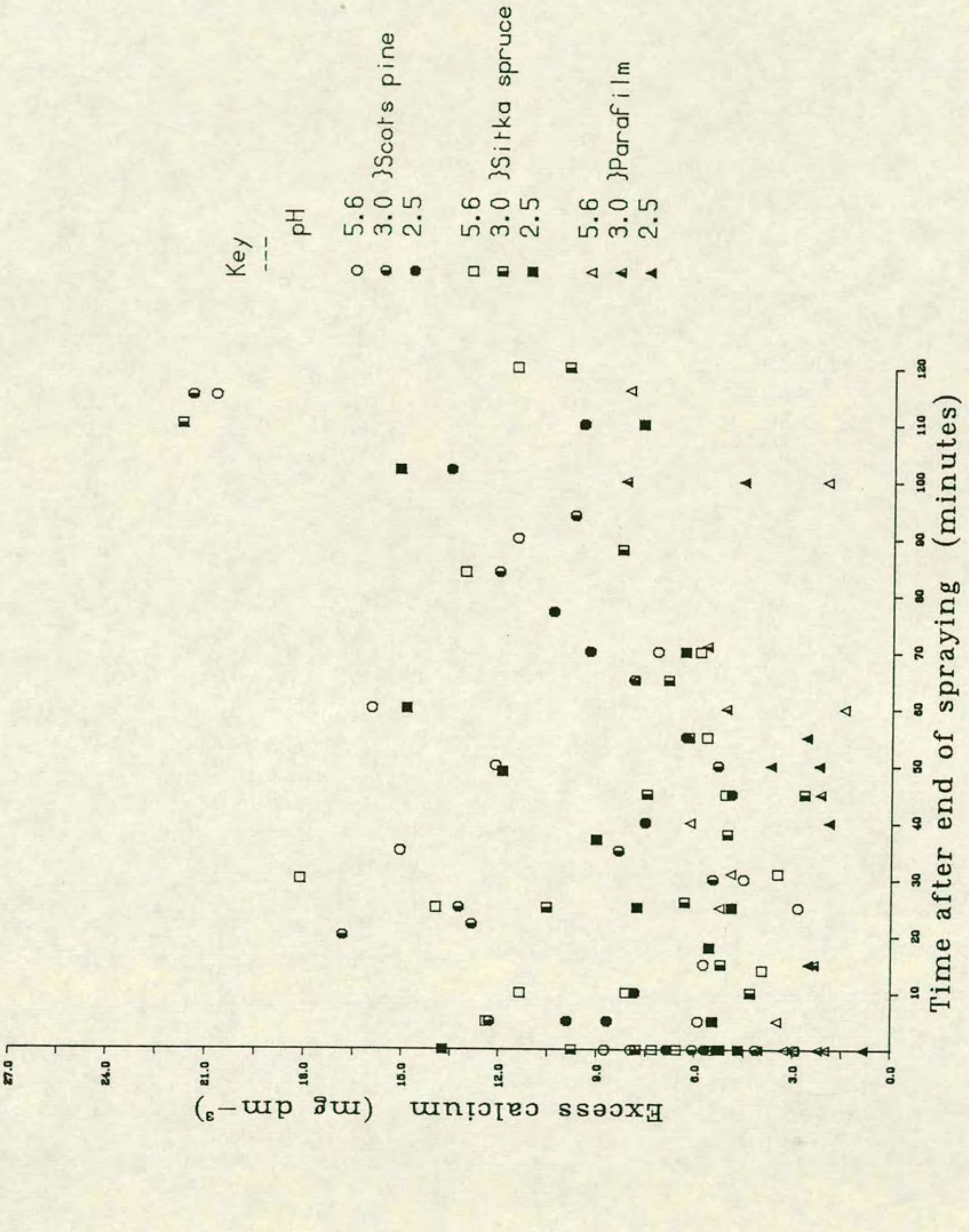


Figure 4.3a Excess (concentration in analysed droplets minus concentration in original spray) calcium in droplets from experiment 4.

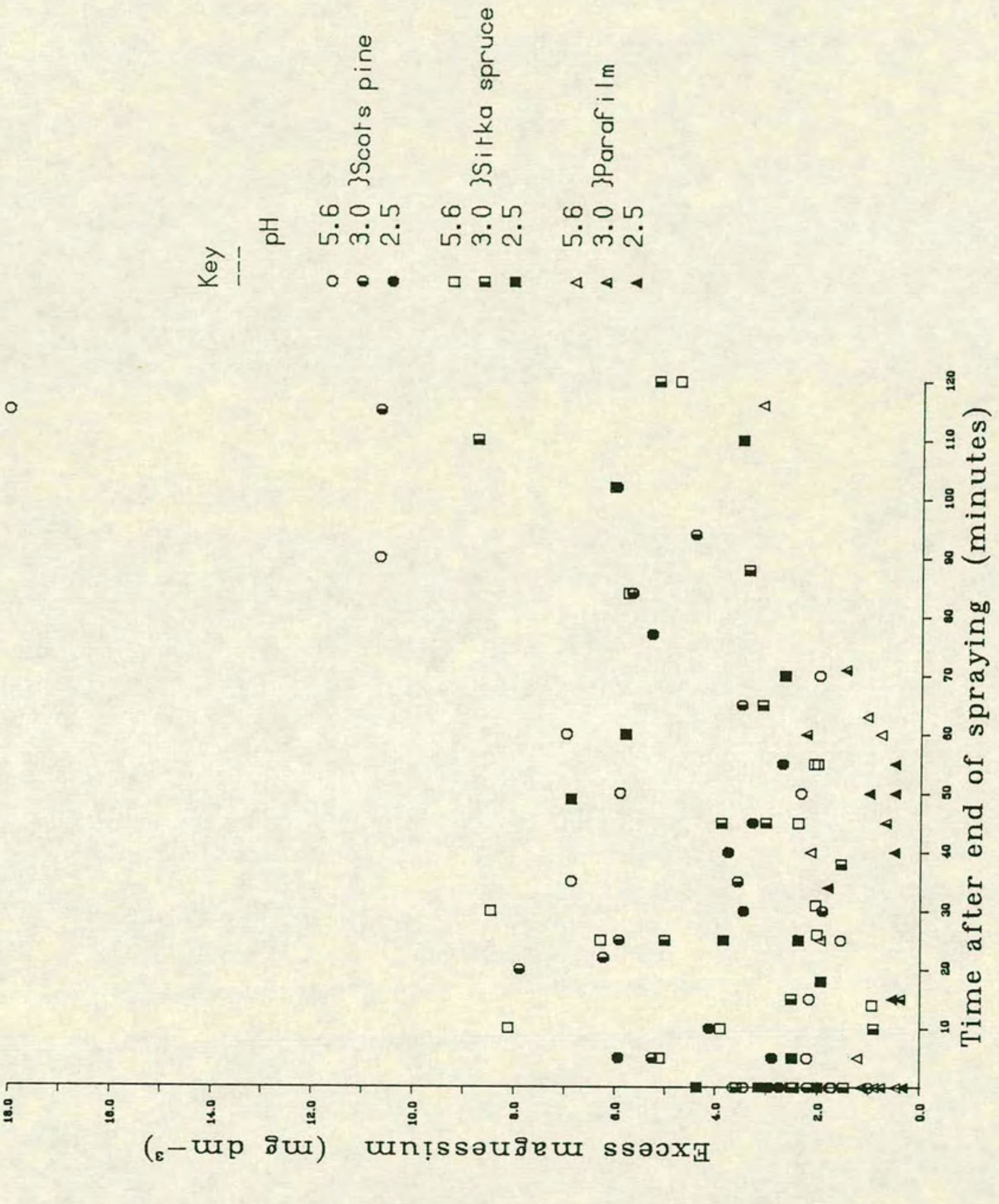


Figure 4.3b Excess magnessium in droplets of experiment 4.

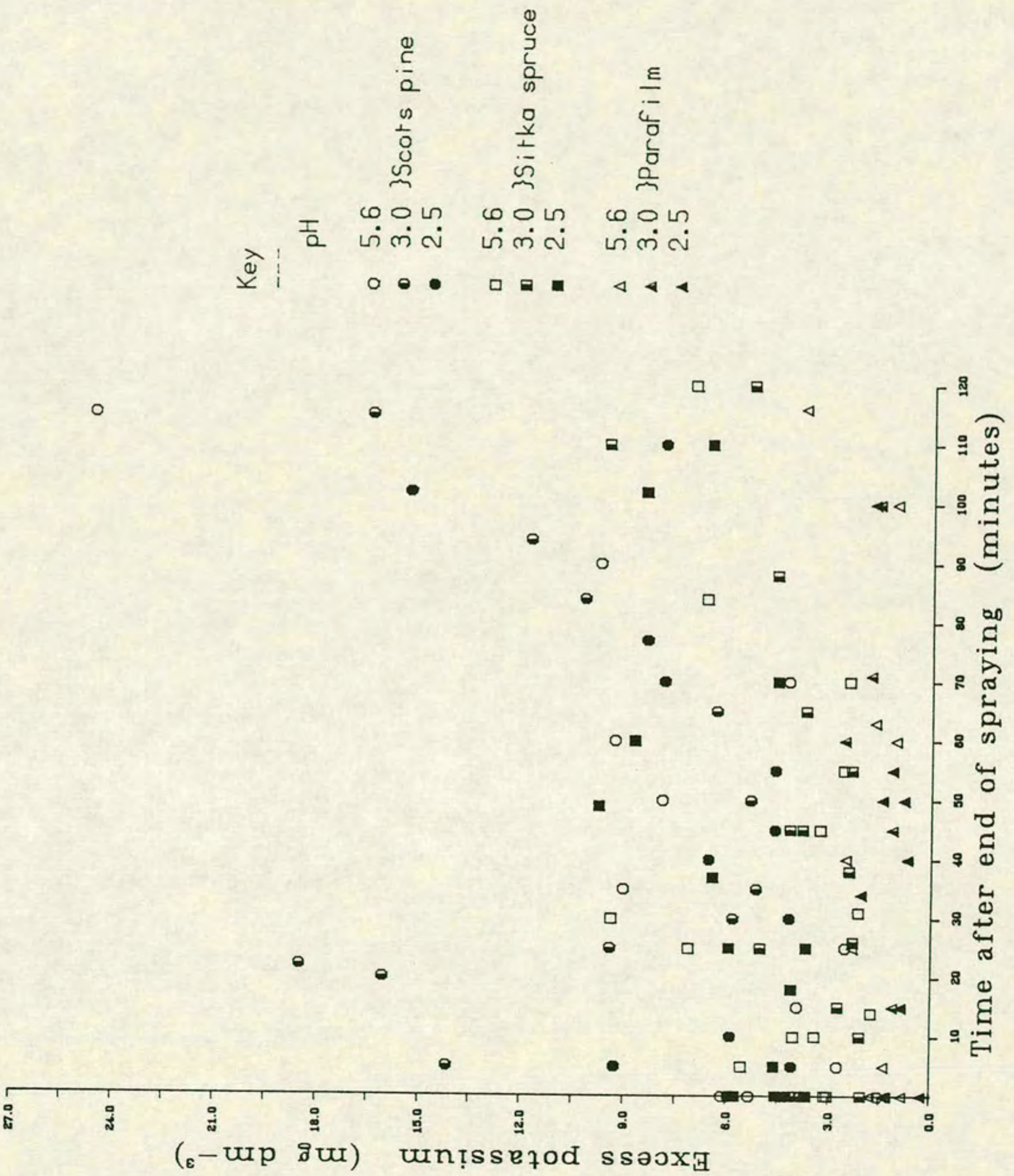
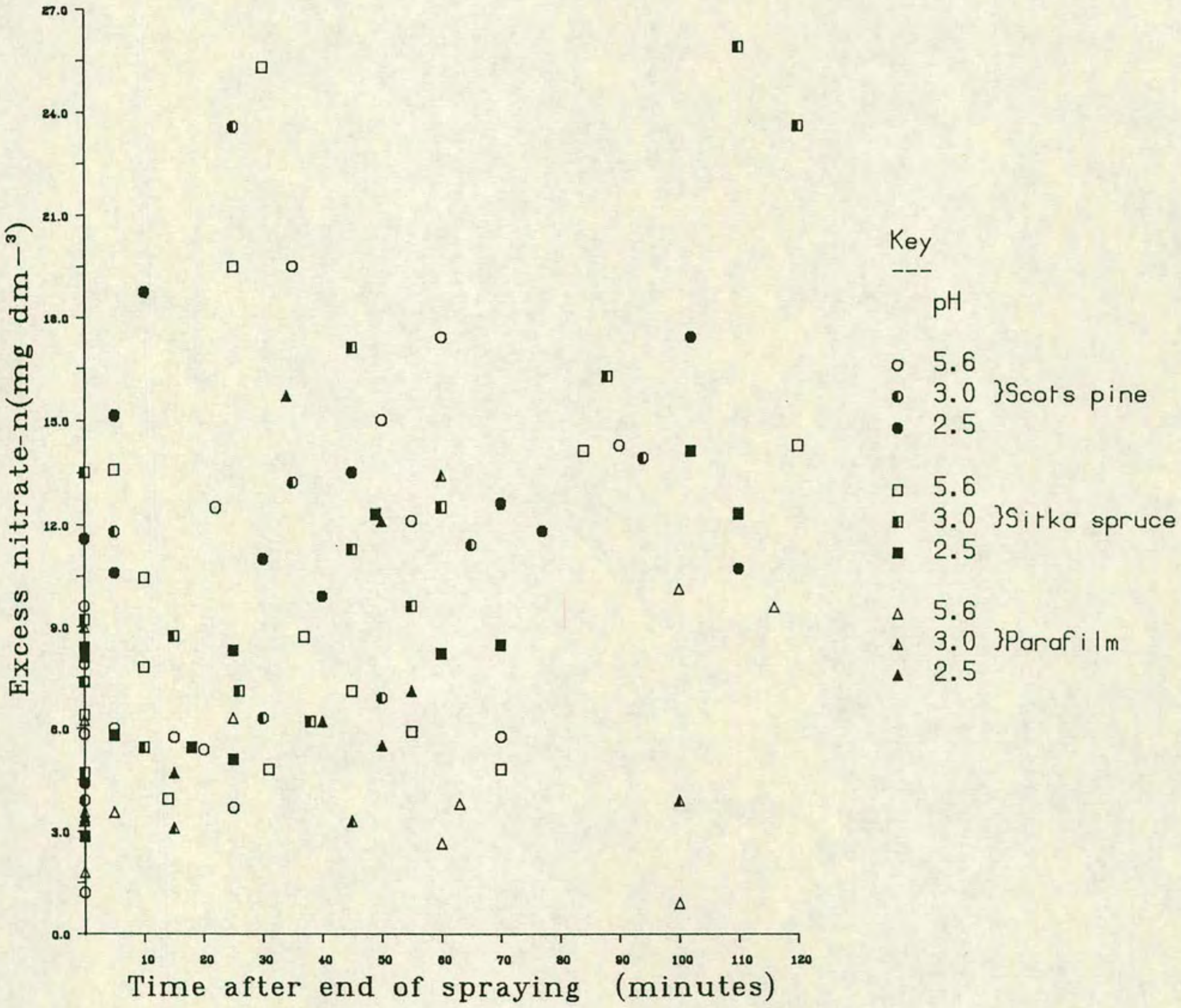


Figure 4.3c Excess potassium in droplets from experiment 4.

Figure 4.3d Excess nitrate-n in droplets from experiment 4.



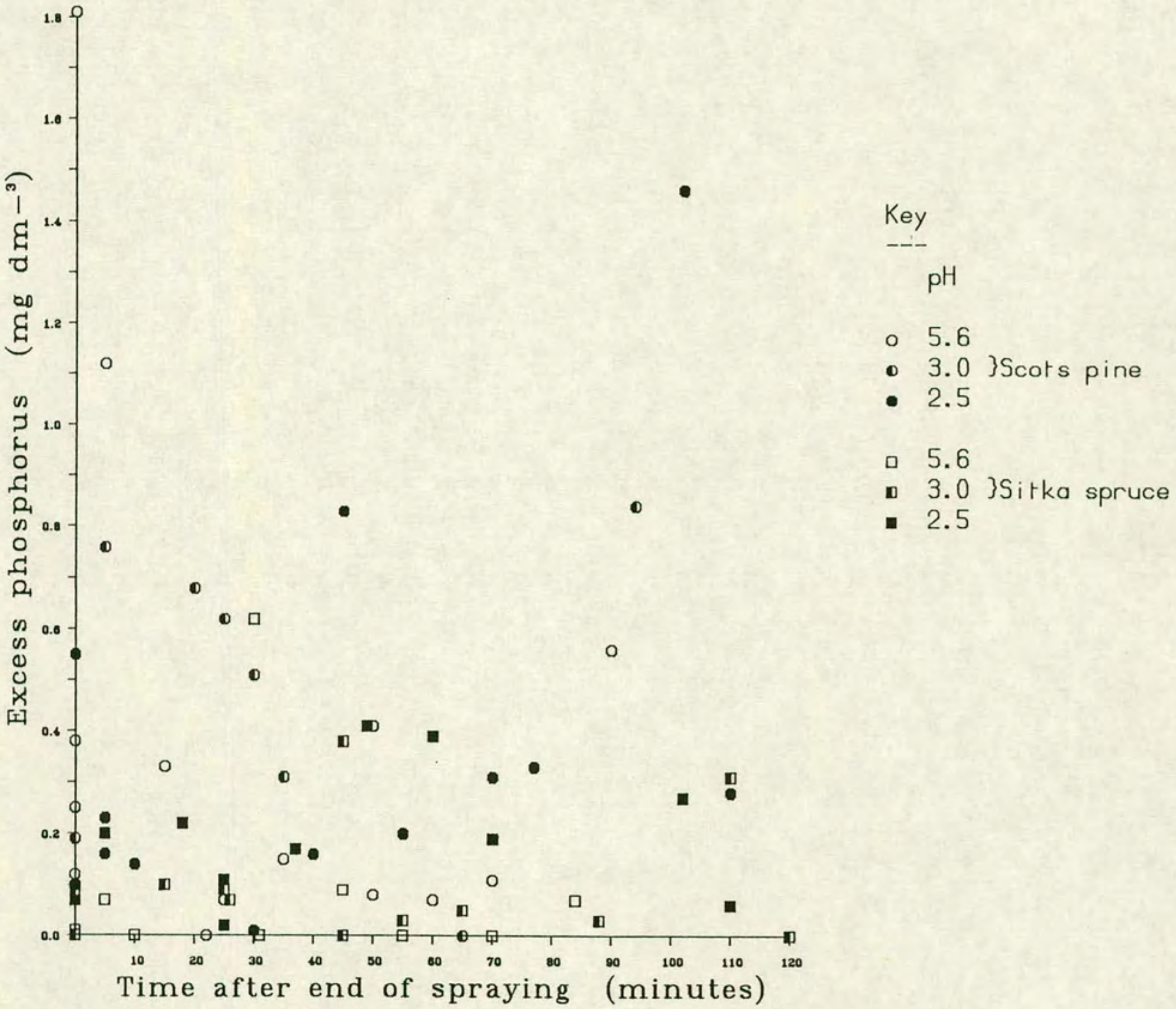


Figure 4.3e Excess phosphorus in droplets from experiment 4.

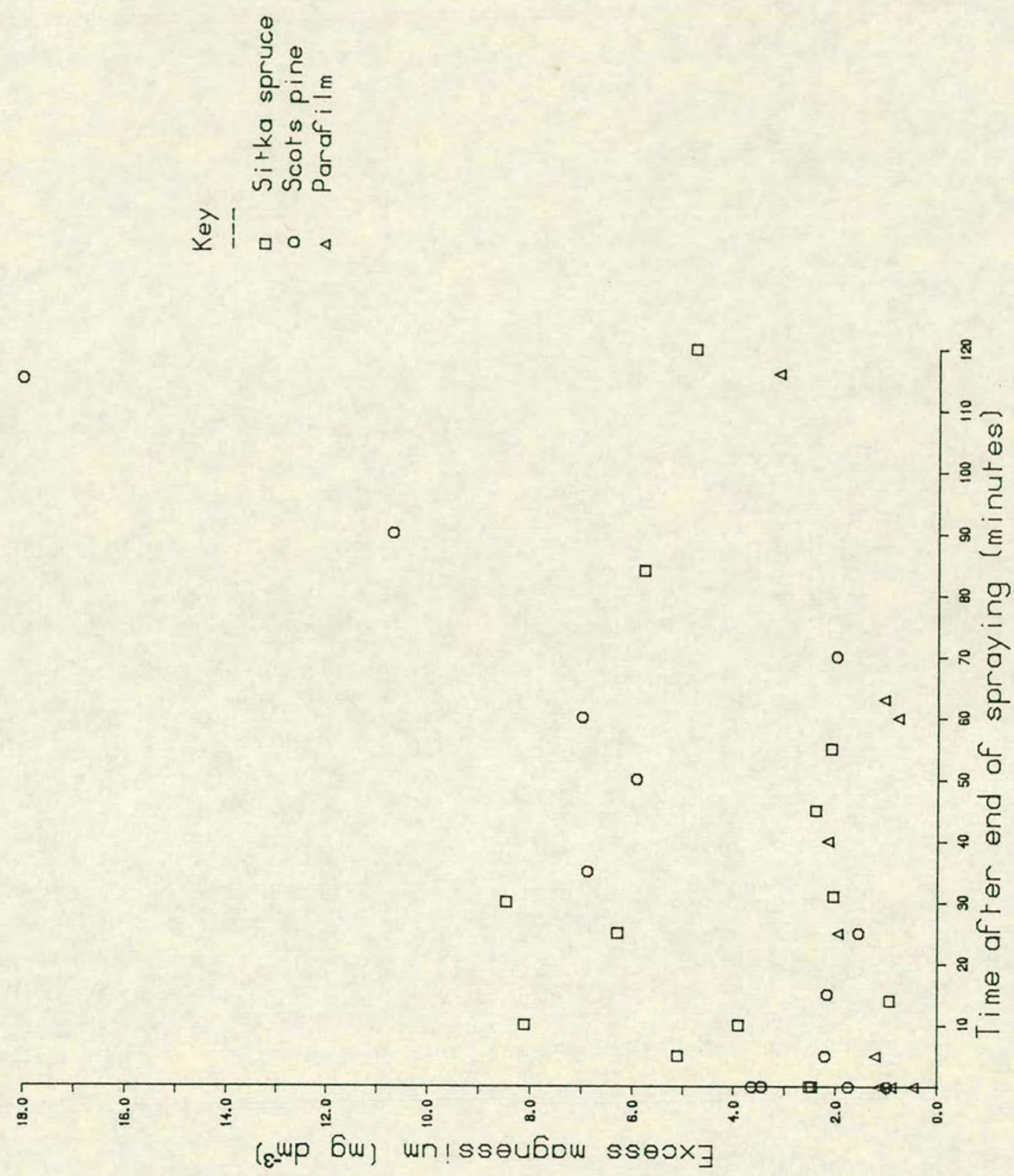


Figure 4.4 Excess magnessium in droplets from experiment 4 broken down into pH treatments.

Figure 4.4a pH 5.6.

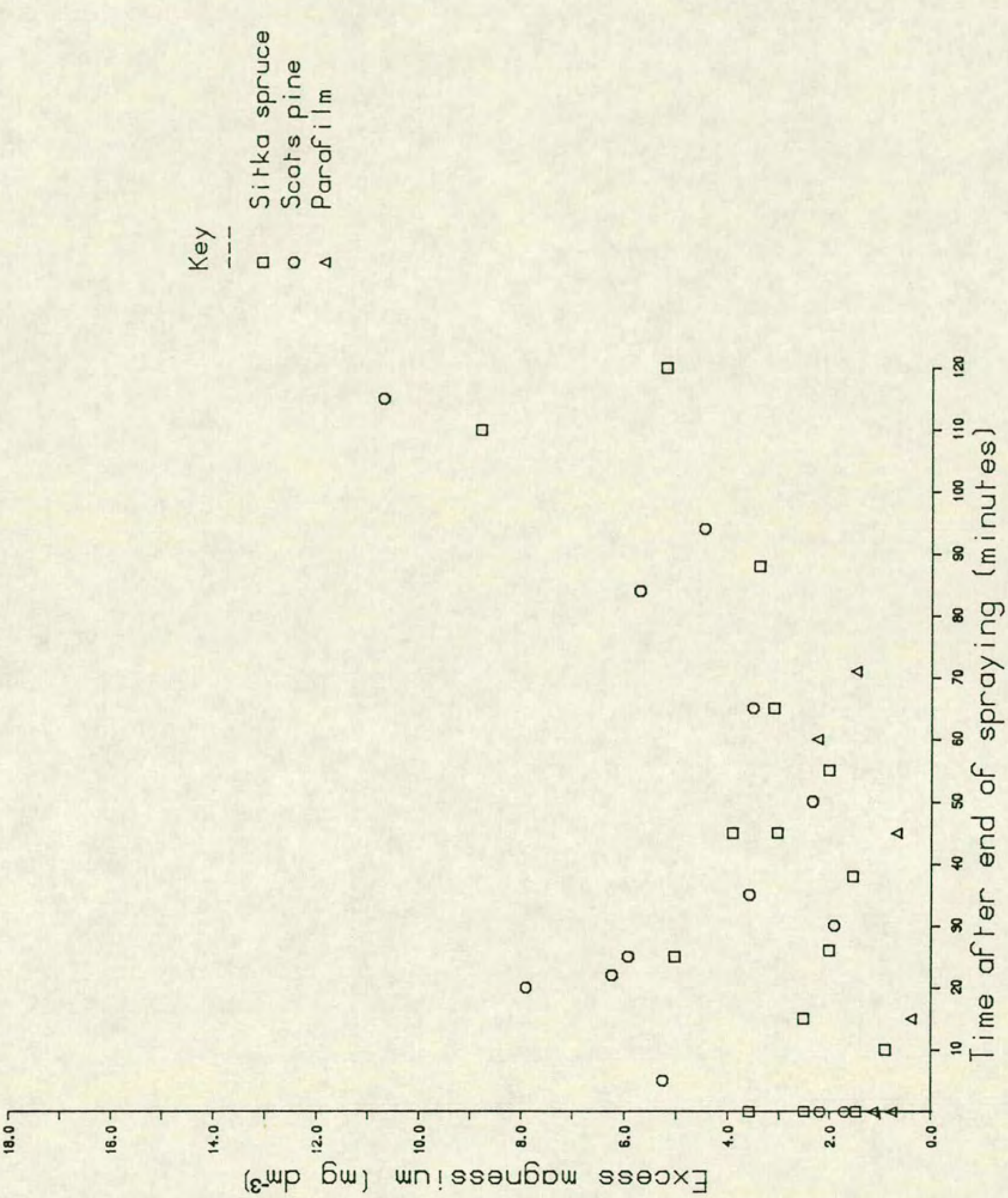


Figure 4.4b pH 3.0.

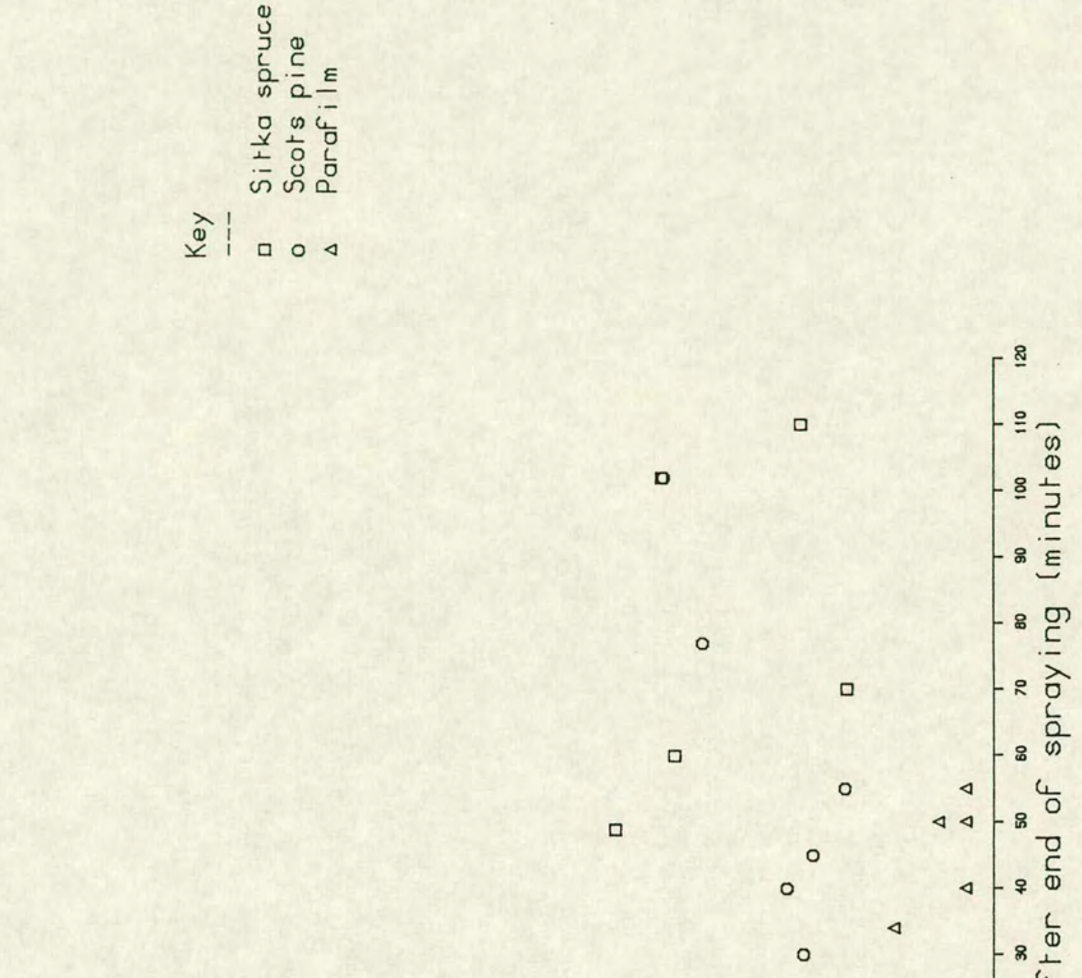


Figure 4.4c pH 2.5.

samples than in those of Sitka spruce. This was not so apparent in the pH 2.5 treatment.

After spraying ceases evaporation (to be discussed in depth in the next chapter) leads to increased concentrations as time progresses. To avoid incorporating this effect only concentrations of ions from droplets collected within the first 10 minutes after the termination of spraying were used to give an indication of the level of mobilization and leaching which had occurred.

Table 4.1 shows a clear trend for Mg concentrations in samples from Scots pine to increase as acidity increased whereas Sitka spruce samples show no clear trend. Pure spray samples had a mean magnesium concentration of 2.07 mg dm^{-3}.

Table 4.1 Excess ions in droplets collected within ten (± S.E.) minutes of the termination of spraying. Values are means of 4-5 samples in mg dm^{-3}. Where no S.E. is included values are means of less than 4 samples.

	Scots pine	Sitka spruce	Parafilm
		Treatment pH 5.6	
Ca	6.21 ± 2.28	9.15 ± 2.57	2.93 ± 0.66
Mg	2.41 ± 1.12	4.42 ± 2.34	0.93 ± 0.34
K	4.03 ± 1.88	3.82 ± 1.05	1.32 ± 0.39
NO_3-N	6.11 ± 3.15	9.12 ± 1.91	3.08 ± 0.85
PO_4-P	0.39 ± 0.42	0.04 ± 0.04	-
		Treatment pH 3.0	
Ca	7.49 ± 4.26	6.78 ± 2.48	3.15 ± 1.29
Mg	3.06 ± 1.92	2.12 ± 1.18	0.96 ± 0.41
K	7.93 ± 5.50	2.98 ± 1.06	1.34 ± 0.06
NO_3-N	7.70 ± 3.96	8.21 ± 4.04	6.17 ± 2.84
PO_4-P	0.92 ± 0.82	0.03 ± 0.05	-
		Treatment pH 2.5	
Ca	7.80 ± 1.63	7.28 ± 4.31	0.83 -
Mg	3.73 ± 1.35	3.00 ± 1.02	0.32 -
K	5.87 ± 2.09	4.64 ± 0.86	0.27 -
NO_3-N	12.10 ± 5.37	6.29 ± 3.57	3.40 -
PO_4-P	0.24 ± 0.18	0.11 ± 0.08	-

The plots of potassium ion concentration (Figure 4.5 a-c) also show that the difference between ion concentrations of "Parafilm" and tree samples became larger as the acidity of the treatment increased. Concentrations of potassium in samples from Sitka spruce were higher in the pH 2.5 treatment compared with the other treatments. Particularly in the pH 3.0 treatment Scots pine concentrations of potassium were higher than those for Sitka spruce. As with magnesium, Table 4.1 indicates increased concentrations of K in Scots pine samples for the two acid treatments compared with the control, but this is not evident in Sitka spruce samples. Pure spray samples contained 2.25 mg dm^{-3} of potassium. Parafilm concentrations of excess potassium were also higher for Scots pine than for Sitka spruce, as indicated by the time series graphs (4.5 a-c).

The increasing separation between "Parafilm" and tree samples with increasing acidity is again evident for calcium (Figure 4.6 a-c), although the high degree of scatter makes it difficult to make direct comparisons of excess concentrations between treatments. Scots pine samples showed higher excess concentrations than those from Sitka spruce in the pH 3.0 treatment. Table 4.1 shows little variation between species or treatments. Pure spray samples contained 4.7 mg dm^{-3} calcium.

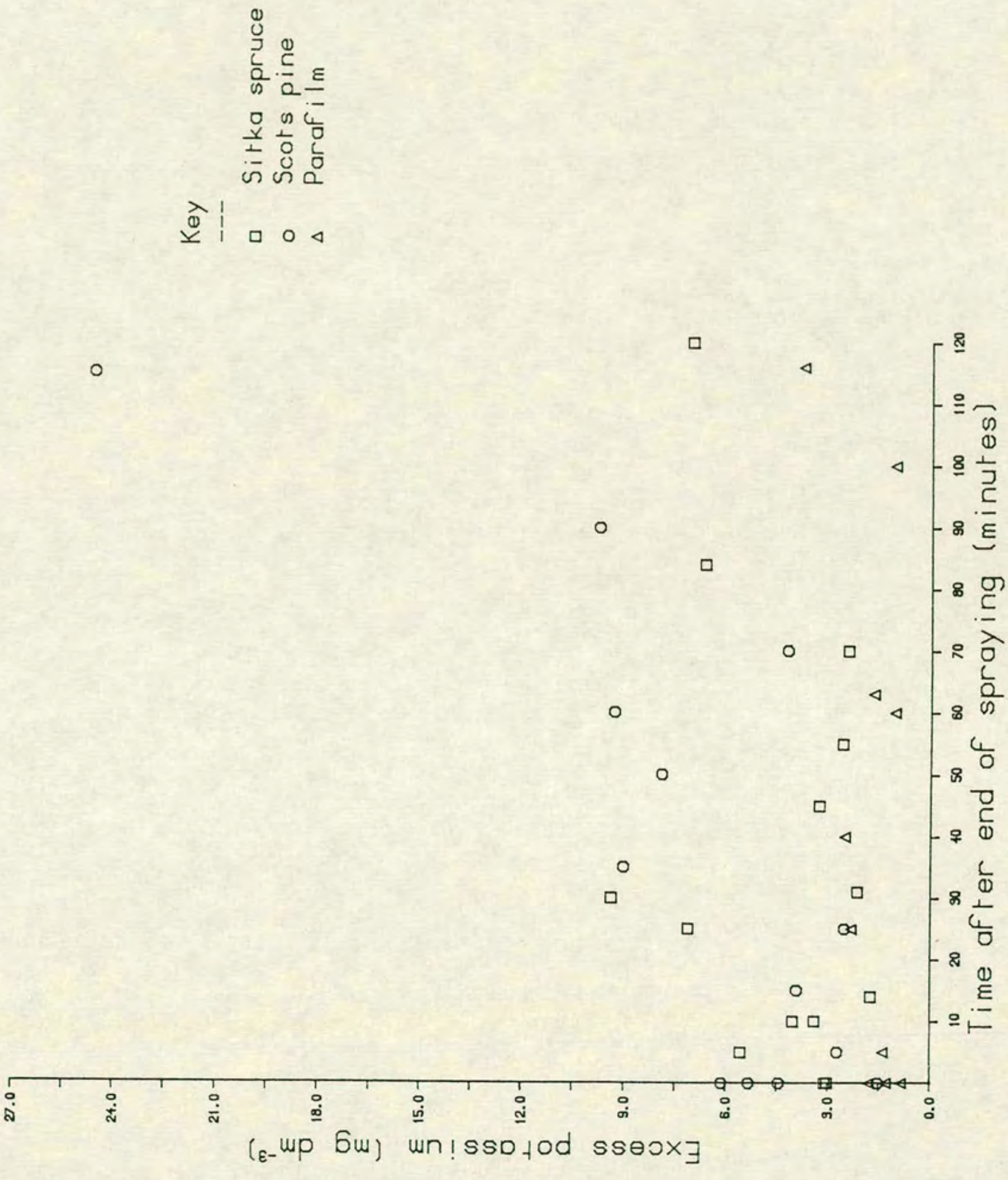


Figure 4.5 Excess potassium in droplets from experiment 4 broken down into pH treatments.

Figure 4.5a pH 5.6.

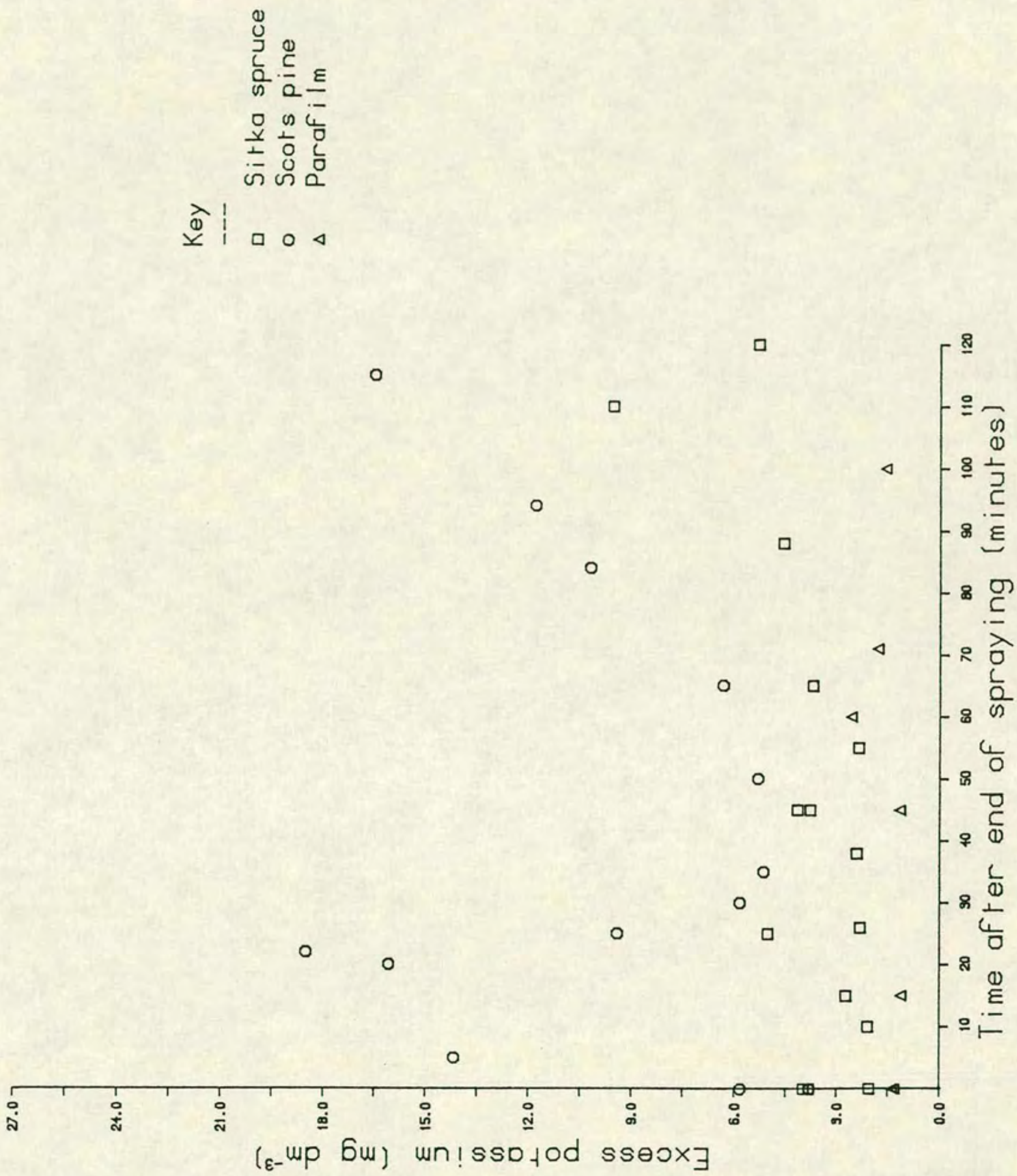


Figure 4.5b pH 3.0.

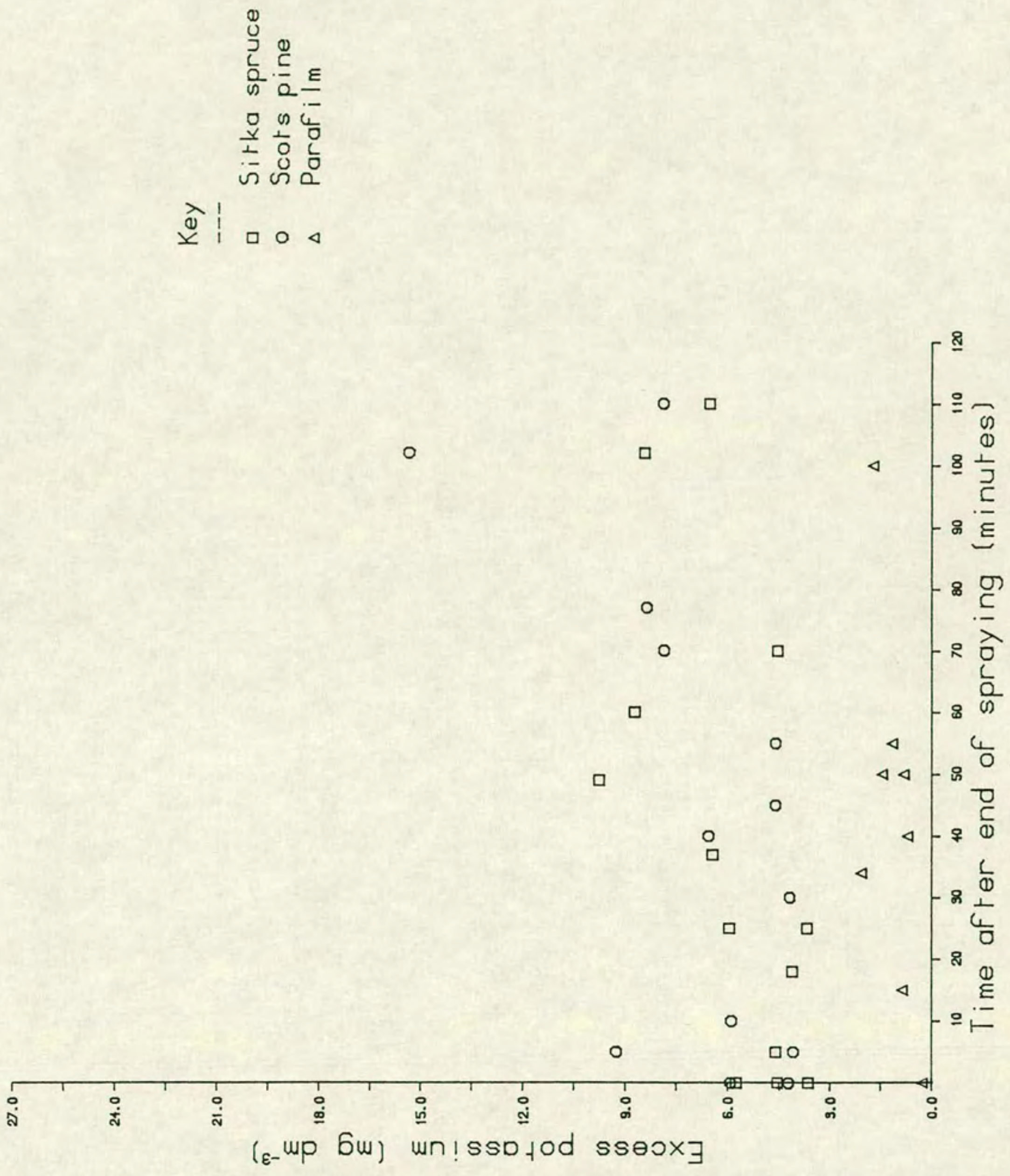


Figure 4.5c pH 2.5.

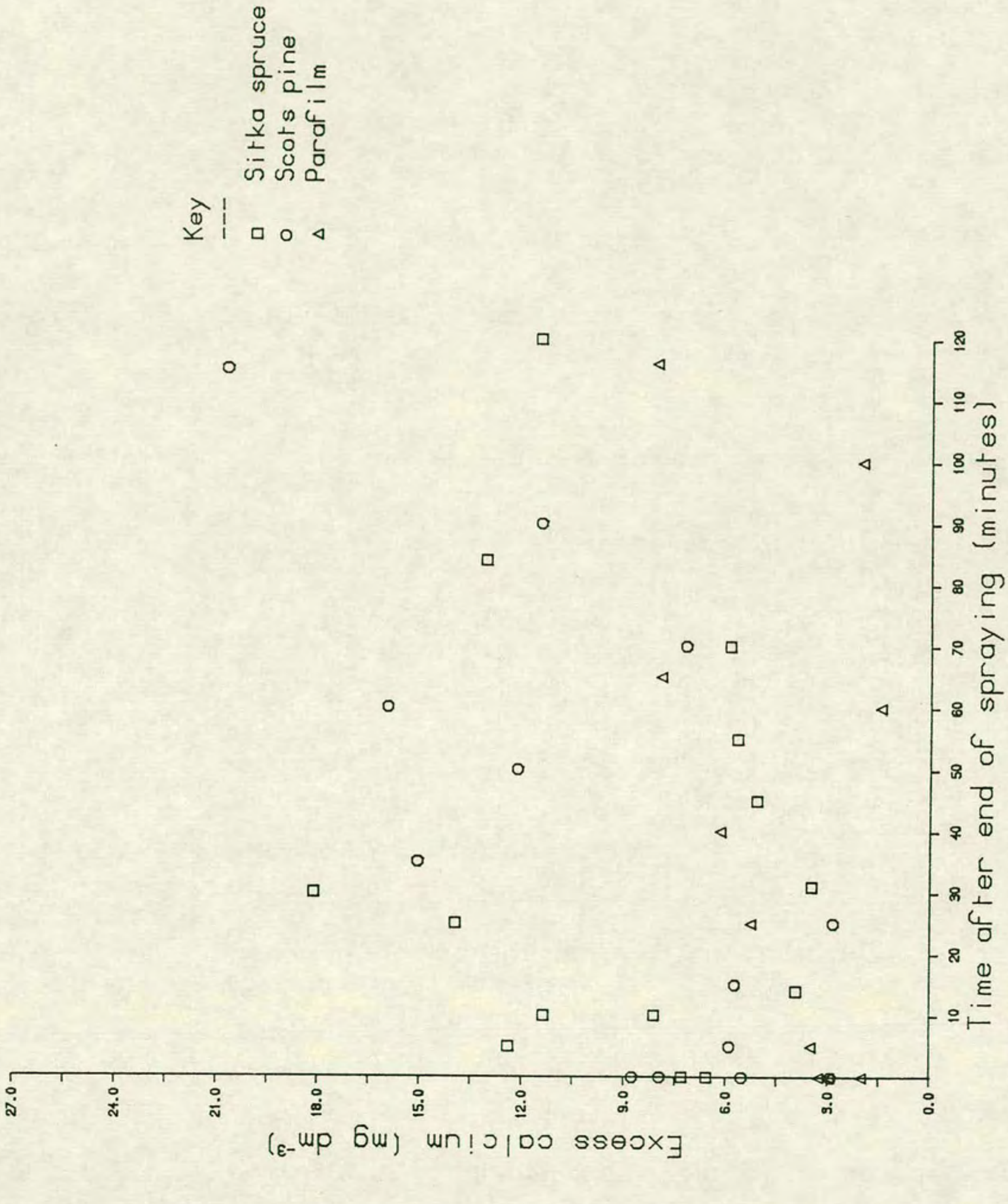


Figure 4.6 Excess calcium in droplets from experiment 4 broken down into pH treatments.

Figure 4.6a pH 5.6.

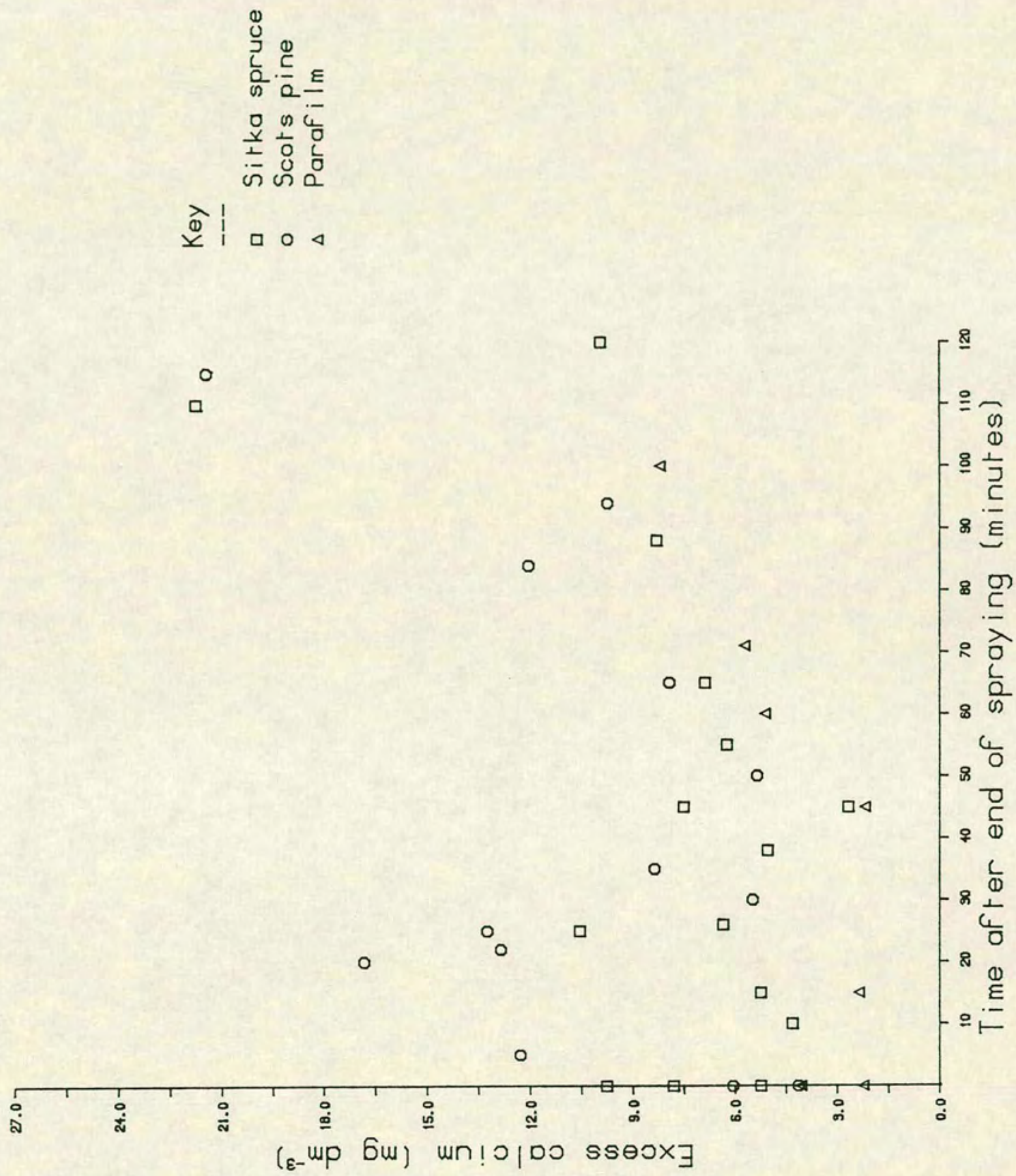


Figure 4.6b pH 3.0.

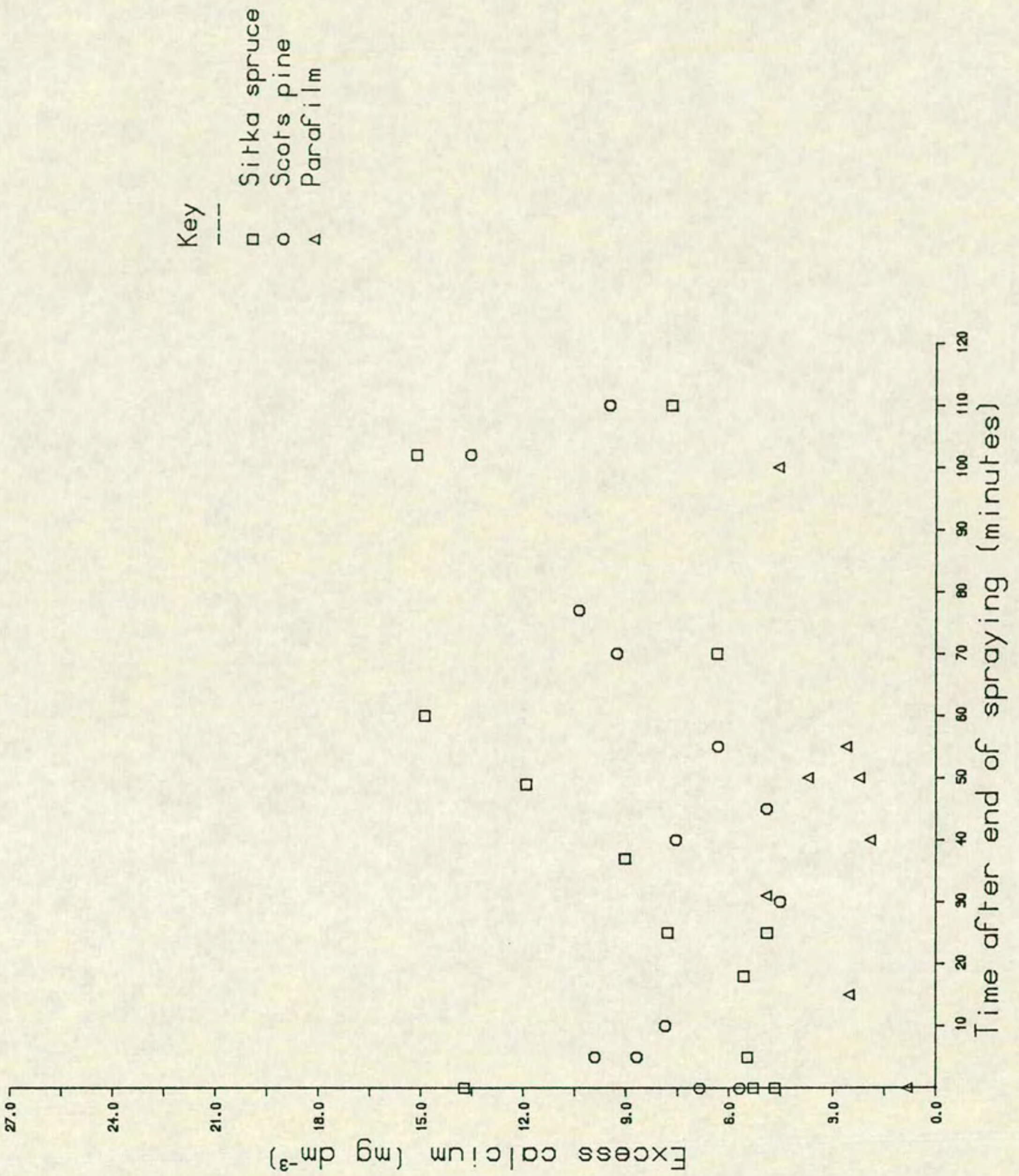


Figure 4.6c pH 2.5.

The graphs of the three cations when compared show similar patterns of excess ion levels. The NO_3^--N plots do not follow this pattern and show much greater scatter. Although the samples from the two tree species were consistently higher in NO_3^--N than those from "Parafilm", there was not the same increasing separation between trees and "Parafilm" with increasing acidity as seen for the cations (Figure 4.7 a-c). In the pH 2.5 treatment Scots pine samples had higher NO_3^--N levels than those from Sitka spruce.

Table 4.1 shows that initial NO_3^--N concentrations declined as pH decreased for Sitka spruce but increased with decreasing pH for Scots pine. Pure spray samples contained 3.94 mg dm^{-3} of NO_3^- at pH 5.6, 6.03 mg dm^{-3} at pH 3.0 and 8.10 mg dm^{-3} at pH 2.5.

PO_4^{3-}-P from shoot samples cannot be compared to "Parafilm" samples as the original spray contained only trace amounts. Thus all PO_4^{3-}-P in samples came from solubilization or leaching of needles (Figure 4.8 a-c). Concentrations of PO_4^{3-}-P from Scots pine samples were higher in treatments pH 3.0 and pH 2.5 than in pH 5.6. Concentrations in Sitka spruce samples were higher in pH 2.5 treatment compared with the pH 5.6 and pH 3.0 treatment. Scots pine samples had higher PO_4^{3-}-P levels than Sitka spruce for the pH 3.0 treatment. Table 4.1 indicates that despite generally low PO_4-P concentration samples from Scots pine contained more PO_4^--P than those from Sitka spruce.

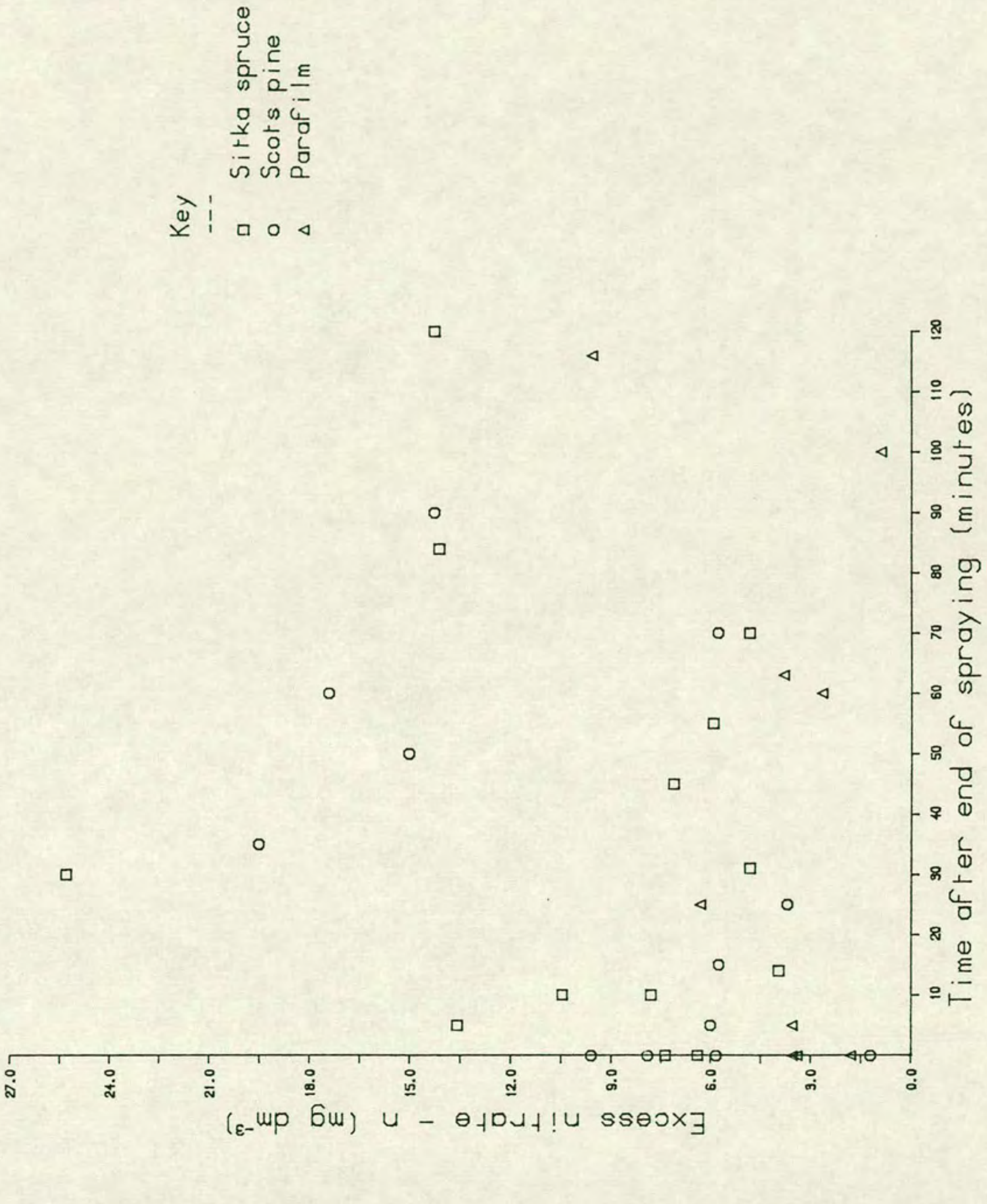


Figure 4.7 Excess nitrate-n in droplets from experiment 4 broken down into pH treatments.

Figure 4.7a pH 5.6.

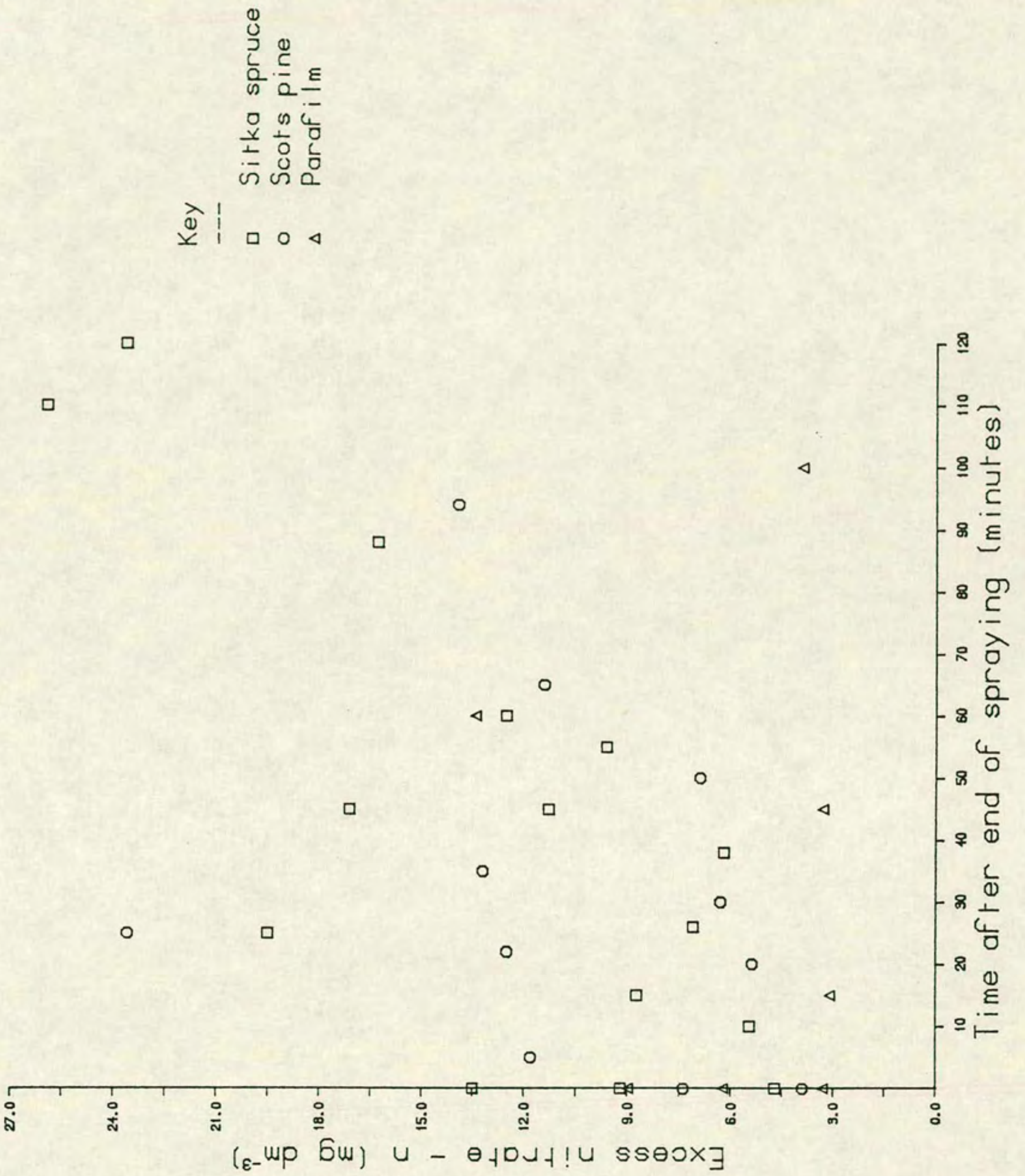


Figure 4.7b pH 3.0.

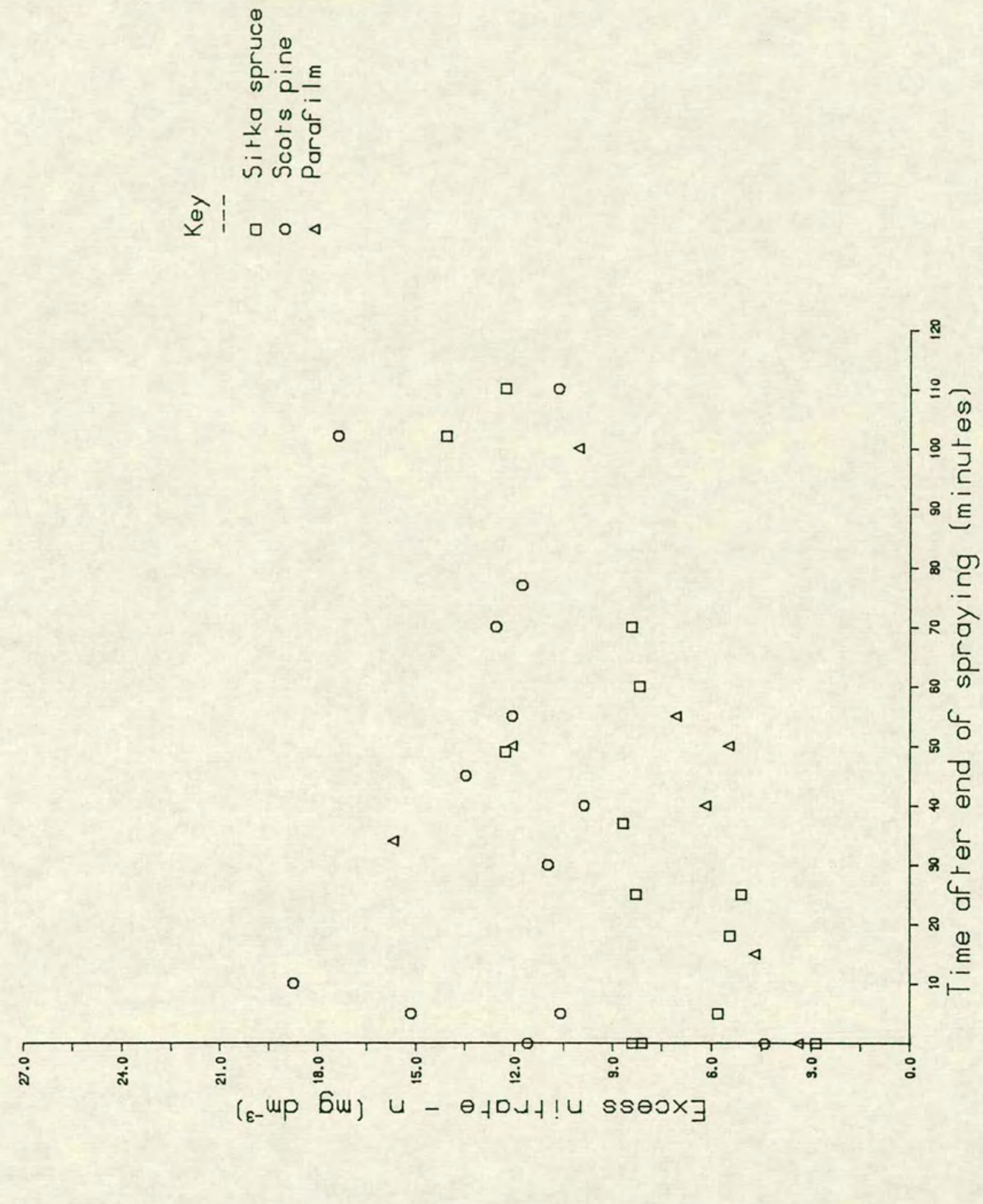


Figure 4.7c pH 2.5.

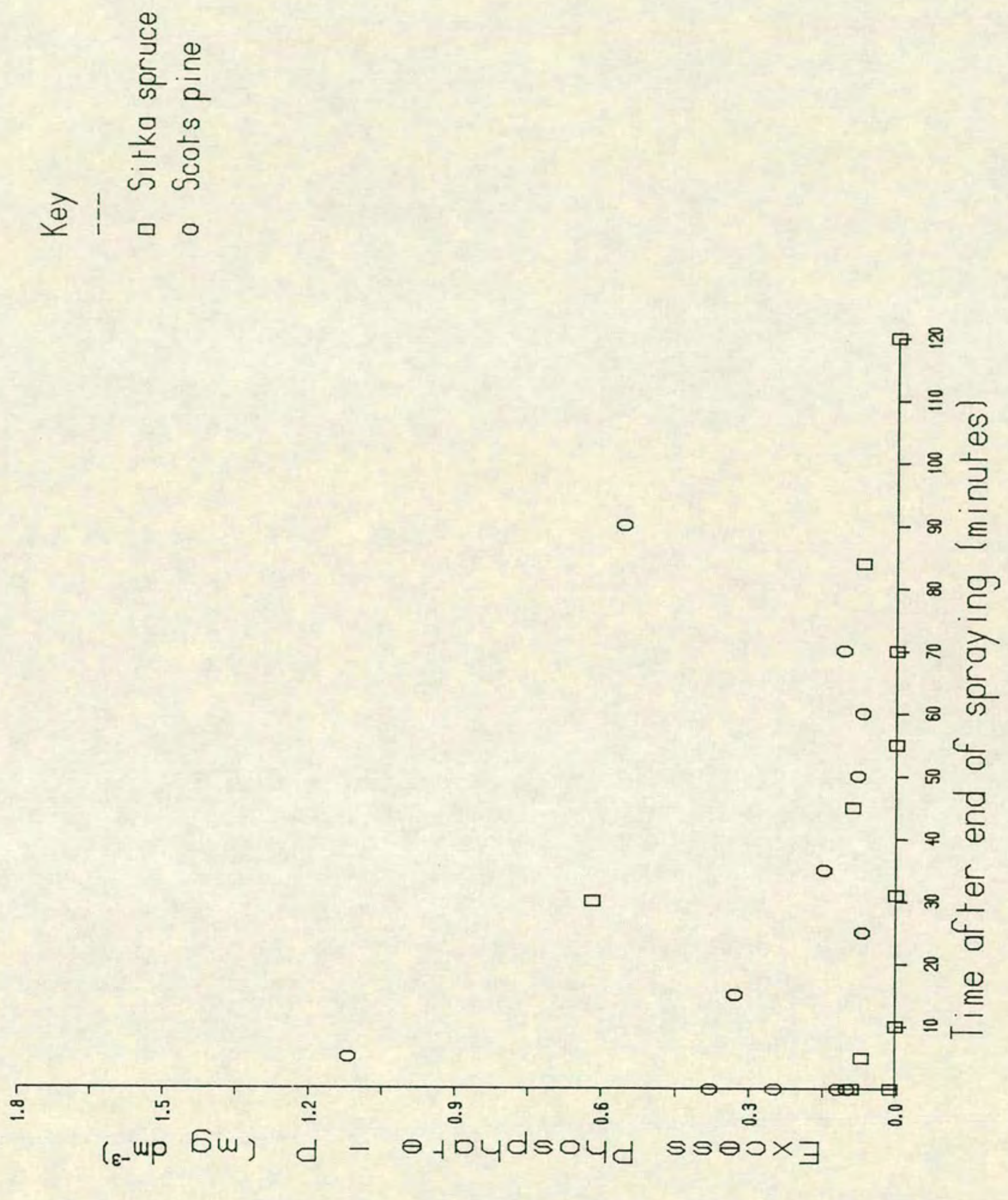


Figure 4.8 Excess phosphate-p in droplets from experiment 4 broken down into pH treatments.

Figure 4.8a pH 5.6.

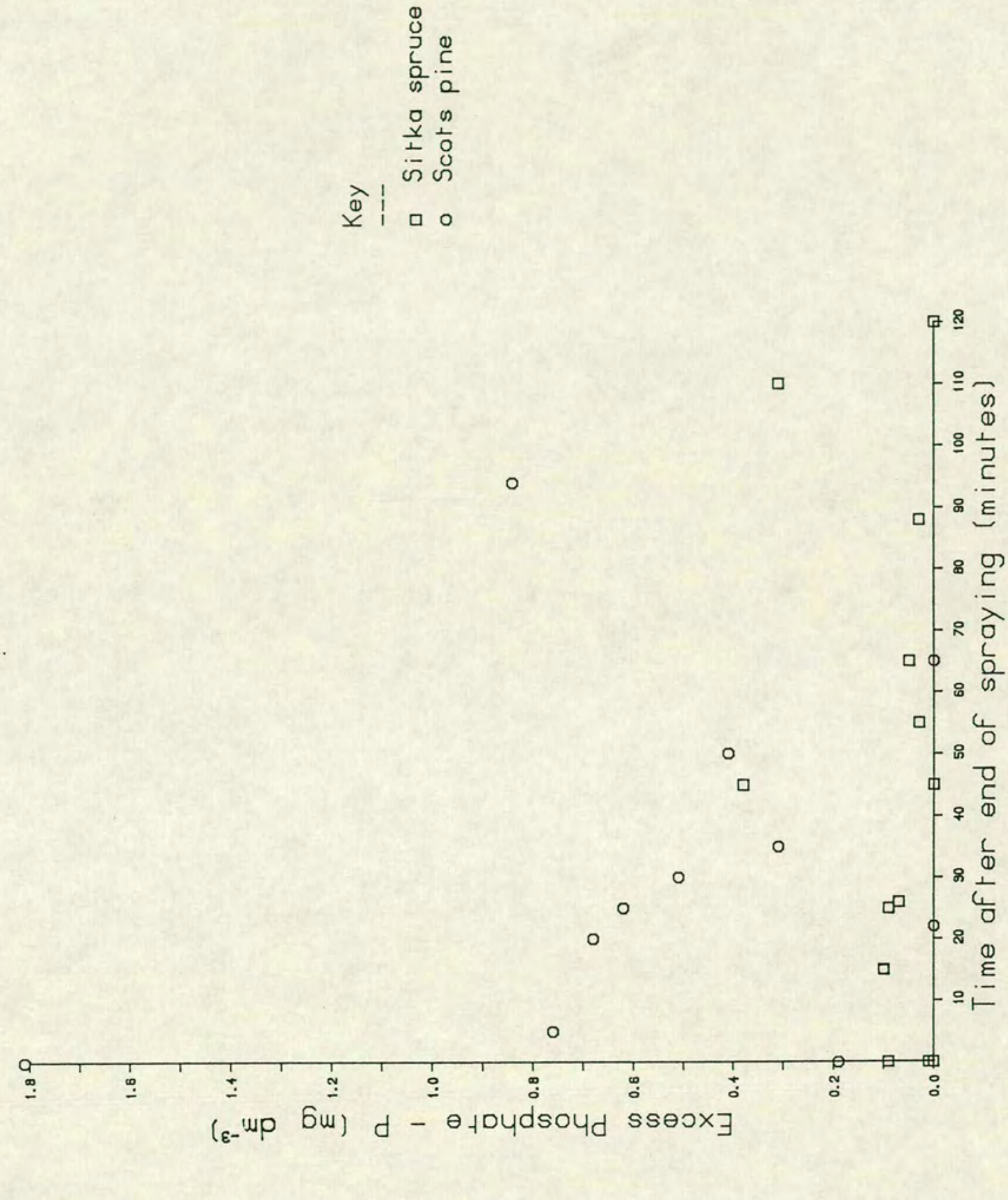


Figure 4.8b pH 3.0.

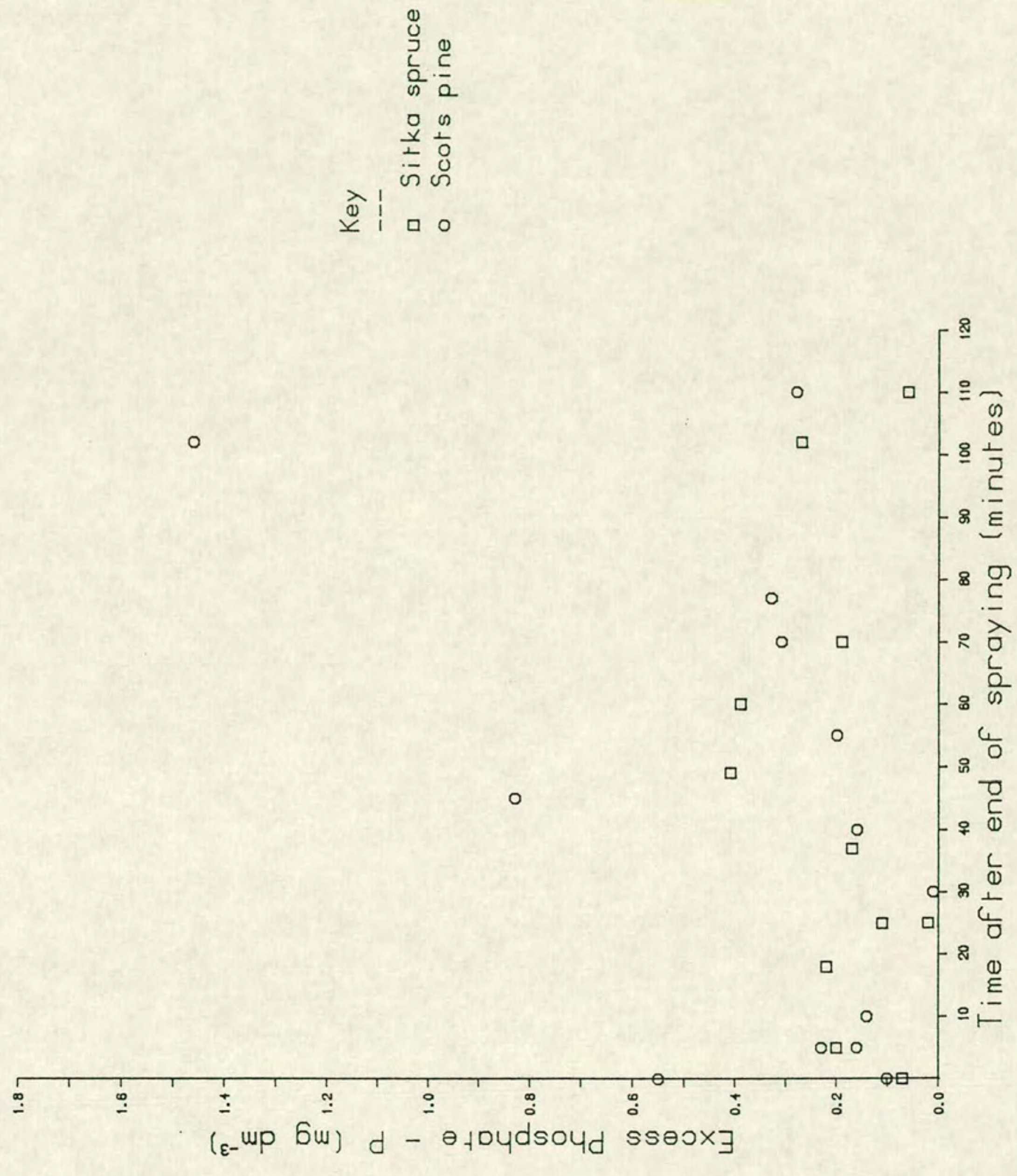


Figure 4.8c pH 2.5.

The leaching behaviour of the cations can also be examined by plotting the excess concentration of the cations against each other. A line corresponding to the increase in concentration of the ions to be expected in evaporating droplets can be drawn. Figure 4.9 shows that when excess potassium concentration is plotted against excess calcium concentration there is a good correlation between the values of the ions in droplets from "Parafilm" and the theoretical evaporation line, as expected. In treatment pH 5.6 (Figure 4.9a) the Sitka spruce samples also follow this line but the Scots pine samples show a tendency towards higher potassium concentrations than expected. This indicates that, after accounting for evaporation, there is a larger increase of potassium in samples from Scots pine, suggesting that potassium is being leached at a faster rate than calcium. In Figure 4.9b the bias towards potassium is larger and clearer and by pH 2.5 (Figure 4.9c) the Sitka spruce samples also show potassium with relatively higher concentrations than predicted by evaporation when compared with calcium.

The graphs of potassium concentration versus magnesium concentration show similar behaviour (Figure 4.10). At pH 5.6 Scots pine samples are weakly biased towards potassium (Fig. 4.10a), this bias becoming stronger at pH 3.0 and pH 2.5 Figs. 4.10 b, c) indicating more leaching of potassium than magnesium. Relatively higher potassium concentrations compared with those of magnesium again become evident for Sitka spruce only at pH 2.5.

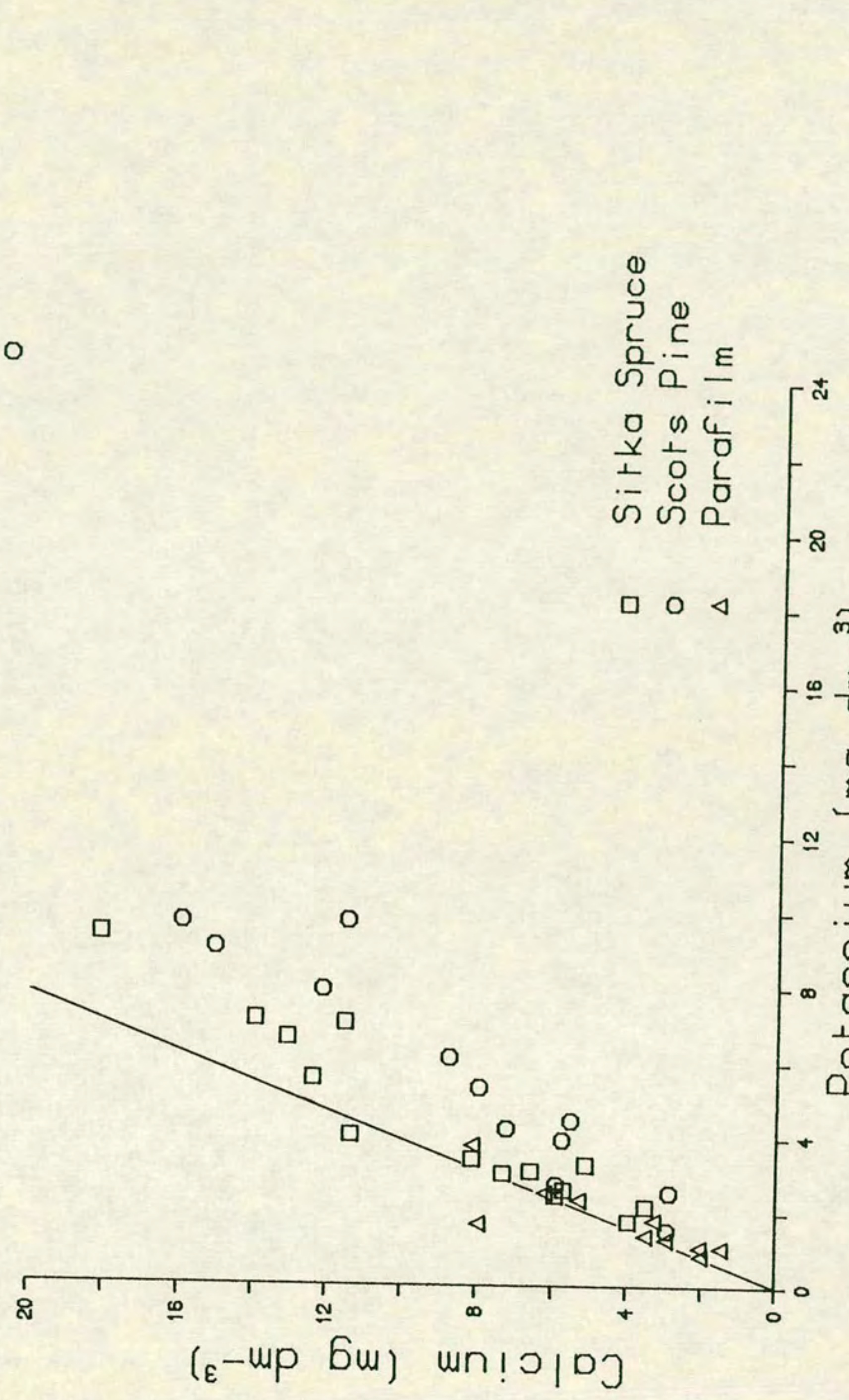


Figure 4.9 Plot of excess calcium concentration verses excess potassium concentration in samples of impacted SAC in experiment 4. The line corresponds to the concentration to be expected through the sole effect of evaporation. Figure 4.9a pH 5.6.

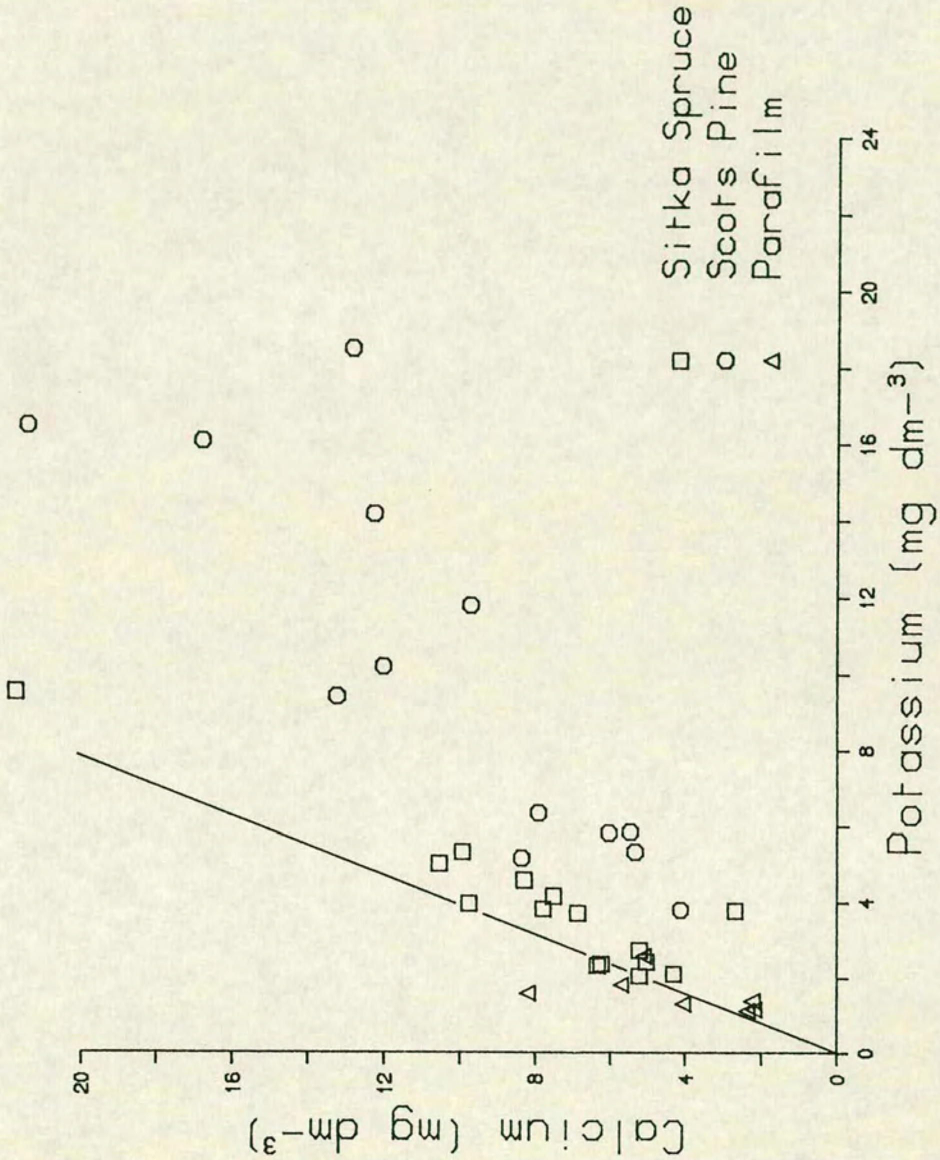


Figure 4.9b pH 3.0.

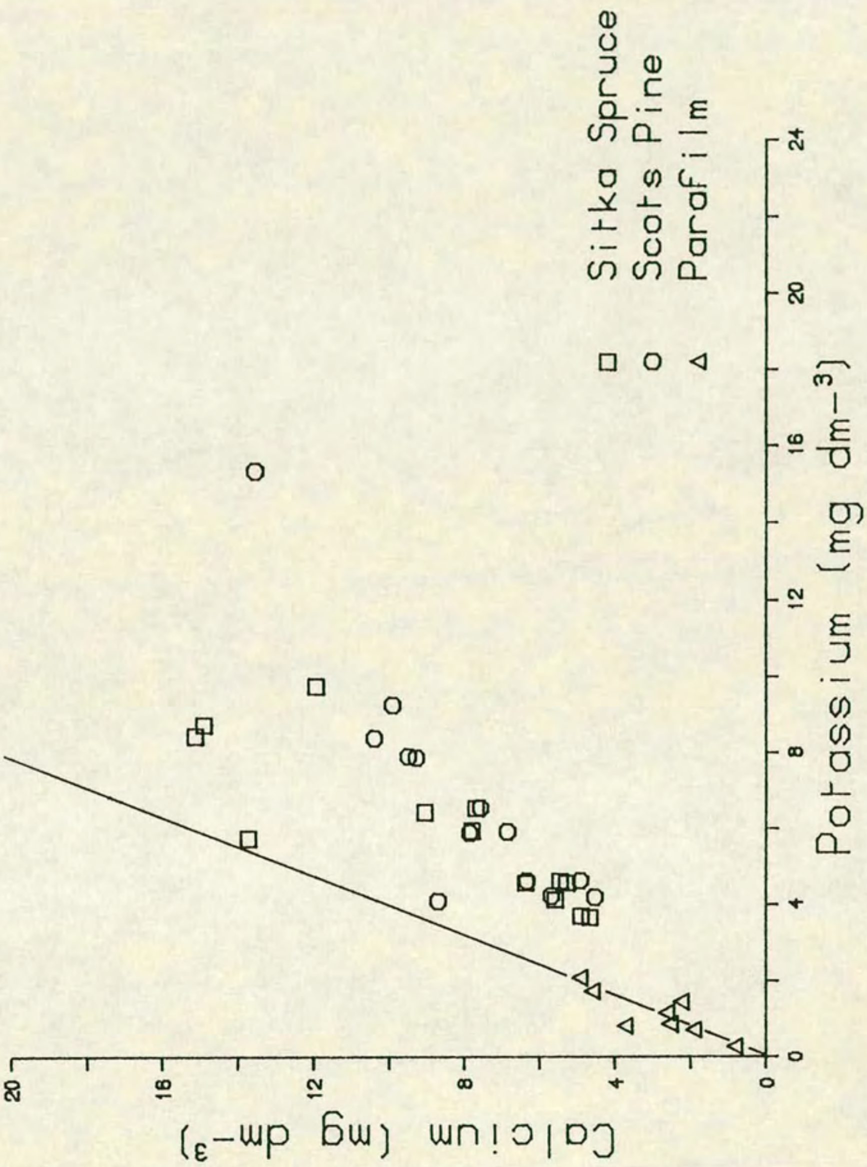


Figure 4.9c pH 2.5.

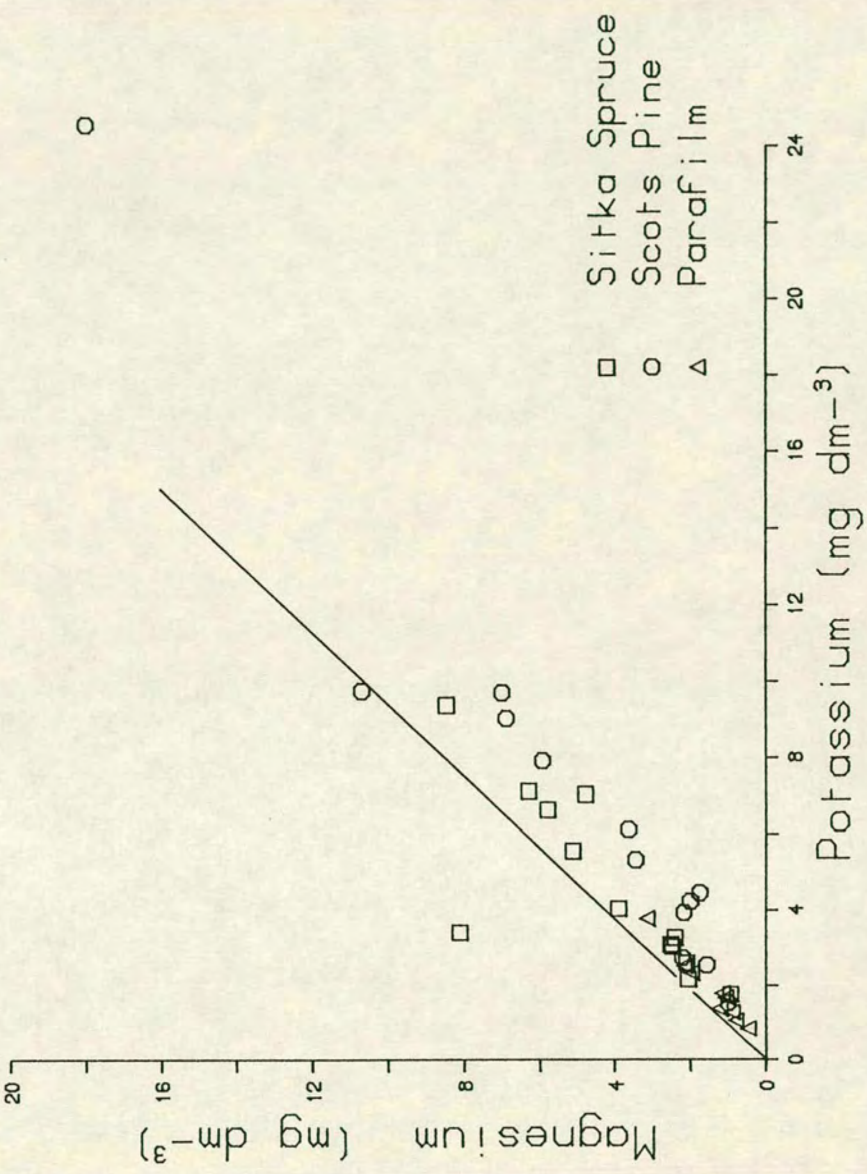


Figure 4.10 Plot of excess potassium concentration versus excess magnesium concentration in samples of impacted SAC in experiment 4. The line corresponds to the concentrations to be expected through the sole effect of evaporation. Figure 4.10a pH 5.6.

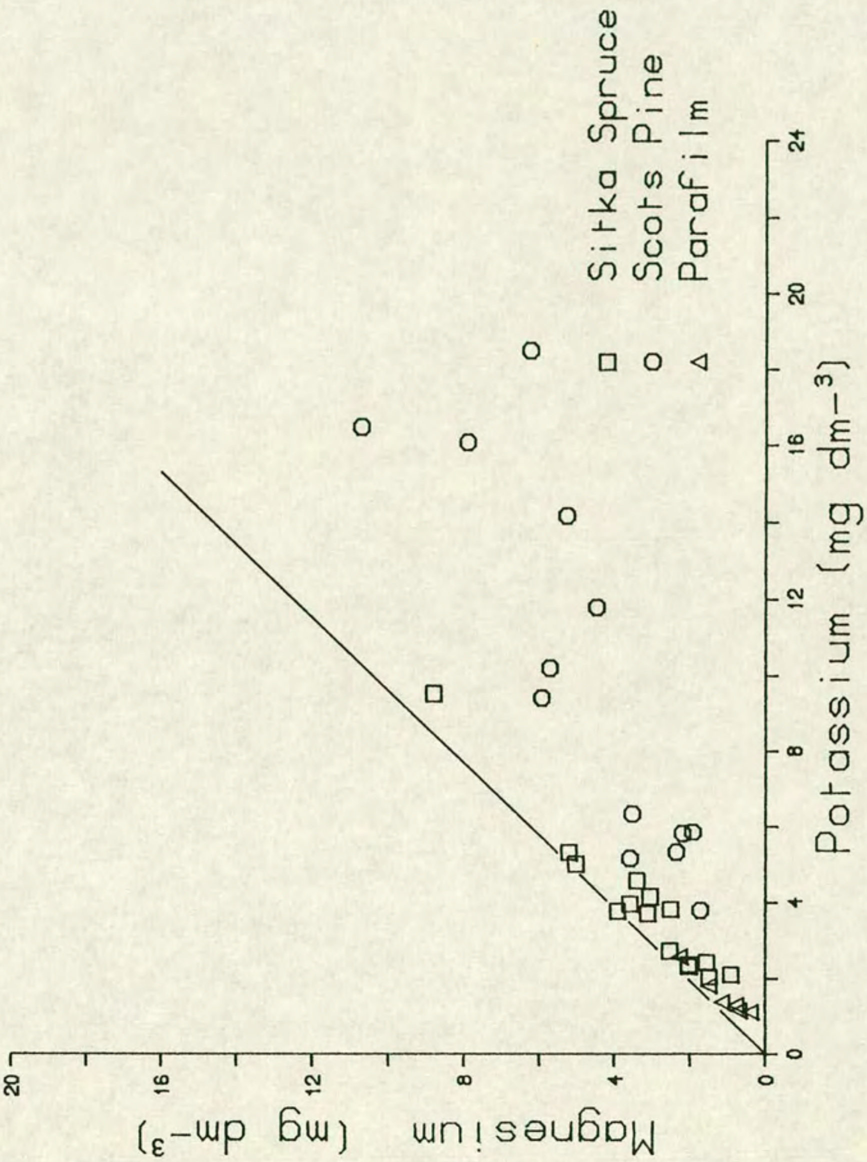


Figure 4.10b pH 3.0.

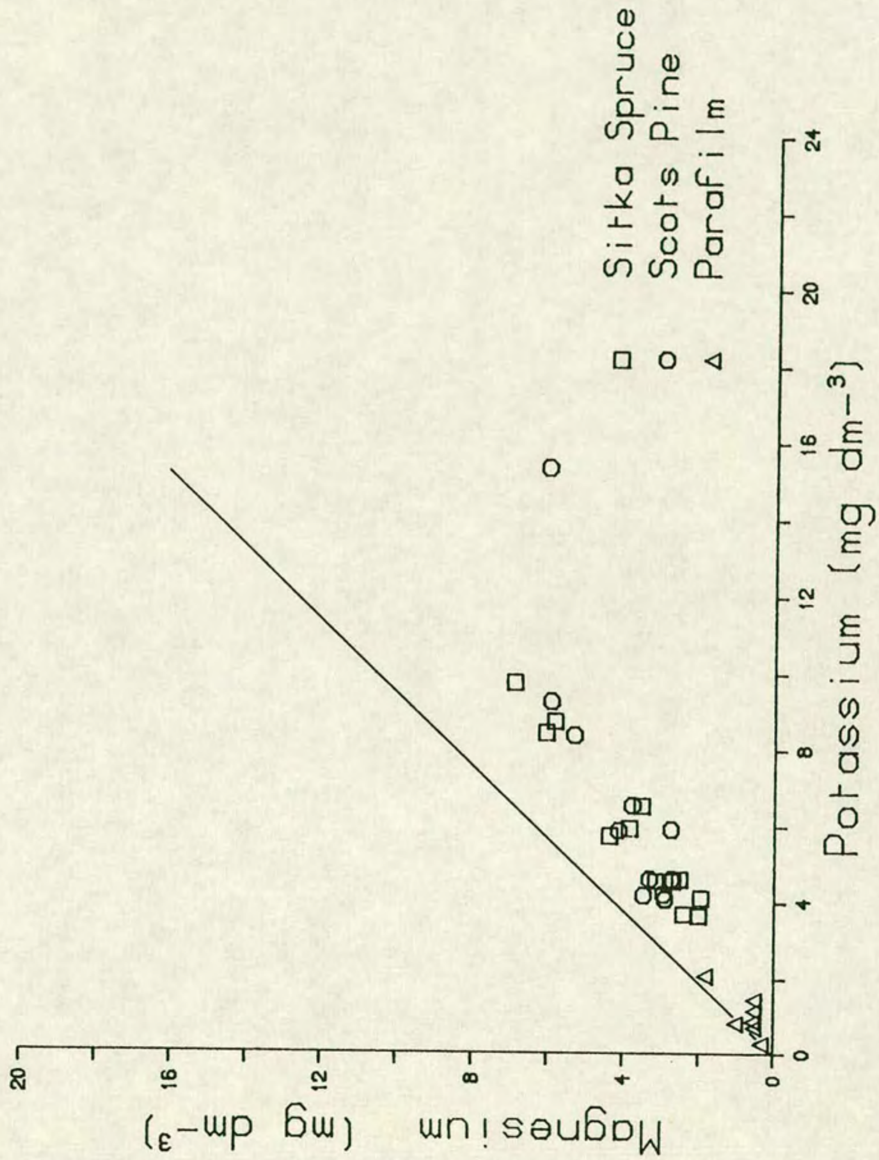


Figure 4.10c pH 2.5.

When the two ions which are leached to a lesser degree than potassium are plotted against each other a close correlation between observed and predicted calcium and magnesium values is seen for Sitka spruce (Fig. 4.11). This does not limit the increase in ion concentrations to evaporation but indicate that neither ion is preferentially leached. Scots pine samples also showed a good correlation between observed and predicted calcium and magnessium at pH 5.6 and pH 3.0. At pH 2.5 (Fig. 4.11c) however there was a slight tendency towards relatively higher magnesium concentrations.

Although there was no clear separation between the pH of the samples from Sitka spruce, Scots pine and "Parafilm", the "Parafilm" sample concentrations were generally higher than those from either tree species, particularly at pH 5.6 (Figure 4.12).

Table 4.2 shows that initial pH values did not vary much between live shoots and "Parafilm" in the pH 5.6 or pH 3.0 treatments. At pH 2.5 although Scots pine samples had a lower mean pH than Sitka spruce and "Parafilm" samples, the difference was not significant. It can also be seen that in the pH 5.6 treatment the mean pHs of all the samples were higher than 5.6. This is possibly the result of CO_2 degassing and traces of NH_3 in the air. In the pH 3.0 and pH 2.5 treatments all sample means were lower than the pH of the SAC.

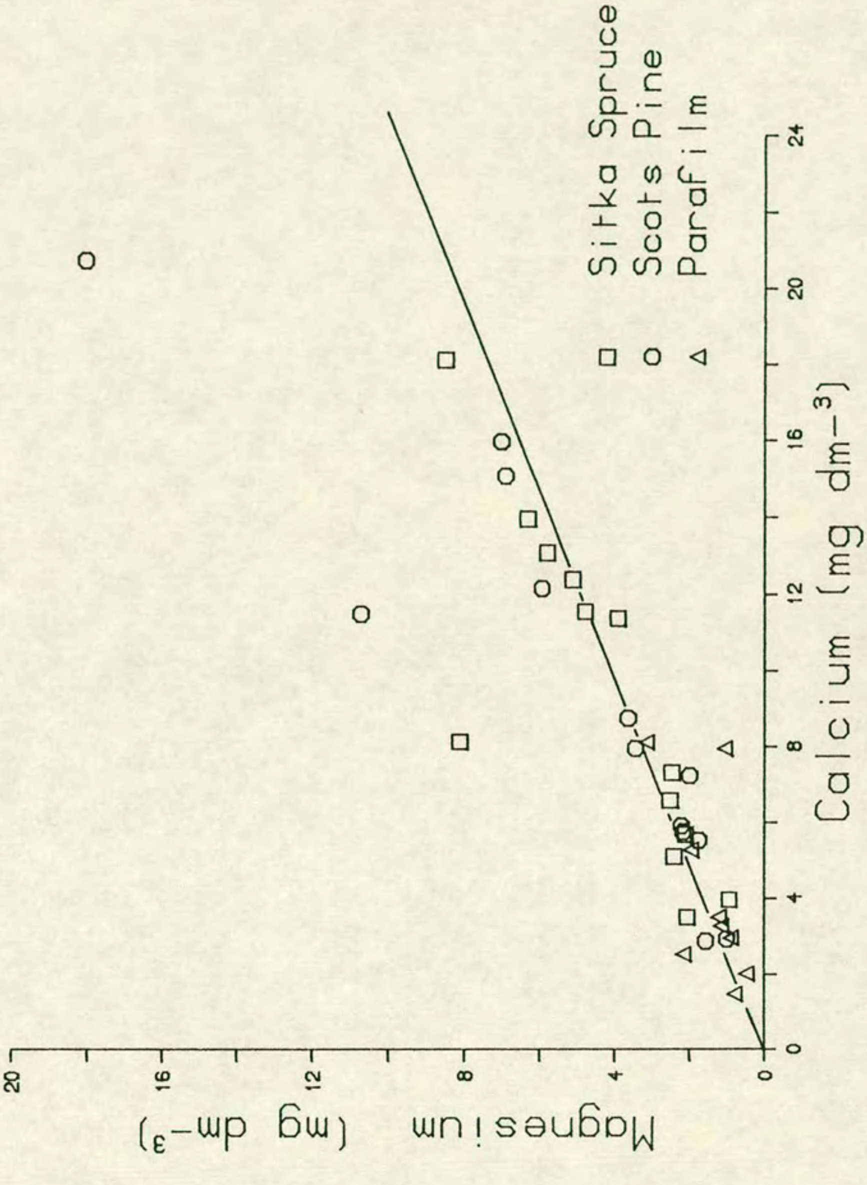


Figure 4.11 Plot of excess calcium concentration verses excess magnesium concentration in samples of impacted SAC in experiment 4. The line corresponds to the concentrations to be expected through the sole effect of evaporation. Figure 4.11a pH 5.6.

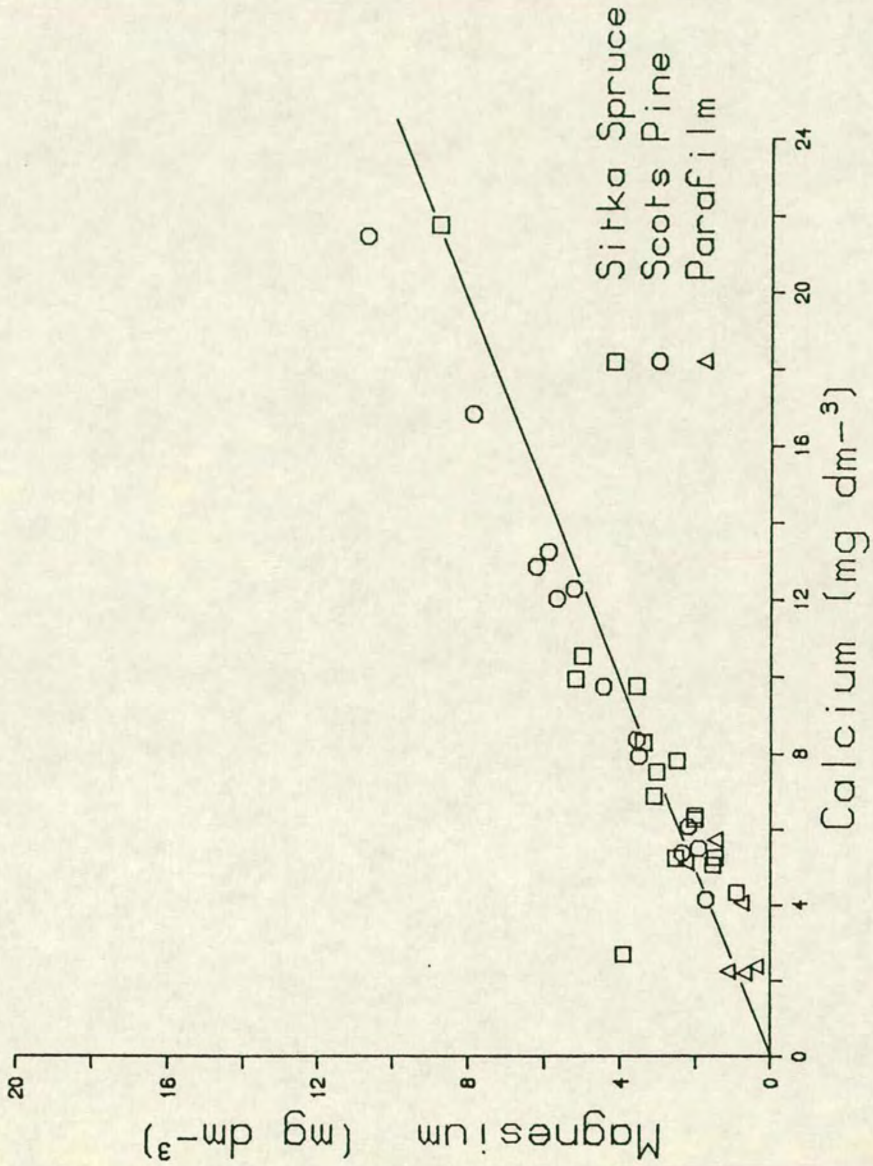


Figure 4.11b pH 3.0.

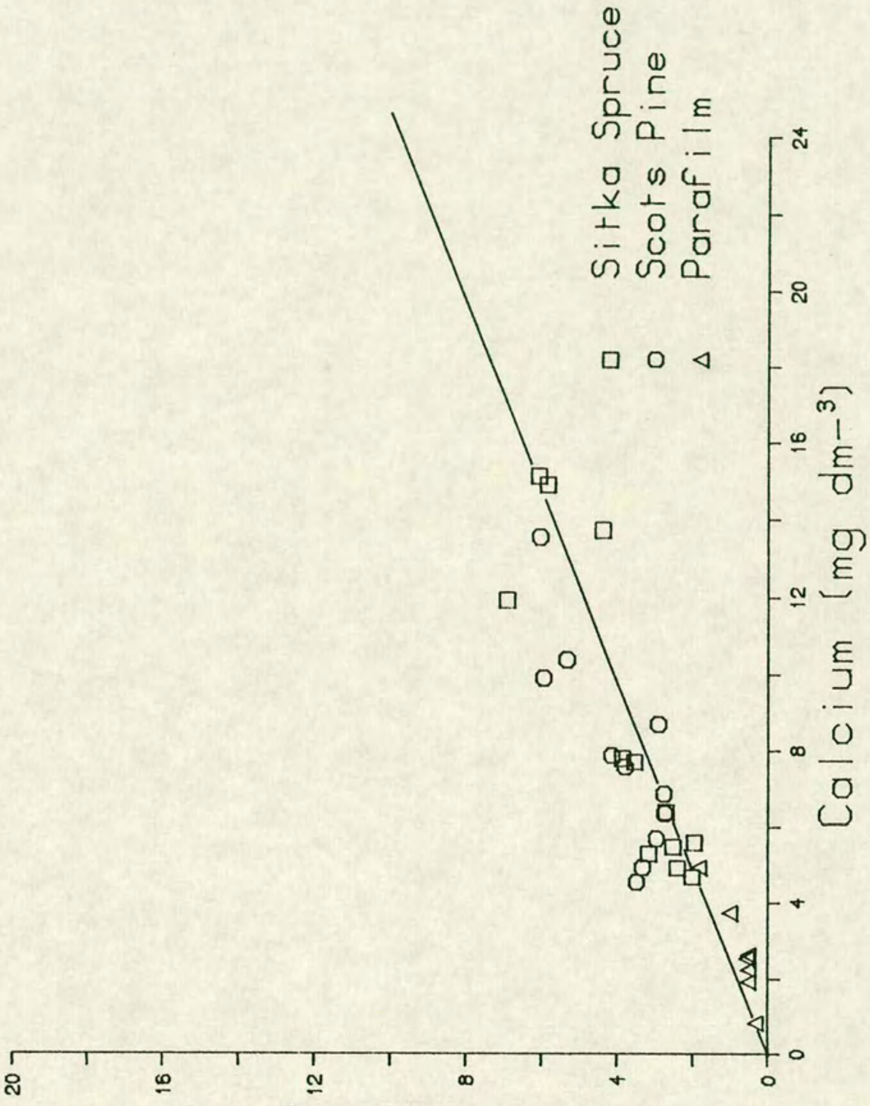


Figure 4.11c pH 2.5.

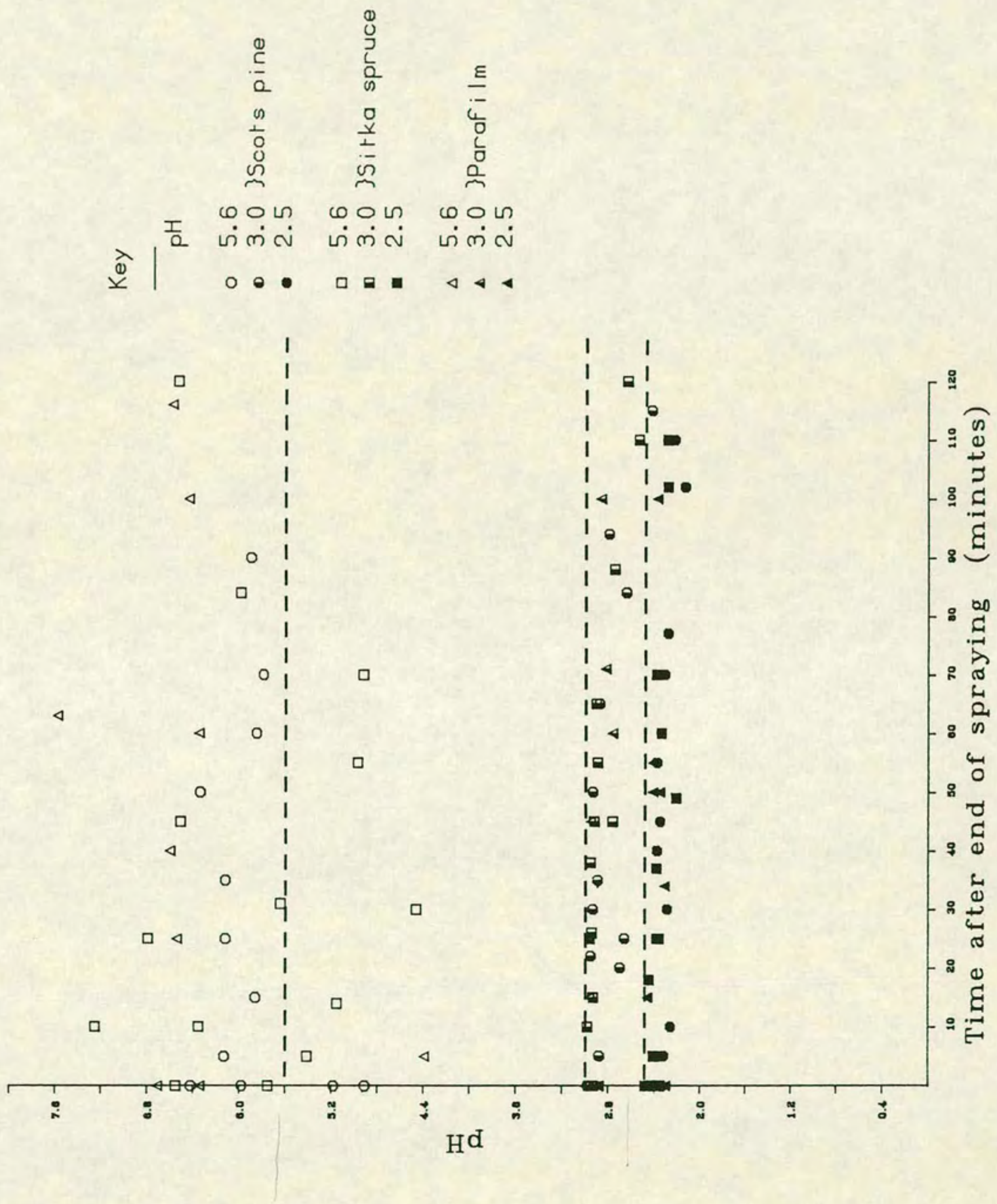


Figure 4.12 pH of samples of droplets from each treatment of experiment 4.

Table 4.2 pH of samples collected within ten minutes of the termination of spraying. Values are means of 4-5 samples. H^+ concentration in µeq dm^{-3} are given in brackets.

Treatment	Scots pine	Sitka spruce	Parafilm
5.6	5.72 (1.9)	6.26 (0.5)	5.94 (1.1)
3.0	2.92 (1202.3)	2.96 (1096.5)	2.92 (1202.3)
2.5	2.34 (4570.9)	2.40 (3981.2)	2.40 (3981.2)

For treatment pH 5.6 there was no decreasing trend with time whereas in both the pH 3.0 and pH 2.5 treatments pH decreased with time.

4.3.2 Simulated cloud acidity and nutrient content of needles

Experiment 1:

The nutritional status of the trees was fairly good (Mg) or good (Ca, K, N, P) (Figure 4.13, c.f. Everard 1973, Binns *et al*. 1980, Miller and Miller 1987). The calcium content of the needles increased with needle age whereas the nitrogen content decreased. For both PO_4^{3-}-P and K current year needles had considerably higher levels than current +1 and current +2 needles. Foliar Mg tended to be highest in current +1 needles.

There were no indications of any effect of acidity on foliar nutrient content (Figure 4.13). Trees in one of the high acidity treatments had lower calcium concentrations and in the other high acidity treatment magnesium concentrations were high. These were exceptions to the strong similarity existing between needles from the different treatments.

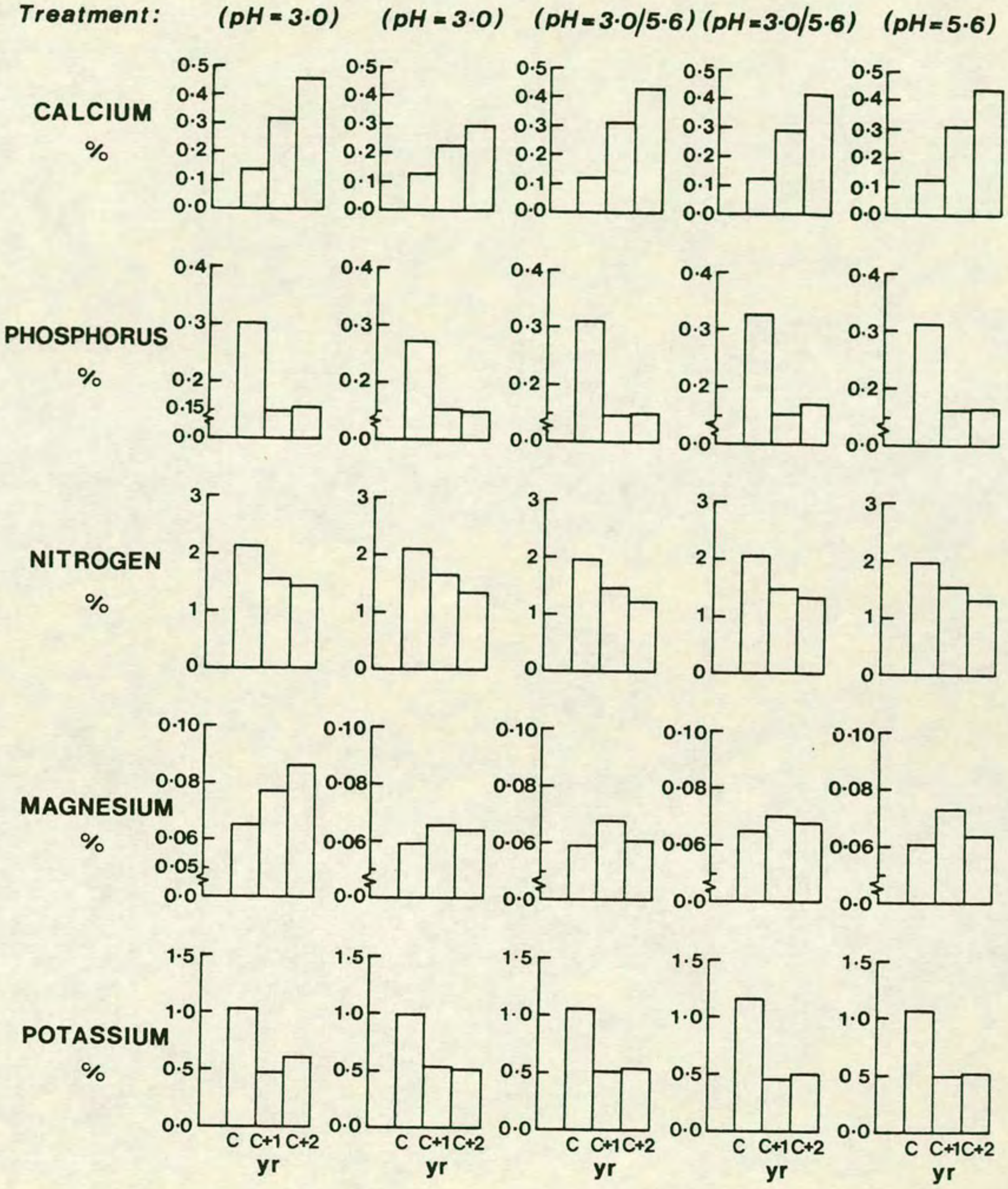


Figure 4.13 Nutrient ion concentrations of the Scots pine needles in experiment 1 for three needle year classes and five treatments.

Experiment 3:

Only current year needles were analysed and the status of the trees was fairly good (N) or good (Ca, K, Mg, -P). There was a strong indication that trees subjected to SAC at pH 3.0 had higher foliar nutrient concentrations than at pH 5.6 (Table 4.3). This was highly significant for -N and PO_4^{3-}-P and significant for Mg and Ca.

Table 4.3 Foliar nutrient concentrations of current year needles of Sitka spruce trees in experiment 3.

	Foliar nutrient concentration (mg g^{-1})	Probability of no difference
Magnesium		
5.6	0.107	0.012*
3.0	0.132	
Calcium		
5.6	0.111	0.011*
3.0	0.180	
Potassium		
5.6	0.746	0.12
3.0	0.819	
Nitrogen		
5.6	1.332	0.0095**
3.0	1.723	
Phosphorus		
5.6	0.229	0.0089**
3.0	0.278	

*Significant at P = 0.05 ** Significant at P = 0.01

4.4 Discussion.

The leaching of nutrients from plants takes place both by diffusion and cation exchange. Leaching by diffusion occurs when ions move directly from the transpiration stream into the leaching solution through areas devoid of cuticle, and cation exchange takes place when cations from exchangeable cation pools are replaced by hydrogen ions (Mecklenberg *et al*. 1966, Tukey 1970, Rentschler 1973, Prinz 1983, Richardson and Dowding 1988). Richardson and Dowding (op. cit.) also suggested that cations from within the leaf may diffuse into the cuticle and displace hydrogen ions bound there, thereafter to be leached out. It is, however, cation exchange, whereby an increase in H^+ in the external leaching solution might lead to an increase in leaching, that is of particular interest in acid precipitation studies.

The exchangeable cation pools are located in the Donnan free space which acts as an ion exchange system. Free carboxyl groups give rise to fixed negative charges in the cell wall which bind cations in preference to anions (Lauchli 1976, Walker and Pitman 1976, Demarty *et al*. 1984). Cuticular cutin may also act as a cation exchanger as a result of non-esterified carboxylic groups of the cutin acids becoming ionized (Martin and Juniper 1970). A cation exchange site preferentially accepts the cation which occupies the smallest volume (Schönerr 1976) so that two H^+ ions would be preferred to one large divalent ion. Adams and Hutchinson (1984) propose that acid neutralization and cation exchange takes place by a reaction such as:

$$\underset{\text{In drop}}{H_2SO_4} \quad + \quad \underset{\substack{\text{On exchange}\\ \text{site in leaf}}}{Ca^{2+}} \quad \leftrightarrow \quad \underset{\text{In leaf}}{2\ H^+R^-} \quad + \quad \underset{\text{In leachate}}{Ca\ SO_4}$$

The experimental results indicate some variation in the effect of acidity on leaching. Results from experiment 1 showed clearly that the leachate from trees treated with acid (pH 3.0) was higher in leached ions than the control (pH 5.6) but the number of samples was too small to draw any firm conclusions.

In experiment 4 there was a considerable amount of scatter. For cations this high degree of scatter is the result of several factors. There may be differences in the leachability of shoots and individual trees, despite samples being collected from several trees for any one sample. Such differences in leachability may arise from different responses to droplet size spectra or vapour saturation deficit. In addition retention of simulated cloudwater may vary as a result of differences in wettability. Differences in the integrity of epicuticular wax structure may also affect diffusion of ions. Differences in functioning of the cation exchange sites, nutrient status and ion replenishment after leaching may also lead to differences in leaching between individual trees. The large driving forces for evaporation combined with the variation in droplet size on needles will, because of surface/volume ratios, lead to differences in the rates of concentration of ions.

Despite this scatter, breakdown of the data showed several strong processes occurring. There was an increase in the concentration of all ions in throughfall compared with the original spray. This in itself is not evidence for leaching as evaporation will be responsible for some of the increase. However ion concentrations were also higher in samples from live shoots than in those from "Parafilm", indicating that leaching had occurred.

This included NO_3^--N which, particularly in field studies, is often absorbed rather than leached. Although the concentration of organic N often increases in conifer throughfall compared with incident rainfall (Verry and Timmons 1977, Henderson et al. 1977), inorganic N, particularly NO_3^--N is usually absorbed by conifers (Verry and Timmons 1977, Horntvedt et al. 1980, Lovett and Lindberg 1984, Ingersoll 1985). However in some cases NO_3^--N has been found to increase in conifer throughfall (Cronan and Reiners 1983). Cape and Brown (1986) found different responses in the behaviour of NO_3^--N in throughfall in the same species at different sites and in different species at the same site. At one site there was a net removal of NO_3^--N and NH_4^+-N from a Sitka spruce canopy into the throughfall whilst Scots pine and larch absorbed inorganic N. Cape and Brown (op. cit.) suggested that the reason for this was that the spruce trees were not nitrogen limited, although the absence of tissue chemistry data prohibits any firm conclusions. However Lang et al. (1982) provided data on the composition of the leaves of Abies balsmam investigated by Cronan and Reiners (op. cit.) and pointed out that the nitrogen content of the foliage was notably high.

In Experiment 4 the needles of Scots pine had been analyzed the previous year and the nitrogen status shown to be good. In addition, prior to the experiment a top dressing including nitrogen was given to the trees. It is possible that the nitrogen content and availability were therefore high enough to preclude nitrogen absorption. There appears to be a need for controlled experiments involving trees of different nitrogen status and with different availabilities of nitrogen where the possibility of a relationship between nitrogen status and the absorption of N by, or leaching of N from, needles could be investigated.

The increase in ion concentrations in samples from live shoots compared with those from "Parafilm" also showed a response to acidity. The increasing separation of control (pH 5.6) and treatment (pH 3.0 and pH 2.5) leachate concentration clearly indicates a trend of increased leaching with increased acidity.

This is in general agreement with results from other controlled experiments on trees (Table 4.4).

Table 4.4 Effect of acidity on leaching of base cations from trees.

Author	Species	Increased leaching at low pH	Increased leaching at high pH	No difference
Leonardi and Flückiger 1989	*Fagus sylvatica* (seedlings)	*Ca** Mg		K
Wood and Bormann 1975	*Acer saccarum* (seedlings)	*Ca* K Mg		
Scherbatskoy and Klein 1983	*Betula alleghaniensis* (seedlings)	K Ca		
Kelly and Strickland 1986	Hardwood/ softwood mixture (seedlings)	Ca		Mg K
Kaupenjohann *et al*. 1988	*Picea abies* (3-year-old saplings)	*Mg* Ca K		
Mengel *et al* 1988	*Picea abies* (5-year-old saplings)	K Mg Ca		
Scherbatskoy and Klein 1983	*Picea glauca* (seedlings)	*Ca* K		
Skeffington and Roberts 1985a	*Pinus sylvestris* (3-4-year-old saplings)	Mg K	Ca	
Skiba *et al* 1986	*Picea sitchensis* (young trees)	Ca K Mg		
Horntvedt *et al*. 1980	*Picea abies* (mature trees)	Ca K Mg		
Kreutzer and Bittersohl 1986	*Picea abies* (mature trees)	*Ca* K Mg		

* underlined elements showed particularly large differences between the high and low pH treatments.

It should be noted that many of these experiments in Table 4.4 were on seedlings or 3 to 5-year-old saplings and that leaching characteristics vary with age (Miller 1987). These experiments provide very little information on Pinus sylvestris and Picea sitchensis and the problems of comparing the experimental results involving these species are severe. For example Skiba et al. (1986) performed some of their experiments on excised twigs and others on small trees and all "simulated" mist lacked background ions. Similarly, Skeffington and Roberts (1985a) used acid mist without background ions, and distilled water as a control.

However from these experiments there appears to be particularly large differences between high and low pH treatments on broadleaves for calcium, whereas potassium tends to show consistently greater leaching at low pH for conifers. This agrees with the experiments of this thesis in which potassium was leached to a greater extent at low pH than either magnesium or calcium.

The magnitude of the increase in leaching of cations resulting from increasing acidity can be estimated by expanding Table 4.1 to show contributions to the increase in ion concentrations from the appropriate source. The excess ions collected from "Parafilm" samples within the first ten minutes of the cessation of spraying (column 3 of Table 4.1), compared with the original spray, can be attributed to evaporation. By subtracting the concentration of ions in samples from live shoots subjected to SAC at pH 5.6 from the concentration in samples from "Parafilm" at the same pH, an estimate of the increase in ion concentration attributable to leaching not involving H^+ ion is obtained. Similarly the differences between concentrations of ions in samples from the control treatment and the acid treatment provides an estimate of leaching by H^+ (Table 4.5).

It is clear that the increase in leached ions attributable to decreasing pH does not match the increase in hydrogen ion concentration. For treatment pH 2.5 the leaching attributed to H^+ was 79.8 µeq dm^{-3} for Ca, 106.9 µeq dm^{-3} for Mg, and 48.6 µeq dm^{-3} for K (the µeq used in cation exchange is dependant on equivalence and not molarity). The increase in H^+ concentration was, however, 3160 µeq dm^{-3} so that only a very small proportion of these ions was consumed by the sum of leached Ca, K and Mg which are the major ions involved in cation exchange. It can therefore, be seen that cation exchange is an important component of SAC leaching but relatively small compared to H^+ input.

This means that any exchange of H^+ for cations has only a minimal effect on neutralizing acid precipitation. Neutralization of acid precipitation has been shown for herbaceous plants (Adams and Hutchinson 1984, 1987) and throughfall of broadleaves is often of lower acidity than incoming rain (Horntvedt and Joranger 1976, Cronan and Reiners 1983, Foster 1985, Ingersoll 1985, Leonardi and Flückiger 1987). However conifers tend to increase rather than decrease the acidity of throughfall (Horntvedt and Joranger 1976, Cronan and Reiners 1983, Cape and Brown 1986).

Table 4.5 Leaching of ions from Scots pine shoots attributable to different sources,

Source Element	Evaporation	Leaching not involving H^{+}	Leaching by H^{+} at pH 3.0	Leaching by H^{+} at pH 2.5
Ca	2.3 mg dm^{-3} 114.8 µeq dm^{-3}	3.3 mg dm^{-3} 164.7 µeq dm^{-3}	1.3 mg dm^{-3} 64.9 µeq dm^{-3}	1.6 mg dm^{-3} 79.8 µeq dm^{-3}
Mg	0.7 mg dm^{-3} 57.6 µeq dm^{-3}	1.5 mg dm^{-3} 123.4 µeq dm^{-3}	0.6 mg dm^{-3} 49.4 µeq dm^{-3}	1.3 mg dm^{-3} 106.9 µeq dm^{-3}
K	1.0 mg dm^{-3} 25.6 µeq dm^{-3}	2.7 mg dm^{-3} 69.1 µeq dm^{-3}	3.9 mg dm^{-3} 99.7 µeq dm^{-3}	1.9 mg dm^{-3} 48.6 µeq dm^{-3}

It has been hypothesised that increased neutralization of acid precipitation reduces foliar damage (Adams and Hutchinson 1984), although more recently, it has been shown that such a simple relationship does not always hold (Adams and Hutchinson 1987). The results here clearly show that very high resistance to injury and low buffering capacity are compatible. The waxy cuticle appears to confer both resistance to injury and to ion movement.

The significance of the leaching rates of Ca, Mg and K can be assessed by calculating the quotient of annual leaching in throughfall (and stemflow where that is significant) divided by the nutrient pool in the foliage. Annual leaching of upland forests by acid clouds is the product of ion concentration in leachate and an estimation of cloudwater deposition. The latter is estimated at 2259 mm a^{-1} for conifers at 600 m in the S. Uplands (Appendix 1). Although the mean pH is higher than 3.0 (Appendix 1), ion concentrations in leachate from the pH 3.0 treatment can be used as an extreme scenario. For Sitka spruce increased loss of nutrients because of acidity would appear to be negligible. For Scots pine ion concentrations in the leachate attributable to the increase in acidity of SAC between pH 5.6 and pH 3.0 (see Table 4.5) were:

Mg	49.4 µeq dm^{-3} (24.7 mmol m^{-3})
K	99.7 µeq dm^{-3} (99.7 mmol m^{-3})
and Ca	64.9 µeq dm^{-3} (32.4 mmol m^{-3})

These values are equivalent, based on 2259 mm of cloud to:

Mg	1.11 keq ha^{-1} a^{-1} (13.48 kg ha^{-1} a^{-1})
K	2.25 keq ha^{-1} a^{-1} (87.75 kg ha^{-1} a^{-1})
and Ca	1.47 keq ha^{-1} a^{-1} (29.5 kg ha^{-1} a^{-1})

These values, which do not include leaching by diffusion, are very large compared with the amount of ions in throughfall collected from conifers in the field (Nihlgard 1970, Foster 1974, Horntvedt and Joranger 1976, Bringmark 1977, Henderson et al. 1977, Alcock and Morton 1981; Cronan and Reiners 1983, Cape and Brown 1986, Alenås and Skarby 1988). This is a result of two main factors.

Firstly, the concentrations of ions in the leachate were higher. Typical concentrations for Mg, Ca and K in throughfall (with concentrations in the rainfall subtracted) published by the above mentioned authors are 80 μeq dm^{-3} (maximum 260 μeq dm^{-3}) 100 μeq dm^{-3} (maximum 380 μeq dm^{-3}) and 75 μeq dm^{-3} (maximum 240 μeq dm^{-3}), respectively. Values from Table 4.2 for leaching of Ca, Mg and K by diffusion plus leaching by H^+ at pH 3.0 are, respectively, approximately 130%, 116% and 125% larger than the above typical concentrations. Cloud is a much more effective remover of nutrients from foliage compared with rain as only a light wetting of foliage is needed for exchange of ions to take place. The same volume of cloudwater, simulated or in the field, takes considerably longer to impact upon a canopy than would that volume of rain, thus increasing markedly the contact time over which leaching can take place. The cloudwater droplets usually remain on the needles at primary impact rather than bouncing of and stay on the needles over a longer period of time.

Secondly, the role of cloudwater in both volume and acidity is often ignored in throughfall studies, or avoided by using forest plots which are below the elevation to which clouds frequently descend. At high elevations the considerable addition to leaching by cloudwater therefore leads to considerably larger amounts of nutrients being leached.

Overstorey foliage amounts of Ca, Mg and K for Scots pine have been estimated as 45 kg ha^{-1}, 12 kg ha^{-1} and 35 kg ha^{-1} (Cole and Rapp 1981).

The amounts of these ions leached by acid clouds over a period of a year at high elevation in the Southern Uplands, based on ion concentrations in leachate from experiment 4 were calculated above as 29.5 kg ha^{-1} a^{-1} for Ca, 87.75 kg ha^{-1} a^{-1} for K, and 13.48 kg ha^{-1} a^{-1} for Mg. This indicates very high levels of leaching compared with overstorey amounts, particularly for Mg and K.

The most important means of compensating for such a loss on nutrients by acid leaching is likely to be an increased uptake through the roots.

Nutrient uptake has been shown to increase dramatically to compensate for increased leaching (Mecklenberg and Tukey 1964) and would appear to be a major contribution to the replacement of leached ions, possibly even involving over-compensation.

In experiment 1 foliar concentration of nutrients remained unaffected by increased leaching in the acid treatments and in experiment 3 foliar concentrations of NO_3^--N, PO_4^{3-}-P, Mg and Ca all increased. Previous experiments in the literature indicate both increases and decreases in foliar nutrient concentration in response to exposure of conifers to acid precipitation (Wood and Bormann 1977, Tveite 1980, Scherbatskoy and Klein 1983, Skeffington and Roberts 1985a,b, Kelly and Strickland 1986, Abouguendia and Baschak 1987, Johnsen et al. 1987, Kaupenjohann et al. 1988, Mengel et al. 1988, Westman and Temple 1989, D. Turner and D. Tingey (pers. comm.)). The experiments which showed a decrease in needle ion concentrations tended to be those lacking background ions in the acid precipitation (Wood and Bormann 1977, Kaupenjohann et al. 1988) or those using other types of acid precipitation which did not simulate background concentration of ions in the precipitation (Tveite 1980). However, other authors have observed increases in needle K, Ca and Mn (Mengel et al. 1987, 1988) and Mg and Ca (Skeffington and Roberts 1985a,b) without background ions in the acid precipitation. Differences occur in the response of needle nutrient concentrations to precipitation acidity between different species in the same experiment (Tveite 1980, Skeffington and Roberts 1985a,b, Westmann and Temple 1989), but it is clear that increased leaching of ions from needles does not necessarily lead to a reduction in foliar concentrations and in some cases increases results.

The effect of the increased leaching on the nutrient concentration of the needles was therefore negligible, probably because leaching losses were adequately replaced from the soil. However, although foliar nutrient concentrations did not appear to be affected, a long term elevated leaching rate may, in certain conditions, lead to a depression in growth. The effect of nutrient loss on a forest system will depend on the foliar leaching rate, the time lapse before replacement (Tukey 1970, Adams and Hutchinson 1984), replacement cost (Amthor 1984, Lovett et al. 1985, Richardson and Dowding 1988), rate of loss of nutrients from the system through soil leaching, replacement through weathering of primary minerals (Johnson et al. 1982), root mineral uptake efficiency (Hindawi et al. 1980, Lovett et al. 1985), immobilization of leached nutrients through incorporation into insoluble salts (Fairfax and Lepp 1975) and Al toxicity to root systems (Ulrich 1980, 1982, 1985). In addition high nitrogen inputs may further exacerbate nutrient imbalances.

Low foliage nutrient concentrations are a prominent feature of declining forests. In particular the "montane yellowing" of Norway spruce (Picea abies) seen over much of central Europe is associated with low foliar Mg concentrations and sometimes also with low Ca concentrations (Bosch et al. 1983, Kandler et al. 1987, Rehfuess

1987, Prinz et al. 1987, Forschner and Wild 1988, Prinz and Krause 1989, Roberts et al. 1989a, Zöttl and Hüttl 1989). Yellowing occurs particularly on needles exposed to sunlight (Bosch et al. 1983, Haughs and Wright 1986, Krause et al. 1986) and is the result of a decrease in the photosynthetic pigments caused by Mg deficiency (Kandler et al. 1987). Magnesium deficiency and associated needle yellowing have also been in Norway spruce in N.E. United States (Ke and Skelly 1989) and in Silver fir (*Abies alba*) in Europe (Zöttl and Hüttl 1989). Less frequently K and Mn deficiency and a yellowing of young needles occur (as opposed to older needles in the classic Mg deficiency syndromes) (Hutchinson 1987, Rehfuess 1987, Forschner and Wild 1988, Roberts et al. 1989b).

There are two main hypotheses concerning these nutrient deficiencies. The first is built around Ulrich's hypothesis of soil acidification (Ulrich 1980, 1982, 1985, Ulrich et al. 1980, 1981). The second involving Prinz and his co-workers is based on photo-oxidants damaging cell membranes, leading to increased permeability to ions which are thereafter leached through these 'leaky' membranes by acid cloud (Krause et al. 1986, Prinz et al. 1987, Prinz and Krause 1989). Although these and other factors may dominate at certain sites for certain species, it is clear that no single hypothesis can account for the spread of novel forest decline in Europe and elsewhere in the world. The importance of inadequate nutrients in above-ground plant parts is however clear and a broader based view of the problem is to approach it as an imbalance of source-sink relationships between the crown and root systems.

The following scenario may approach reality. Nutrients may be leached by the combined (or separate) effect of photo-oxidants and acid precipitation, particularly acid clouds, leading to lowered nutrient concentrations in the needles, a reduction in photosynthetic apparatus and a shortage of assimilates which lead to a reduction in the growth of the root system. Meanwhile the soil, possibly already low in one or more of the nutrients Ca, Mg, K or Mn becomes progressively more acidic and a low Mg/Al ratio, coupled with a high concentration of toxic aluminium salts, inhibits root uptake of a low supply of nutrients that have already been reduced by acid leaching of cations from the soil. Nutrient uptake is this disturbed and a cycle of steadily decreasing vitality results. This cycle can often be reversed by a fresh supply of nutrients in the form of quick release Mg or K fertilizers resulting in the regreening and renewed health of the trees (Zöttl and Hüttl 1986, 1989).

The above is by no means the only mechanism of forest decline but is consistent with much of the available evidence. Although acid-related effects on soils may be an important predisposing factor, the increased loss of nutrients by leaching with acid cloudwater is likely to play a major role in forest decline. Thus where acid clouds are frequent, as at high elevations, forest damage would be expected to increase. This is indeed so and many reports indicate that forest decline does increase markedly at high elevation sites (Roberts and Blank 1985, Hutchinson 1987, Bucher 1989, Prinz and Krause 1989). This is not proof of cause and effect but further support of a strong link between acid clouds and forest decline.

CHAPTER 5

The exchange of elements between cloudwater and the needles of Scots pine and Sitka spruce in the field

5.1 Introduction.

The experiments described in the previous Chapter have the advantage, because of their controlled nature, of allowing measurable comparisons between treatments of simulated acid cloud (SAC) of different pH. However, although controlled comparison between real cloudwater of different pH is not possible, field measurements have the advantage of allowing direct conclusions to be drawn concerning the interactions between cloudwater and conifer foliage. The influence of natural variations in the weather, variations in cloud event pH, duration and intensity, and the use, at least partially, of trees which have grown *in situ* under the prevailing conditions at the field site help to ensure that the results of measurements in the field reflects a true picture of the interaction of cloudwater and conifer foliage. Whilst the interactions between cloudwater and conifer foliage are being investigated, the temporal and spatial variation of cloud episodes, which are poorly understood, can also be examined.

Field observations were thus made to examine the variation of cloudwater pH within cloud episodes and the modification of cloudwater by drizzle or rain. To achieve this, intensive sampling of cloudwater during cloud events was undertaken with both pH and cation concentration being measured. Cloudwater samples were obtained by collecting impacted cloudwater from artificial shoots of conifers.

The interaction of the cloudwater during these episodes with the needles of Scots pine and Sitka spruce was examined simultaneously to the collection of cloudwater samples from the artificial shoots. This allowed direct comparisons to be made between impacted cloudwater on live and artificial shoots. These comparisons reveal the modifying effect of live shoots on cloudwater, the movement of cloudwater ions into the foliage and the leaching of ions out off the foliage. For this purpose, samples of impacted cloudwater were collected from live shoots of Sitka spruce and Scots pine.

The investigation also aimed to determine if the original nutrient status of trees affects ion exchange between the foliage and cloudwater. For this purpose, foliar nutrient concentrations of Sitka spruce trees were measured and the cloudwater impacted during a series of cloud episodes was collected.

Lastly the importance of dry deposition was investigated. The deposited substances may be basic such as Ca^{2+} or NH_3, or acidic such as most other nitrogen and sulphur compounds. Transfer takes place by turbulent diffusion (Cape and Unsworth 1988) and depends on windspeed, temperature stratification and surface roughness

(Fowler 1984). Deposition is thus strongly affected by prevailing weather conditions and topography. For coniferous forests, much of the resistance to transfer is across the needle boundary layer because atmospheric turbulence is high,

Dry deposition is washed off needle surfaces by precipitation. Such removal depends on canopy structure, needle size and shape, precipitation intensity, duration and droplet size spectrum, and the physical and chemical characteristics of the deposited substance (Lauver and McCune 1984). The pattern of washing is thus complex and these dry deposits, when mobilized, may form an important constituent of throughfall. In many of the more densely populated areas of Europe, dry deposition of N and S compounds probably exceeds that deposited in rain and snow and even in remote areas dry deposition is still substantial (Semb and Dovland 1986, Cape and Unsworth 1988). To assess the contribution of leaching of ions to throughfall dry deposition must therefore be measured.

A large variety of methods and analytical techniques have been used to measure dry deposition, including sulphur enrichment of throughfall (Mayer and Ulrich 1978), stream water output (Eaton _et al_. 1978), regression analysis (Lakhani and Miller 1980, Lovett and Lindberg 1984), chloride ratios above and below the canopy (Miller _et al_. 1987), radioactive tracers (Graustein and Armstrong 1978, Garten _et al_. 1988, Lindberg and Garten 1988), canopy modelling (Lovett _et al_. 1982, Lovett 1984, Reiners and Olsen 1984, Reiners _et al_. 1987), micrometeorological flux measurements (Bache 1977, Cape et al. 1987), and exclusion of dry deposition (Banaszak 1975, Höfken et al. 1983).

Many of the above methods are prone to major assumptions, some of which are certainly not tenable (see e.g. Galloway and Parker 1980. The method in this series of experiments was to use artificial surfaces. The major advantage of using artificial surfaces is that it allows a direct measurement to be taken and avoids most of these assumptions. The major drawback is the possibility that deposition to artificial surfaces differs from deposition to live foliage. This may occur through a wrong choice of shoot material (Schlesinger and Reiners 1974), or by using surfaces which do not simulate the shape of conifer shoots (Nihlgärd 1970, Hart and Parent 1974, Lakhani and Miller 1980, Lindberg and Harriss 1981, Lovett et al. 1985). The following conditions should therefore be met in the use of artificial surfaces.

1) The material of which the artificial shoot is made should be inert and non-leachable.
2) The artificial shoots should be as similar as possible to the foliage with regard to aerodynamic properties and durations. This should be quantified.
3) The artificial shoots should be thoroughly clean and free from contaminants before use.

Tests to verify that these conditions were met, are detailed in the Methods and the Results sections. Dry deposition was assessed by allowing some of the artificial shoots to accumulate dry deposits between cloud events whilst other shoots were exposed only to the cloud events themselves.

5.2 Methods.

5.2.1 The Field site.

The field site was situated at Dunslair Heights, Glentress Forest in the Moorfoot Hills at a height of 600 m a.s.l., grid reference NT 287 436. Glentress forest is planted with a large variety of broadleaves and conifers but in the field site area the forest is composed only of Sitka spruce planted in 1964. Fifteen eight-year-old Scots pine, growing in pots, were transferred from Bush Estate to the field site and the pots were sunk into the ground in close proximity to the spruce trees. A series of meteorological instruments was situated 20 metres away and gave 5 minute readings of wind direction and speed, wet and dry bulb temperature, solar and net radiation, rainfall and cloudwater amounts (Figure 5.1). Cloudwater was collected by an E.T.F.E. (ethyl-tetra-fluoroethylene) stringed, lidded cloudwater gauge which excludes rainfall when the windspeed is less than 5 m s^{-1} (Figure 5.2). Cloudwater samples were collected weekly and analysed for pH, various ions (including Ca, Mg and K), and particulate matter. A small concrete building nearby contained equipment for a radio mast and provided an electricity supply for the

Figure 5.1 The field site and meteorological station. The cloudwater samples were collected from Sitka spruce and Scots pine trees on the lower right hand side of the photograph.

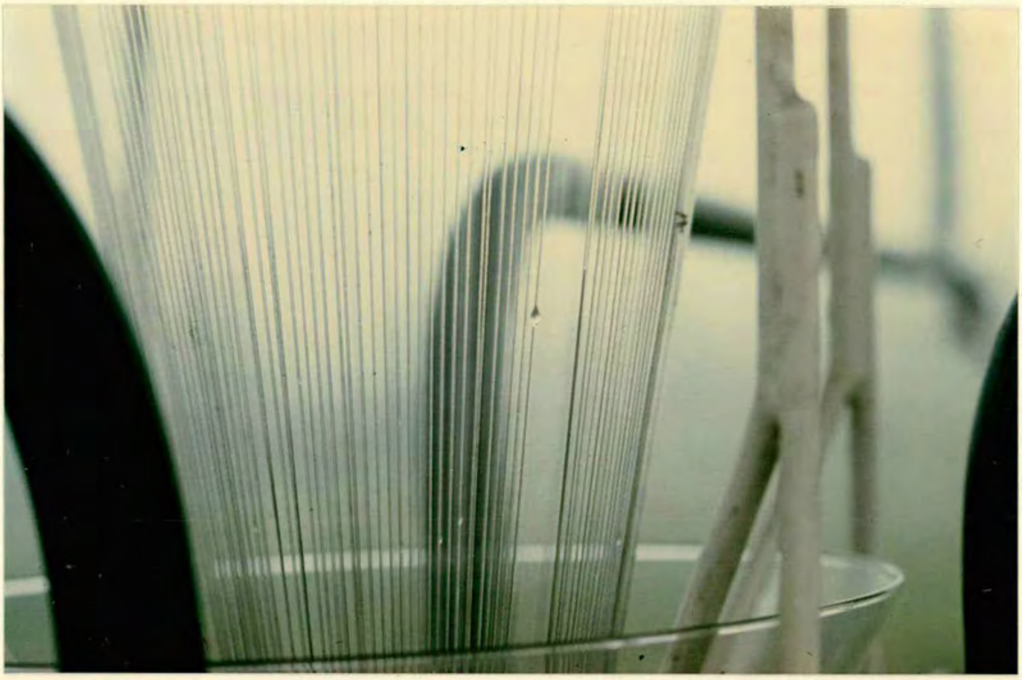


Figure 5.2 Cloudwater collection. Top photograph shows an accumulated droplet running down an E.T.F.E. string. The lower photograph shows the gauge immersed in cloud.

field site. The building and mast were 50 m from the field site. The topography of the site was a fairly flat and exposed plateau on the hill top and the trees used in measurements were near the edge of the forest (see Figure 5.1).

5.2.2 Artificial shoots

The material chosen for artificial shoots was polypropylene. A sheet, 40 cm long and 3.5 cm wide was cut with a razor blade to form a strip of attached "needles" 1-2 mm wide and 3 cm long. This strip was then twisted round the upper 12 cm of a 25 cm polypropylene rod to form a spiral representing the whorls of coniferous needles. Shoots were enclosed in polyethylene bags filled with deionised water, shaken and left for 24 hours. Differences between samples of that water and control deionised water were not detectable for all ions studied, with all ions being below the detectable level.

Aerodynamic resistance of the artificial shoots was evaluated and compared with that of Sitka spruce and Scots pine shoots. One shoot at a time was placed in a wind tunnel of cross-sectional area 280 cm^2. A solution of known Na^+ content was sprayed (droplet size 5-20 μm (Grace pers. comm.)) at various wind speeds measured with a hot wire anemometer. Shoots were then rinsed in a cylinder with 100 cm^3 distilled water. This was repeated and the resultant 200 cm^3 analysed for Na^+ content by an atomic absorption spectrophotometer. The cross sectional area of the shoots was measured with an area meter (Licor Inc., Linoln, Nebraska) and the aerodynamic resistance calculated as the concentration in the air in the wind tunnel divided by the flux to the shoots (Table 5.1)

Table 5.1 Aerodynamic resistance of live and artificial shoots.

Windspeed	Shoot	Aerodynamic resistance ($s\ m^{-1}$)	S.E.
1 $m\ s^{-1}$	Artificial	8.5	2.21
	Scots pine	5.7	1.195
	Sitka spruce	7.0	0.765
3 $m\ s^{-1}$	Artificial	4.15	0.865
	Scots pine	1.35	0.06
	Sitka spruce	1.2	0.215
5 $m\ s^{-1}$	Artificial	1.85	0.145
	Scots pine	1.2	0.05
	Sitka spruce	1.15	0.115

The results show that the artificial shoots had slightly higher aerodynamic resistance than the live shoots. Deposition velocities to these shoots would therefore be slightly lower than to live shoots but the same order of magnitude. Whilst these differences can be taken into account, the aerodynamic similarity is strong enough to accept the results as a true picture of the processes involved in cloudwater impaction and ion exchange.

Twelve shoots were tied to the Scots pine trees by cable glands and left exposed. These were termed DD (dry deposition) shoots. A similar number were put out at the start of cloud events, having been thoroughly washed in DECON, hot water and deionised water and stored in sealed polyethylene bags. These were termed NDD (no dry deposition) shoots.

5.2.3 Sample collection

Two methods were used to sample impacted cloudwater droplets. Firstly, a vacuum-pipette system was used to draw accumulated cloud droplets off the shoots. The system is described fully in Chapter 4, section 4.2.1. Approximately 2 cm^3 were needed for sample analysis and this took from 5-15 minutes to collect, depending on how abundant droplets were. Samples were collected from NDD and DD artificial shoots, and current and current +1 shoots of both Sitka spruce and Scots pine. Because of the large temporal variation in incoming nutrients during cloud events and the time needed to collect and label samples, it was not possible to collect from each of these six shoot types during each event. Collection was, therefore, made from two or three types (e.g. DD artificial shoots and current year shoots of Scots pine and Sitka spruce) during any particular cloud event. A total of 21 partial or full cloud events were sampled. Samples were stored at 2-3 °C before ion analysis and pH measurement took place as described in Appendix 4.

Secondly, a microelectrode (MI-410, Microelectrodes, Inc., New Hampshire, U.S.A.) with an outer tip diameter of 1.2 mm was used to measure the pH of individual droplets. Full details of the use of the microelectrode are given in Appendix 4. The microelectrode did not measure cations but had the advantage of allowing H^+ to be measured in a large number of drops (10 per sample) and standard errors to be calculated. Figures with data from the microelectrode rather than the vacuum system are therefore identifiable by the standard error bars.

5.2.4 Examination of the relationship between needle and leachate nutrient concentration.

Six Sitka spruce trees at the field site were chosen to examine the relationship between needle nutrient content and leachate nutrient content. Over several months 18 sets of samples of impacted cloudwater were taken from these six trees within a time span of 15-20 minutes, sealing and labelling taking a further 15-20 minutes. Samples were stored and analysed within 24 hours as described in Appendix 4.

From the six trees, ten needles from each of four shoots from each tree were taken and analysed as described in Appendix 4.

5.3 Results.

5.3.1 The nature of cloud episodes with respect to pH.

Temporal variation of pH within cloud deposition episodes was strong. In particular the effect of in-cloud drizzle was marked. The pH of droplets collected from artificial shoots increased sharply during periods of drizzle and decreased when cloudwater only was deposited (Figure 5.3). The pH of droplets was therefore a compromise between the pH of cloud and rain, with drizzle diluting the droplet concentrations and cloudwater increasing concentrations. Although this effect was slightly dampened on live shoots the trend of increasing pH with drizzle was clear.

Variation within events was not limited to the interaction of cloud and drizzle. Often considerably lower pHs were observed during cloud events than for weekly bulk cloudwater samples collected by the E.T.F.E. filament cloudwater collectors (Table 5.2). The table also shows the consistent, almost invariably higher pH observed in rain sample than in cloudwater samples.

Certain patterns of change were seen in pH during cloud episodes. Commonly pH rose as the cloud base dropped below the site and fell again as the clouds rose (Figure 5.4). On occasions this pattern was absent or severely modified. The

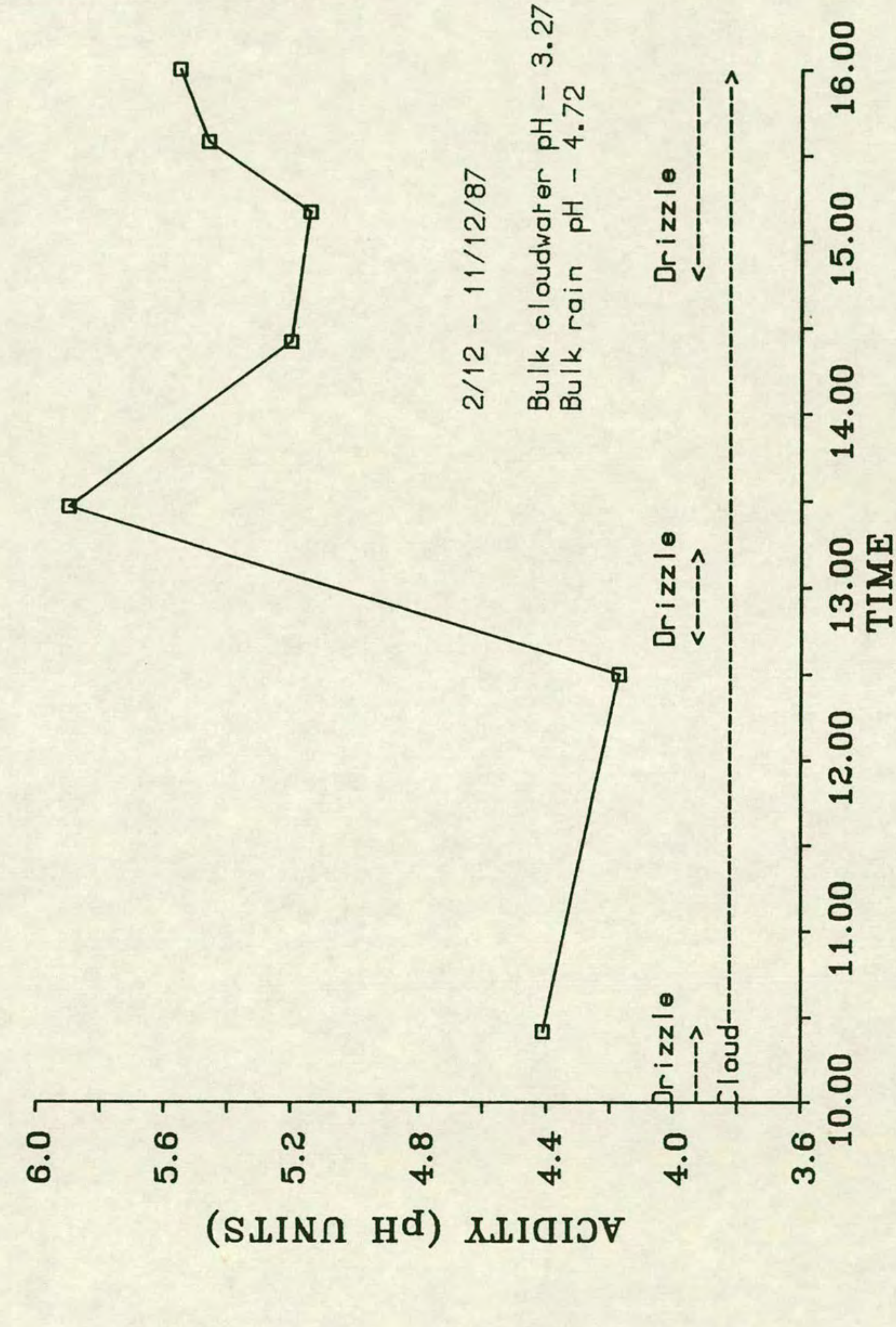


Figure 5.3 The influence of in-cloud drizzle on pH (11/12/87): artificial shoots with dry deposition.

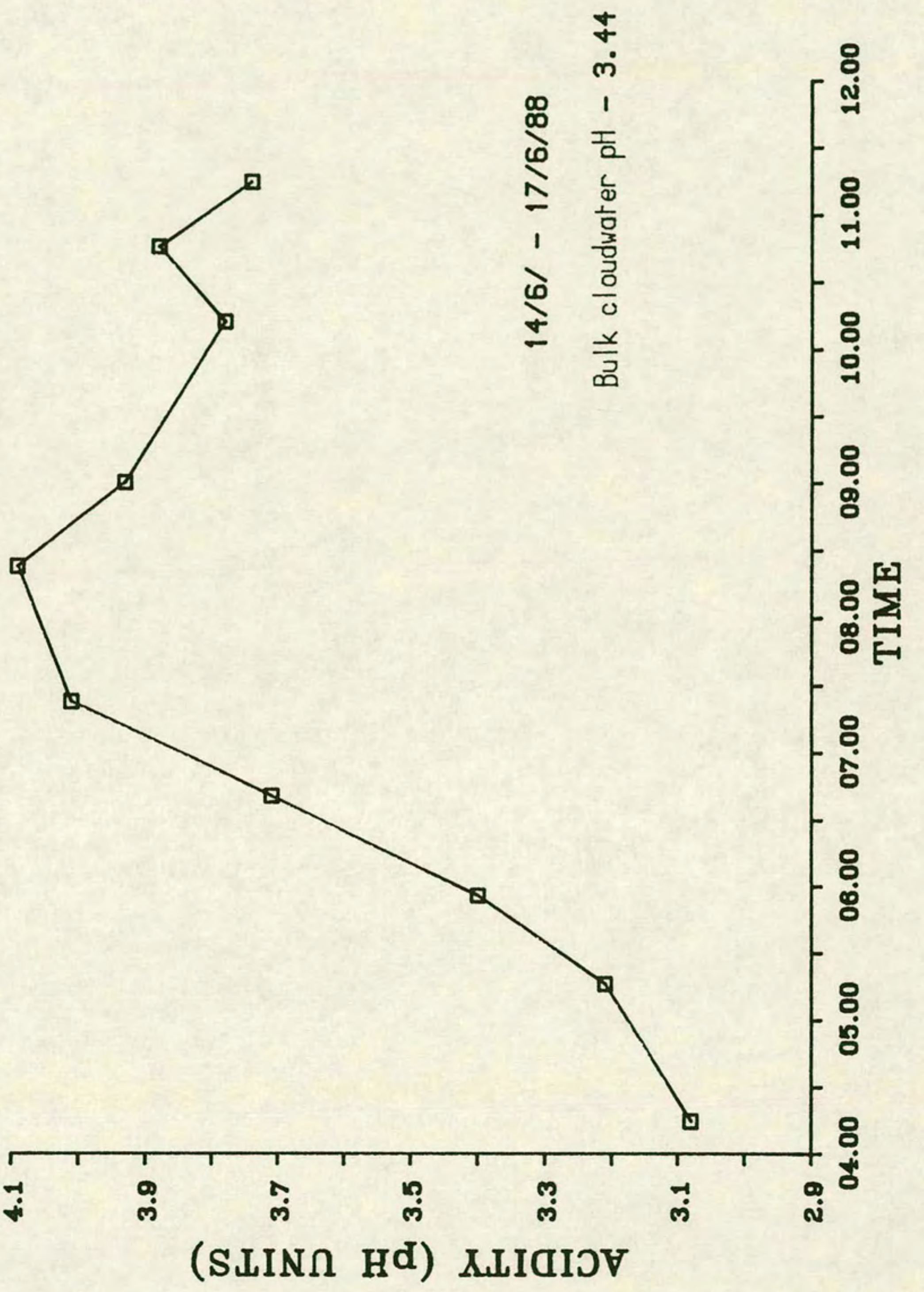


Figure 5.4 Comparison of cloud episode extremes and bulk pH (17/6/88). Artificial shoots.

fall in pH was not as marked as the initial rise because of the diluting effect of droplets remaining from previously impacted cloudwater. When the results of analysis of samples from live shoots during the same episode are super-imposed (Figure 5.5), the same pattern, slightly dampened, can be seen.

Table 5.2 Comparison of event and bulk pH

Date of sampling	Bulk rain pH over sampling period	Bulk cloud-water pH over sampling period	Minimum pH on artificial shoots with no dry deposition	Minimum pH on artificial shoots with dry deposition
27.10.87	4.16	4.11	3.93	3.59
9.11.87	3.92	2.90	-	3.09
19.11.87	4.77	5.10	6.14	-
1-2.12.87	5.44	3.75	-	4.19
11.12.87	4.72	3.27	-	4.17
16.12.87	4.83	4.04	3.88	3.70
26.1.88	4.38	3.49	-	4.10
30.3.88	6.40	5.99	4.98	-
11.5.88	4.10	3.37	-	2.86
12-13.5.88	3.75	3.56	3.45	3.34
2.6.88	4.49	4.34	4.08	4.04
16.6.88	4.08	3.44	3.19	3.12
17.6.88	4.08	3.44	3.08	3.09
21.4.88	4.52	4.15	-	5.13
3.5.88	4.11	3.51	-	3.75
4.5.88	4.11	3.51	-	3.82

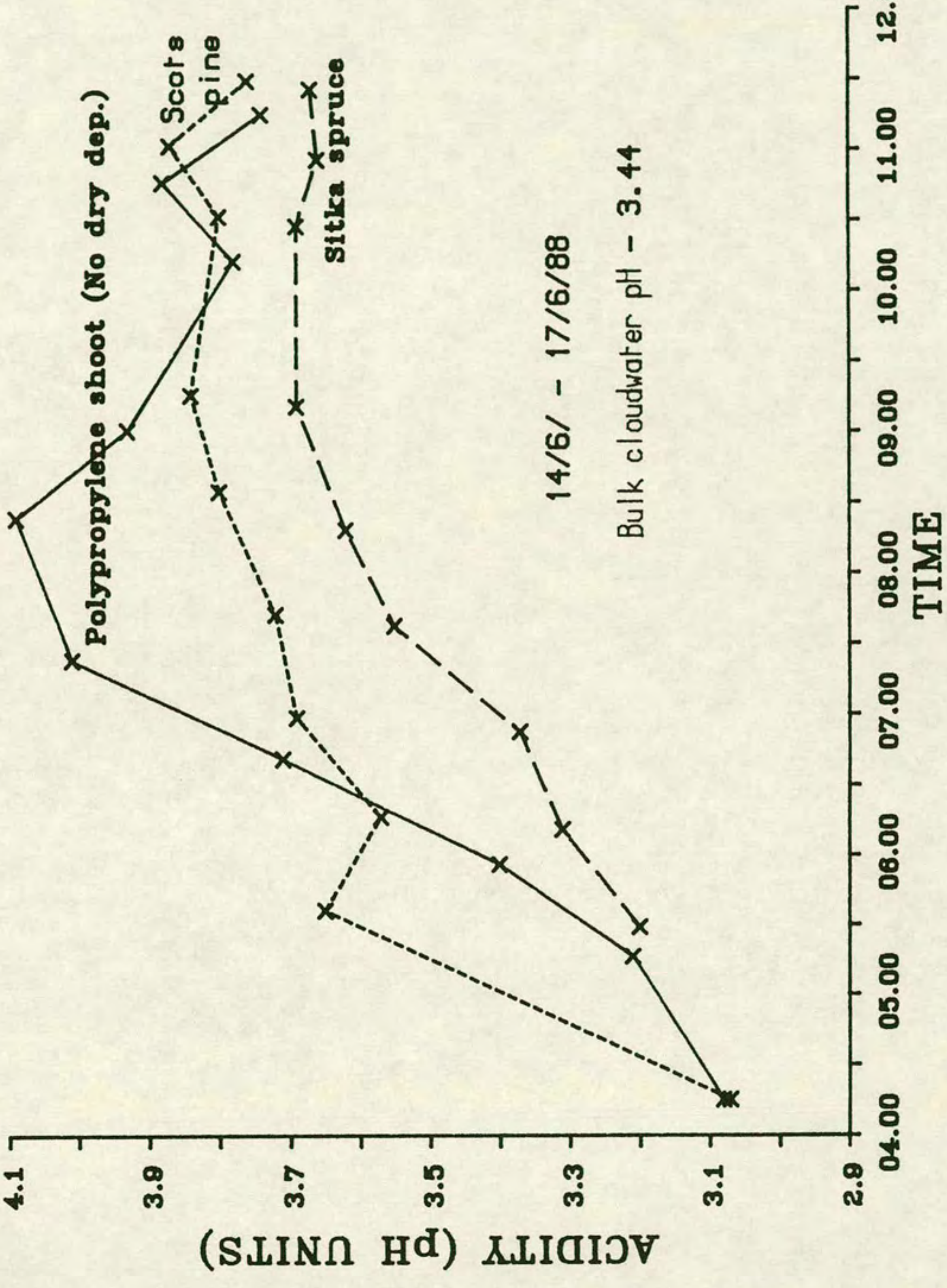


Figure 5.5 Comparison of cloud episode extremes and bulk pH (17/6/88). Comparison of live tissue and artificial shoots.

5.3.2 Differences in pH of impacted cloudwater between different shoot types.

Differences in the pH of the droplets collected from shoots with and without dry deposition and live shoots varied from event to event. This variation tended to be in the amplitude of the difference whilst the direction of the differences (higher or lower pH between the different types of shoots) was generally similar. Table 5.2 showed that droplet samples from artificial shoots with dry deposition had lower pH than those from shoots without dry deposition. When several cloud-free and rain-free days had elapsed before a cloud event, initial differences in droplet pH between artificial shoots with and without dry deposition were large, becoming less marked as the event progressed (Figure 5.6). If occasional intermittent cloud or drizzle preceeded the cloud event, the initial difference in droplet pH was reduced (Figure 5.7); if consistent cloud or drizzle had preceeded a cloud event, pH of samples from the shoots with and without dry deposition tended to be similar with only a slightly higher pH value for those without dry deposition (Figures 5.8 and 5.9).

As seen earlier in Figure 5.5 droplet pH was generally lower on live shoots compared with artificial shoots. Between the two tree species Sitka spruce droplet samples consistently showed lower pH values than those from Scots pine. In eight

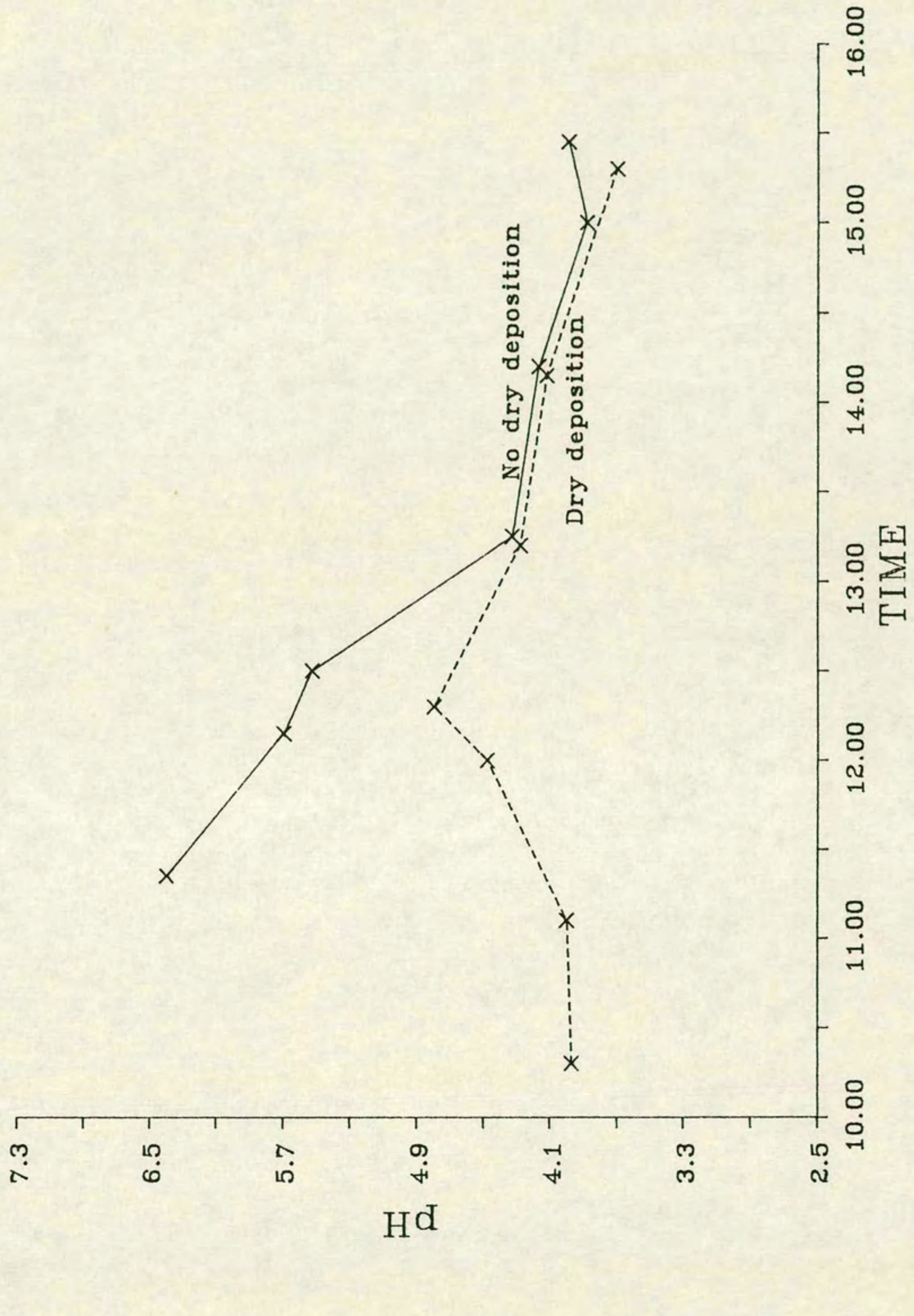


Figure 5.6 pH of droplet samples from artificial shoots with and without dry deposition. The days preceeding the episode sampled had been dry. (15-16/12/87)

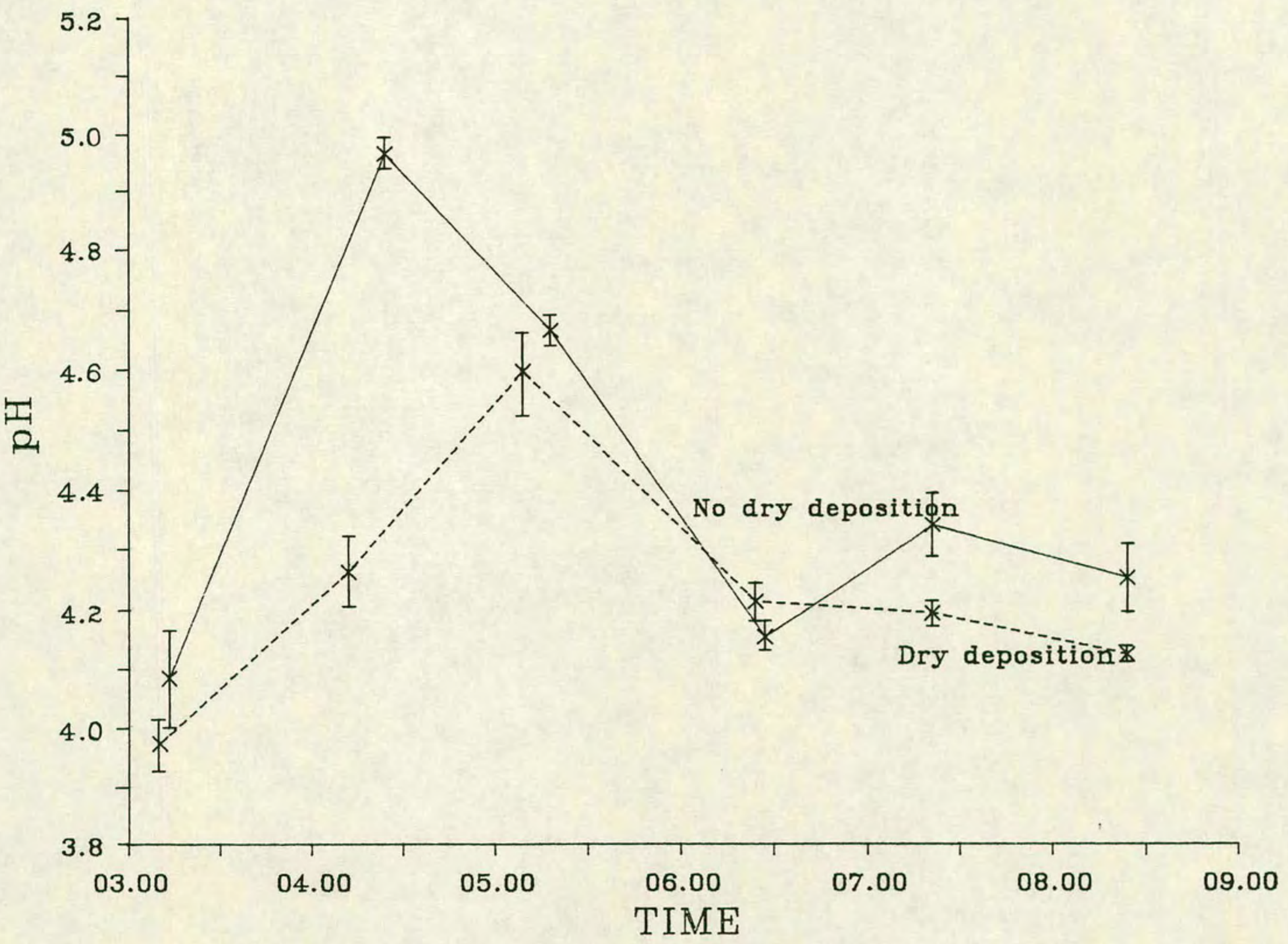


Figure 5.7 pH of droplet samples from artificialshoots with and without dry deposition. Intermittent cloud/drizzle preceeded the cloud episode sampled. (2/6/88)

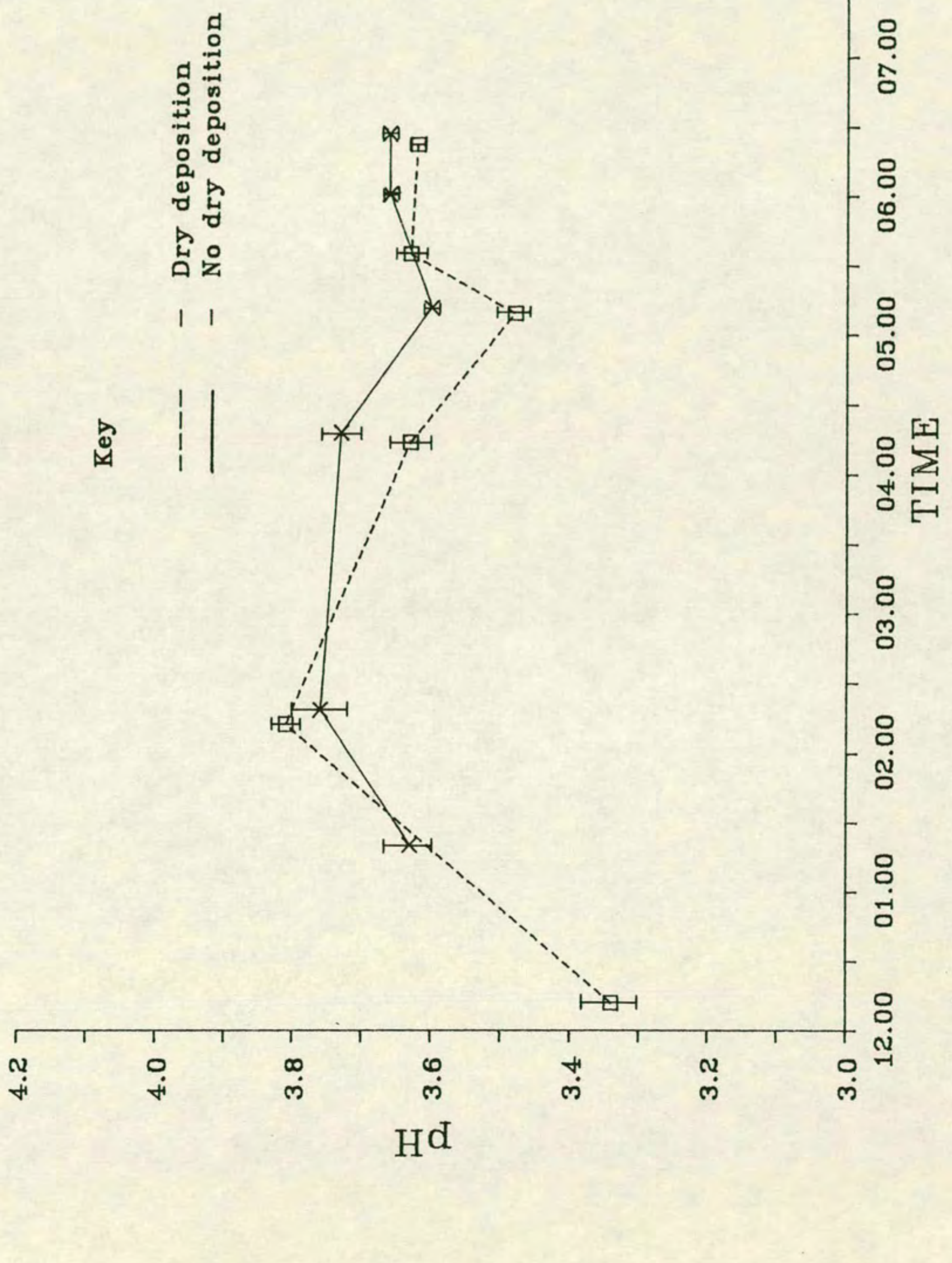


Figure 5.8 pH of droplet samples from artificial shoots with and without dry deposition. Shoots had recently been subject to consistent cloud/drizzle. (13/5/88)

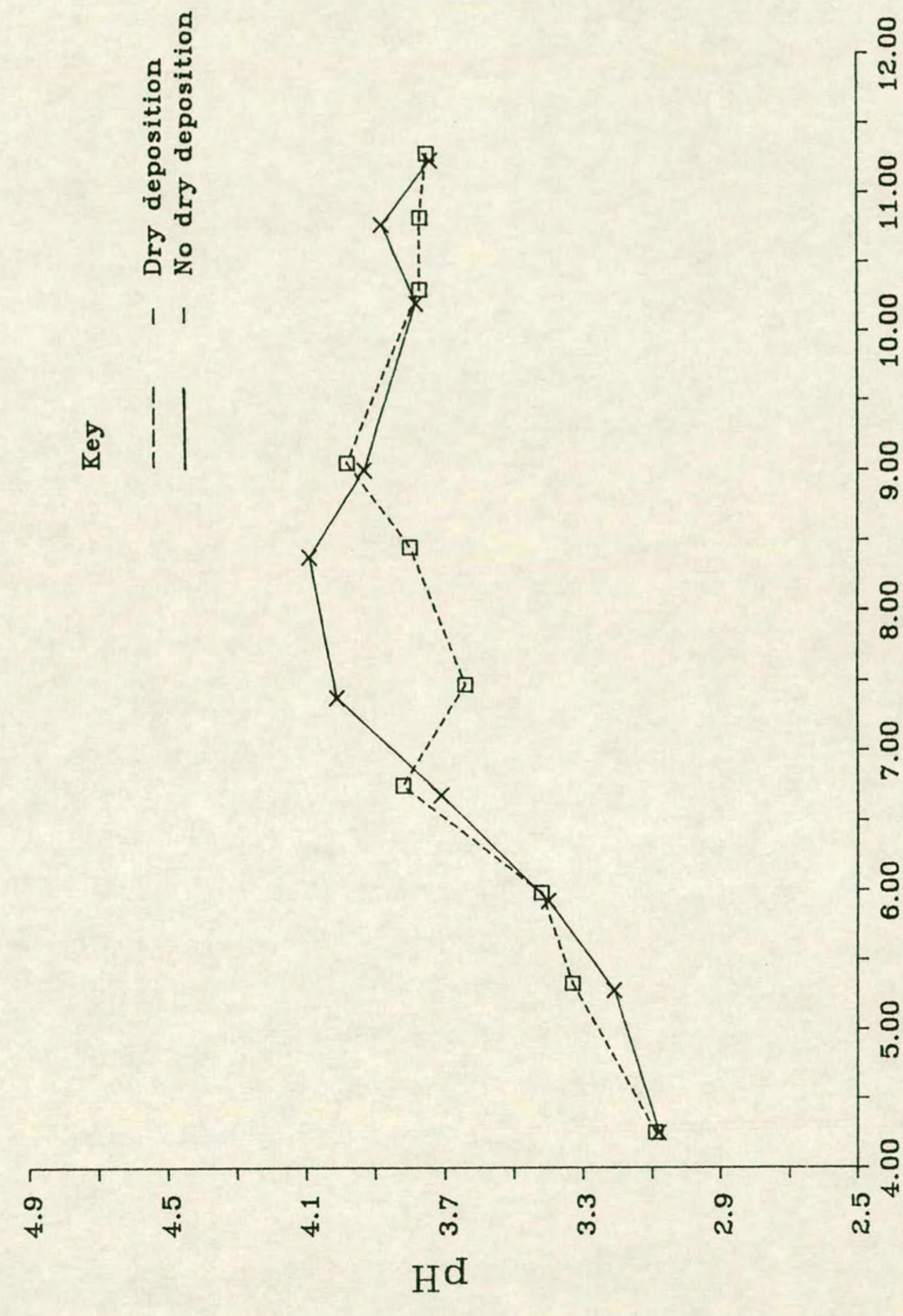


Figure 5.9 pH of droplet samples from artificial shoots with and without dry deposition. Shoots had recently been subject to consistant cloud/drizzle. (17/6/88)

of the cloud events, pH of cloudwater on current and current +1 needles of Scots pine was compared. Two sets of samples showed very similar values for both ages of needles, whilst current year needle samples had higher pH in three events and current +1 needles had higher pH in three. The direction of this difference did not depend on the time of year.

5.3.3 Differences in cation concentrations in impacted cloudwater between different shoot types.

Because of the relevance of the equivalent rather than the mole in cation exchange, as well as to allow easier comparison with the literature which tends to use the µeq, the µeq is used here. Differences between the two tree species in cloudwater droplet samples of Ca^{2+} and Mg^{2+} concentrations were not consistent . Concentrations of K^+ in droplet samples were, however, generally higher in Scots pine samples than in Sitka spruce samples (Figure 5.10). Cation concentrations in samples from live shoots were higher than those from artificial shoots, and samples from artificial shoots with dry deposition had higher ion concentration than those without. The size of this latter difference was very variable and not directly related to the length of period of previous dry weather, as for pH.

The pattern of ion concentration in samples was usually similar for the three base cations (Figure 5.11).

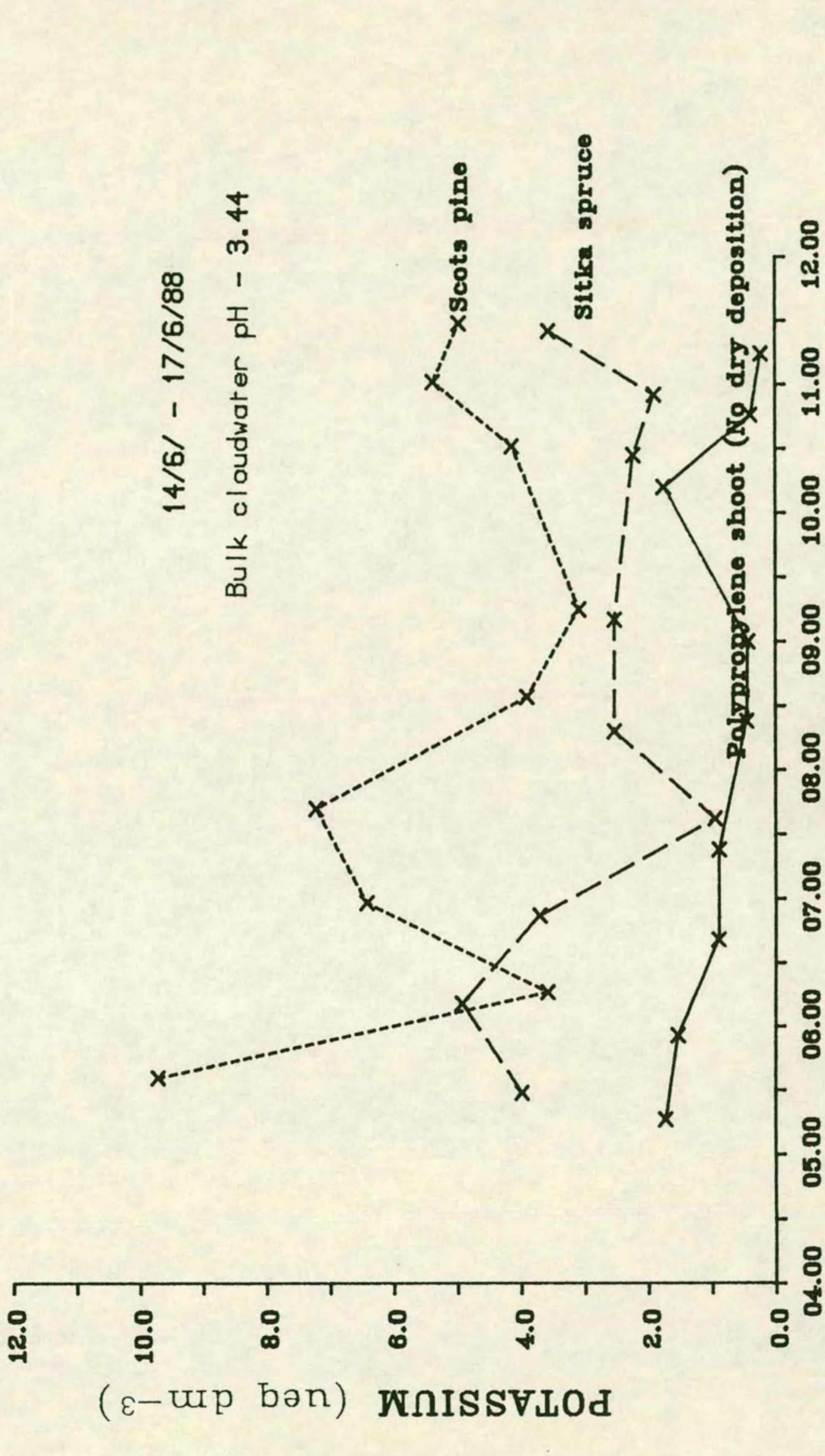


Figure 5.10 Comparison of potassium concentration in droplet samples from Scots pine, Sitka spruce and artificial shoots. Measured cloud episode - 17/6/88.

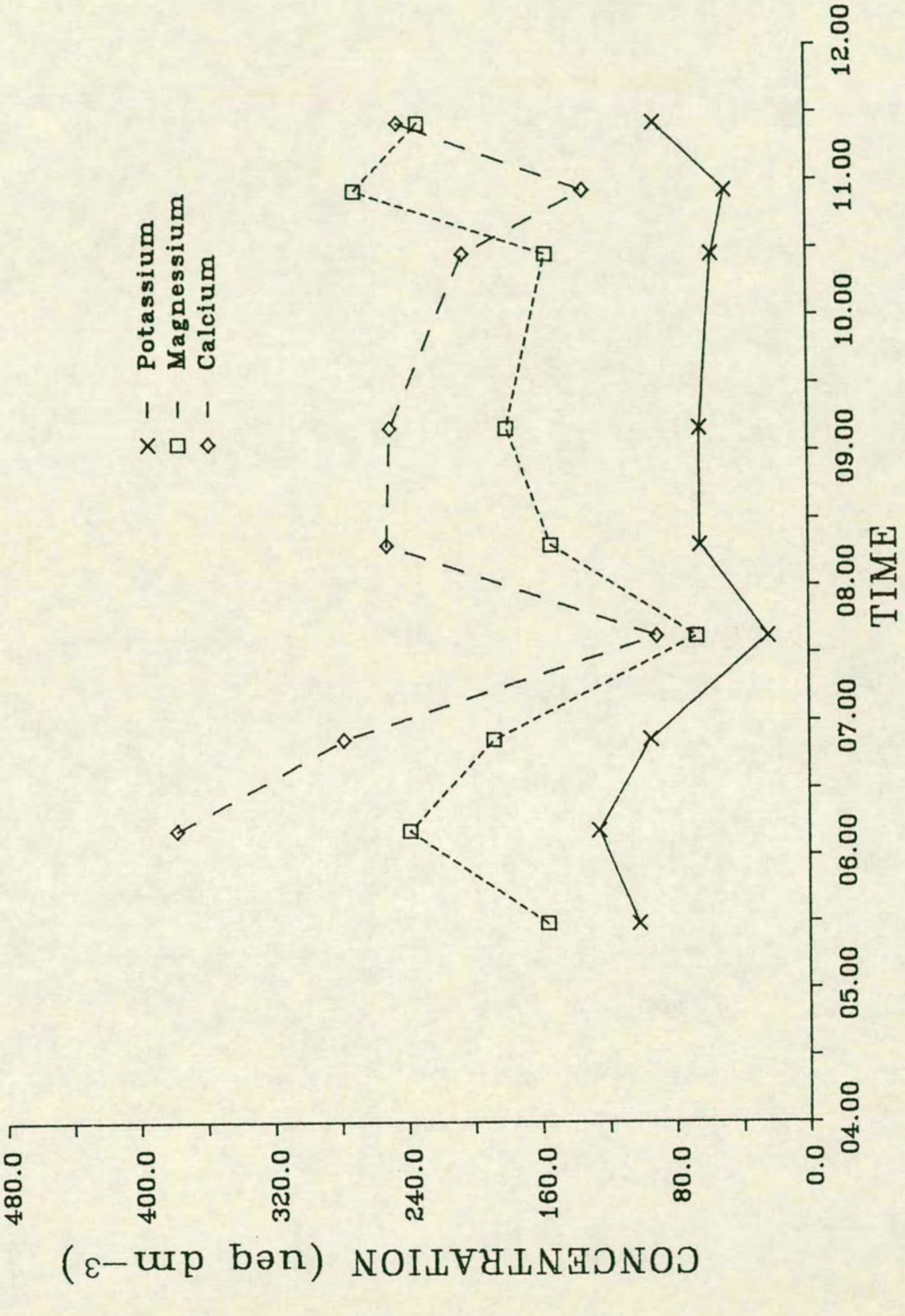


Figure 5.11 Similarity of cation concentrations of Sitka spruce samples during a cloud episode. (17/6/88)

5.3.4 Cation concentration range, 'typical' values and comparison with bulk cloudwater samples from the field site.

As mentioned earlier, because of the time necessary to collect a sample it was relatively rare that samples were taken from both tree species and both sets of artificial shoots during a single event. However, on a few occasions comparisons of a quantitative nature between samples taken from all four shoot types during the same event are possible. These values can also be compared with those from other events and what J.N. Cape (pers. comm.) calls a 'typical value' can be estimated. Means or medians would give the appearance of precision but variability between events and the effect of extreme events suggests a more subjective estimate of 'typical' concentrations to be as valuable. This estimate can then be compared with the mean of the bulk samples collected over the eight month period of sampling.

For potassium the range of droplet concentrations from samples on artificial shoots without dry deposition was 5.8-203.6 μeq dm^{-3} (0.225-7.96 mg dm^{-3}) and typically 19 μeq dm^{-3} (0.75 mg dm^{-3}). The eight month volume-weighted mean (V.W.M.) for the weekly bulk samples was 21.38 μeq dm^{-3}. Over those events where droplet samples from all types of shoot were taken,'typical' values were 20 μeq dm^{-3} for artificial shoots without dry deposition, 50 μeq dm^{-3} for artificial shoots with dry deposition and, with considerable variation, 100-150 µeq dm^{-3} for the live shoots.

With a 'typical value' of 175 µeq dm^{-3} (3.5 mg dm^{-3}), the calcium droplet concentrations on artificial shoots for the events measured were higher than the eight month V.W.M. of 123.9 µeq dm^{-3} (2.5 mg dm^{-3}). For those episodes where all shoot types were sampled the Ca^{2+} droplet concentrations were typically 205 µeq dm^{-3} for artificial shoots without dry deposition, 300µeq dm^{-3} for those with dry deposition and 300-400 µeq dm^{-3} for live shoots.

Although there was considerable variation within and between events, sample values were rarely greatly different from typical values. However, on two occasions extremely high ion concentrations were measured, particularly for magnesium. During the first event, which was the night of the 20th and morning of the 21st of November (1987), samples were being taken only from Scots pine to assess if there was any difference between current and current +1 needle samples and artificial shoots were, therefore, not included in the comparative analysis. Concentrations of 3472 µeq dm^{-3} for Ca^{2+} and 8712 µeq dm^{-3} for Mg^{2+} were reached. The weekly bulk sample concentrations were 125 µeq dm^{-3} (Ca^{+}), and 254 µeq dm^{-3} (Mg) but these included heavy rain on the previous day and snow with ion concentrations of around 50 µeq dm^{-3} for both Ca^{2+} and Mg^{2+}.

The other extreme event occurred on the 11th of December (1987) when droplets from all shoots were sampled for Ca^{2+} and K^{+}. Values at the start of the event on artificial shoots with and without dry deposition were around 450 μeq dm^{-3} and 230 μeq dm^{-3} respectively, falling to 150 μeq dm^{-3} and 6 μeq dm^{-3} as the event progressed. They were included in the typical value analysis without biasing the final value because of the lower values later in the event. However, with magnesium the original droplet concentration from artificial shoots without dry deposition was <u>ca</u>. 1400 μeq dm^{-3} and when the event is included despite the lower values of around 65 μeq dm^{-3} later in the event, the typical value is increased from <u>ca</u>. 100 μeq dm^{-3} (1.22 mg dm^{-3}) to 285 μeq dm^{-3} (3.46 mg dm^{-3}). The eight month V.W.M. was 185.5 μeq dm^{-3} (2.25 mg dm^{-3}). For those events in which droplets from all shoots were sampled the Mg^{2+} concentrations were typically 325 μeq dm^{-3} for artificial shoots without dry deposition, 415 μeq dm^{-3} for those with dry deposition and 450 μeq dm^{-3} for live shoots.

5.3.5 Comparison of base metal cation concentrations and H^{+} concentrations

For droplet samples from live shoots the pattern of ion concentration was generally similar not only for Ca^{2+}, Mg^{2+} and K^{+} but also for H^{+}. Figure 5.12 shows that both Mg^{2+} and H^{+} exhibited a similar pattern of change during one of the particularly concentrated cloud events and this correlation was evident in most of the other events.

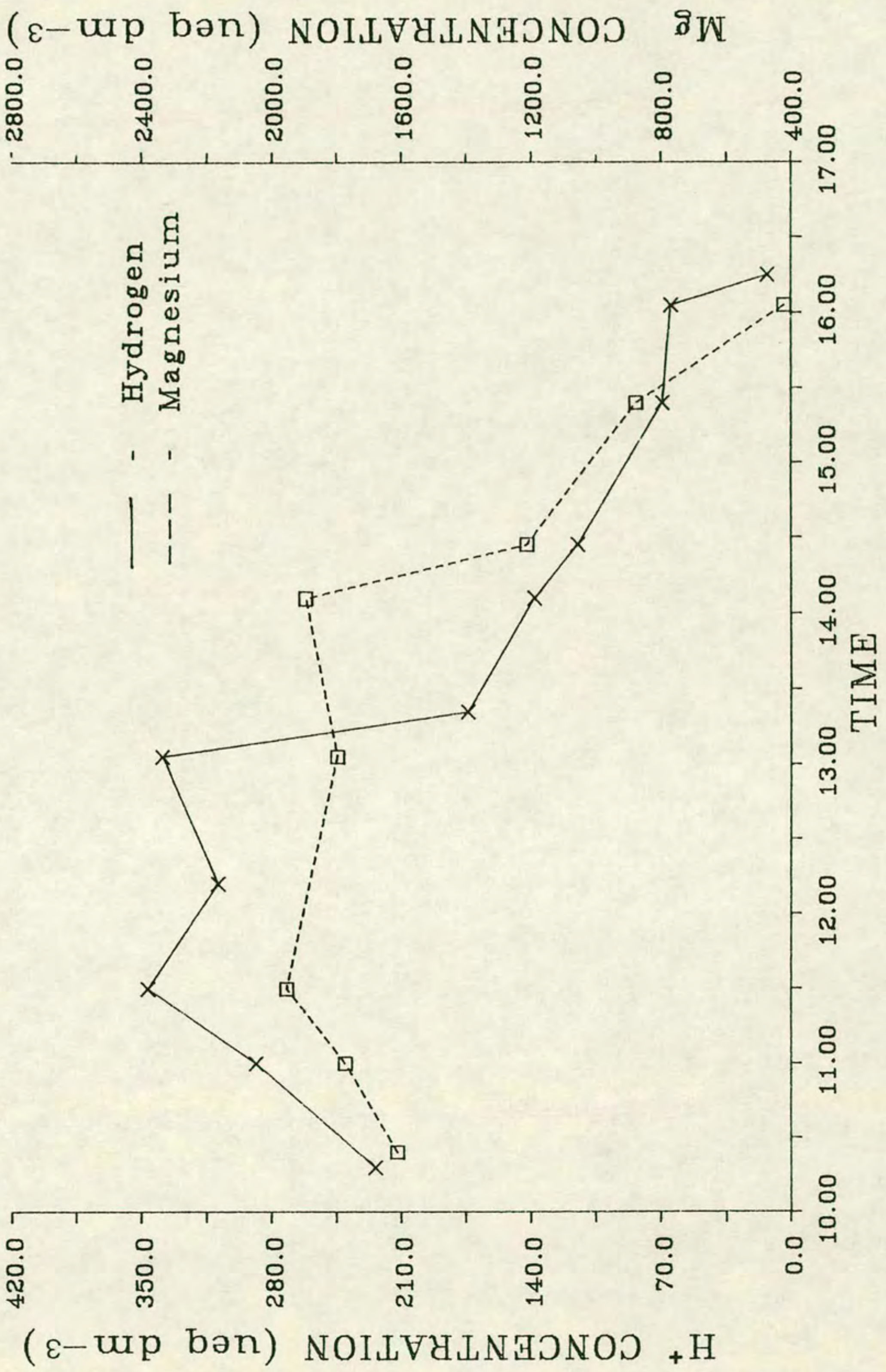


Figure 5.12 The similarity between the concentration of hydrogen ions and cations in droplet samples from Sitka spruce (11/12/87)

5.3.6 Needle nutrient concentration and sample ion concentrations.

The concentrations of nutrients in the needles of six trees chosen to monitor the relationship between needle nutrients and leaching are shown in Table 5.3. The nutrient status of the trees is poor with potassium being slightly deficient, but the distinctive chlorotic symptoms of K deficiency were not evident (Miller and Miller 1987). Calcium concentrations were variable whilst potassium and magnesium concentrations were generally similar for the six trees. The exceptions were a higher concentration of potassium in tree 3 and of magnesium in tree 6.

Cloudwater droplet samples from the six trees did not show any correlation between the nutrient concentration of the tree foliage and the ion concentration of the droplet samples (Table 5.3). Trees 1 and 3 showed positive aggregate differentials against the means of all three ions, yet tree 3 was generally high in foliage nutrients whilst tree 1 was generally low. Conversely, trees 5 and 6 both showed negative aggregate differentials with tree 6 being high in nutrient concentration and tree 5 being low. The higher concentrations of potassium in tree 3 and magnesium in tree 6 were not reflected in higher concentrations of these ions in the cloudwater droplet sample.

Table 5.3 Comparison of shoot nutrient content and the aggregate of the differences between cloudwater samples taken from each tree and the mean of these samples. The aggregate mean is shown in brackets.

Tree	K		Mg		Ca	
	Shoot nutrient concentration	Aggregate differential (38.526)	Shoot nutrient concentration	Aggregate differential (50.89)	Shoot nutrient concentration	Aggregate differential (54.781)
1	0.372%	1.691	0.0475%	4.559	0.173%	7.010
2	0.340%	7.152	0.0435%	-3.233	0.150%	-3.548
3	0.530%	0.081	0.0490%	1.496	0.309%	2.306
4	0.360%	-4.126	0.0550%	-0.155	0.261%	8.397
5	0.390%	-2.380	0.0475%	-2.334	0.127%	-5.211
6	0.420%	-2.425	0.0690%	-4.843	0.215%	-9.009

5.4 Discussion

The principles of cloud chemistry and microphysics indicate that cloud pH would be lower than rain pH (Lee 1986, Isaac and Daum 1987). Field and aircraft measurements confirm that to be true (Isaac and Daum 1987, Kins et al. 1988, Munzert 1988). Recent data from the Dunslair Heights field site also follow this pattern (A. Crossley pers. comm.).

The results of this series of measurements showed clearly that during cloud events outbreaks of rain or drizzle reduce acidity and that cloudwater alone contains much higher concentrations of H^+. This confirmed the higher potential for acid induced damage at high elevations than rain pH would indicate.

The results also showed the importance of assessing cloudwater pH on an event basis rather than on bulk samples collected over several days during which dilution of extreme events occurs. Furthermore, variation within events exposes trees to even higher concentrations of acidity over short periods. These variations within events are a combination of horizontal spatial inhomogeneity and vertical inhomogeneity. It is vertical inhomogeneity that leads to the pattern of an initially low pH gradually rising and then falling again at the end of the event. This results from the lower liquid water content at cloud base compared to higher up in the cloud (Schmitt 1988). As the droplets rise and are cooled they increase in water content and are thus diluted. Horizontal spatial inhomogeneity results from the incorporation of pollution plumes into the cloud and also from the strong horizontal spatial inhomogeneity of cloud liquid water content found down to a scale of $<$ 1 km (Ogren *et al*. 1988).

The variations within the cloud events led to very low pH values of cloudwater compared with weekly or even single event bulk cloud samples. Although continuous monitoring of cloud pH is possible by temporal active sampling procedures (procedures that actively collect a time series of cloudwater samples), these fail to take account of the diluting or concentrating effects of previously impacted droplets. As it is the acidity of droplets on the shoots that is vital and not the cloudwater acidity, the method used in this set of studies was advantageous as it repeatedly measured *in situ* droplet pH. Such intensive measurement is therefore instrumental in pinpointing the likelihood of threshold values for damage being exceeded.

Although there were no obvious damage symptoms attributable to acid clouds at the site, visible damage is likely to be the end product of invisible metabolic or physiological damage. Studies seeking to estimate whether current levels of acidity in precipitation are damaging to conifers should be careful not to use pH values of rain or bulk pH values of cloudwater as an indication of the severity of extreme events. If certain types of damage may be caused by extreme rather than chronic acidity the potential of cloud episodes to inflict that damage is high.

Droplet samples taken from live shoots reflected cloudwater ion concentrations, rather than ion exchange processes. If ion exchange processes had dominated, increasing base metal cation concentrations would have been accompanied by decreasing H^+ values. The similarity of H^+ and cation concentrations is indicative of the domination of cloudwater ion concentrations in these samples. That the nutritional status of individual trees did not have an obvious effect on sample ion concentrations is partly to be expected because of the overriding influence of the background cloudwater ions on droplet sample ion concentration compared with leached ions.

Cation exchange with H^+ therefore, had only a small effect on impacted cloud pH. The higher pH values (lower H^+) found in Scots pine samples compared with Sitka spruce samples were accompanied by higher K^+ concentrations. It is therefore possible that $K^+ - H^+$ exchange occurs in Scots pine to a greater degree than in Sitka spruce and, to a small extent, raises the pH.

Opinion is divided as to whether species which have a higher neutralizing ability are more susceptible to damage (Adams and Hutchinson 1984, Larsen 1988). In the glasshouse misting experiment both trees were very resistent to damage but slight traces of damage were evident on Sitka spruce at pH 2.5 but not on Scots pine. It is possible that increased neutralization of H^+ by K^+ in Scots pine may have been partly responsible for its greater resistance to damage. In the case of these two species, neutralization of acidity is not a major means of reducing damage, but, all other factors being equal, increased neutralization may increase resistance.

The small effect of cations in neutralizing acidity was not only seen in the similarity of H^+ and cation concentration patterns but in a reduction in pH of droplets on live shoots compared with artificial surfaces. This was consistently found in both species but was more extreme for Sitka spruce. This reduction of precipitation pH by conifers is in agreement with the major part of the literature on this subject (Henderson et al. 1977, Cronan et al. 1980, Alcock and Morton 1981, Cronan and Reiners 1983, Johannes et al. 1985, Cape and Brown 1986, Reiners et al. 1987, Kaupenjohan et al. 1988), although increases in pH have been found (Cole and Johnson 1977, Miller, et al. 1981, Mahendrappa 1983, Reiners and Olsen 1984) and certainly compared with broadleaved canopies, coniferous canopies acidify precipitation (Cronan and Reiners 1983, Johannes et al. 1985, Cape and Brown 1986).

Some of this reduction in pH is the result of acidic dry deposition onto live shoots. The results showed a clear effect of dry deposition on the pH of deposited cloudwater.

Two factors govern the differences in sample pH between no dry deposition (NDD) and dry deposition (DD) artificial shoots. Firstly, the length of time previous to a cloud event that the field site was dry determines the period of dry deposition prior to that event. Secondly, the wind direction during that time provides an indication of the likely pollutant load in that air. Figures 5.8 and 5.9 showed minimum dry deposited ions after heavy cloud over the previous days has washed the DD shoots clean of dry deposition. Where the wind was variable and cloud and rain intermittent over the previous few days, a small difference between NDD and DD shoots occurred (Figure 5.7). Other data showed that on the 11th December, 1987, even with a fairly long dry spell previous to the cloud event, a northerly air stream did not lead to heavy dry deposition and the difference between NDD and DD shoots was small, from pH 6.8 to 5.3 - a decrease of only 4.8 μeq dm^{-3}. However, on the 16th December, 1987, after four dry days during which the air stream had changed to a southwesterly from industrial Europe, the initial difference between NDD and DD shoots was 100 μeq dm^{-3}.

Thus long spells of dry weather during which time a polluted air stream is flowing across the field site predisposes trees to a high level of acidity at the onset of cloud events. Initial increases in acidity may be at least 2 pH units or 100 μeq l^{-1}. Combined with the possibility of low pH levels at cloud base experienced at the start of a cloud event the combined damage potential of dry plus occult deposition at the onset of cloud events is high.

Bulk or even event collection would not pick up this short term effect and the sampling method used here allows such extremes to be pinpointed. Once again, if damage occurs at acutely acidic levels rather than due to chronic deposition of acidity such extremes are important.

However, the results showed that dry deposition was not responsible for all of the reduction in throughfall pH and neither was the reduction similar in both species. Differences in the effect of the foliage of coniferous species on throughfall pH may be partly the result of soil conditions: Yawney _et al_., (1978) found that K fertilized stands of _Pinus_ _resinosa_ were subject to greater leaching of P, K and Ca, and Mahendrappa (1983) found N-fertilized plots neutralized rainfall to a greater extent than control plots. The Sitka spruce in the controlled experiments in this study were moderately supplied with nutrients whereas the field site was nutrient poor, yet both sets of experiments produced similar reduction in pH by Sitka spruce shoots.

Cape and Brown (op. cit.) also found that differences in the extent of throughfall modification with respect to H^+ were apparent between different years. The same species at different locations may produce very different effects on throughfall: Miller _et al_. (1981) found that Sitka spruce acidified precipitation at one location in Scotland, whilst at five other locations, pH increased.

There are also effects of different components of the canopy on precipitation pH. The effect of old or new needles, twigs or bole on throughfall pH may all differ (Reiners and Olsen 1984). In addition, the uptake of H^+ by young needles decreases (throughfall acidity increases), as the length of time of the cloud episode increases (Reiners and Olsen op. cit.). The authors suggest this may be a result of the saturation of exchange sites by hydrogen ions.

It is possible that differences in the effects of canopies on throughfall may be a result of differences in canopy structure and needle retention (leading to differences in the exposure of precipitation to the canopy components), inherent differences in neutralizing ability which may be genetically governed (Scholz and Reck 1977), differences in buffering capacity in response to air pollution climate, and the availability of exchange sites in the foliage. Particularly at high elevation sites where extended periods of acidic cloud occur, exchange sites for H^+ may be generally less available than for drier nutrient-rich sites with less acidic precipitation.

Although those processes above may be responsible for differences in throughfall composition, they do not explain an actual reduction in pH. Where dry deposition and leaching are not separated this reduction is often ascribed to acidic dry deposition, but where the reduction in pH is greater for live shoots than DD artificial shoots, dry deposition cannot be the cause. The use of artificial shoots in comparisons also eliminates the uncertainty associated with evaporation. Where evaporation is high, increased H^+ concentrations may not indicate an acidifying effect of the canopy but a concentrating effect of evaporation and this point is often overlooked in comparisons of incident precipitation and throughfall. The use of artificial shoots closely resembling live shoots in this experiment provides valuable information that allows live and artificial shoots to be compared without evaporation complicating the picture, as impacted cloud evaporated at similar rates on all shoots. The results showed clearly that neither evaporation nor dry deposition could account for the increased acidity in samples from live shoots.

It is however, unknown whether the excess H^+ is liberated from organic substances or transported from symplastic or apoplastic water (Reiners and Olsen 1984). The H^+ increase may be related to the leaching of organic acids (Alcock and Morten 1981, Cronan and Reiners 1983, Reiners et al. 1987), although Cape and Brown (1986) found no large anion deficit in their ion balance and concluded that strong inorganic acids rather than leached organic acids must have been responsible for the drop in pH. In addition, because pH falls as well as total filtrated acidity increasing, strong acids appear to be involved. Hoffman et al. (1980) suggest that peroxycarboxylic acids, derived from the exposure of leaves to ozone may oxidise weak acids such as H_2SO_3 and HNO_2 and produce strong acids which could then be washed out.

Further work is needed to tease out the contributions of soil status, species differences, canopy components, acid formation and leaching, and organic acid leaching to the canopy effect on precipitation as this work clearly shows that internal sources of H^+, and not only evaporation and dry deposition, are important contributions to the acidity level of impacted cloudwater on live shoots.

The increase in cation concentration samples from live shoots compared with those from artificial shoots (with dry deposition) was proportionally larger for potassium than calcium and magnesium. This, along with the evidence from the plots of the cation against each other which showed a bias towards potassium indicates that potassium was leached from the needles to a greater extent than the other two cations. Potassium binds less strongly to carboxylic acid binding sites than calcium and magnesium and is therefore more easily leached (Schonerr 1982, Richardson and Dowding 1988). Potassium, being a monovalent ion, is also less subject to charge interaction and passes more easily through the cuticle than divalent ions such as calcium and magnesium (McFarlane and Berry 1974).

The relative importance of leaching compared with dry deposition for K ions compared with Ca and Mg is also increased because Ca, derived from dust, and magnesium from dust and sea-salts, are dry deposited onto shoots, whereas atmospheric sources for potassium are small (Cape and Brown 1986).

Leaching of cations took place at similar rates in current and current +1 needles. Cation exchange takes place from mobile ions held in free space and leaching therefore tends to be greatest in older leaves or needles when the nutrient pool is most mobile (Adams and Hutchinson 1984) as opposed to growing leaves or needles when available nutrients are quickly metabolised within cells (Tukey 1970).

However wettability also affects leaching and although older needles, with a degraded wax structure have lower contact angles and are more easily wetted, field observations showed that these droplets tended to quickly amalgamate and run down the needle to form one large droplet (Figure 5.13). The total surface area covered by droplets was thus often greater in younger needles. The net result was no consistent difference in the acidity or ion content of droplets on needles in different year classes.

The amount of cation leaching from coniferous needles, compared with leaves from herbaceous species which have much lower acidity thresholds for damage, was small. It is apparemt that resistance to injury in Scots pine and Sitka spruce is not the result of high concentrations of leached cations neutralizing acidity but of cuticular properties and possibly of internal cell resistance to high concentrations of H^+ ions.

Whether or not the rates of leaching caused by cloudwater are likely to lead to nutrient deficiencies and possible chlorosis and needle shedding, as in the 'Waldsterben', is difficult to ascertain. Both glasshouse and field site results show that large

Figure 5.13 Droplet retention pattern on Sitka spruce needles. On young needles droplets are smaller, more numerous and discrete, whereas on older needles they have amalgamated, forming one large droplet. This droplet frequently falls off, whereas those on younger needles are more generally retained.

amounts of nutrients would be leached out of the needles over a long term. Although British forests, including Scots pine and Sitka spruce plantations, have recently shown signs of poor health, research has focussed on visual signs of damage rather than on nutrient status (Innes and Boswell 1987, 1988). Widespread Mg deficiency as seen in Norway spruce in central Europe has not been thought likely to affect conifers growing in north-west Europe because of the plentiful supply of magnesium from oceanic fronts. The results here indicate that whilst magnesium is not leached in very large amounts, potassium is not only leached more readily but is also slightly deficient in the shoots. If this deficiency is the results of poor soil conditions, then further leaching by acid cloudwater may enhance the possibility of serious deficiency and resultant physiological damage. With respect to the recent worsening in the health of British forests and the lack of any clear cause, it would be wise that nutrient concentrations are carefully monitored, particularly at high elevation sites subject to acid clouds. It may be that, as in central Europe, the recent downward trend in forest health could be arrested by additions of nutrients.

CHAPTER 6

The resupply of leached nutrients to trees

6.1 Introduction.

The previous two chapters have shown that cloud episodes can leach large amounts of nutrients from Scots pine and Sitka spruce trees and that this process is exacerbated by high acidity levels. It is, therefore, important to discover whether these leached nutrients are resupplied to the plant. If they are not resupplied, then either litter leaf production must be reduced (Johnson *et al*. 1985), or foliar nutrient concentrations must be lowered. Foliar nutrient concentrations of K, Ca and Mg in *Pinus strobus* (Wood and Bormann 1977) and of Mg in Norway spruce (*Picea abies*) (Kaupenjohann *et al*. 1988) fell after increased leaching by simulated acid rain, suggesting nutrients were not replaced. Foliar concentrations of Ca and Mg in *Pinus jeffreyi* and *Sequoiadendron gigantea* (Westman and Temple 1989) and Norway spruce (Johnsen *et al*. 1987) also fell in response to acid precipitation. As the number of misting episodes on *Picea glauca* and *Betula alleghaniensis* increased, the amount of K and other nutrients and metabolites leached fell, suggesting inadaquate resupply (Scherbatskoy and Klein 1983).

However increased uptake of ions in response to leaching (Mecklenburg and Tukey 1964), and similar or elevated foliar nutrient concentrations after exposure to acid precipitation

(Horntvedt et al. 1980, Skeffington and Roberts 1985a,b, Kelly and Strickland 1986, Abouguendia and Baschak 1987, Mengel et al. 1988, Roberts et al. 1989b, D. Turner and D. Tingey pers. comm.) suggest that compensating mechanisms may operate at least in some species under certain conditions.

Opinion is, therefore, divided as to the effectiveness of resupply to plant parts after acid leaching. Uptake may be hindered by low availability of nutrients from the soil and there may also be differences between species and even clones, as leaching losses themselves have been shown to be subject to genetic variability (Percy 1987, Pfirrmann et al. 1988). To understand the nutrient balance of conifers subjected to acid precipitation a clearer understanding of the links between leaching of foliage, resupply and depletion of reserves is needed. Although the use of hydroponic solutions restricts the applicability of results to field situations, it offers a valuable first step at understanding plant nutrient uptake in response to leaching.

The free supply of nutrients to the root system avoids the complication of uptake being dependant on availability rather than demand. Availability could be looked at as a second step. By measuring changes in the nutrient concentrations of the growing solution, increase in uptake in response to acid precipitation can be identified. So far very little work has been done in this way and none with Scots pine or Sitka spruce.

Most water-culture methods used in the study of nutrient uptake are based on the resupply of one or several nutrients in proportion to their continually, or periodically, monitored depletion. Continual monitoring of cations can be done by using ion selective electrodes (Clement et al. 1974) or, in the case of K^+, by a flame photometer (Woodhouse et al. 1978, Wild et al. 1979). pH monitoring linked to a control system is more commonly used than cation monitoring but as it is cations that are the focus of this experiment it is their depletion that should be measured. The cost of setting up a continuous flowing system with either an assembly of ion selective electrodes or a flame photometer was greater than the funds available so that a periodically monitored system was used.

6.2 Method.

144 3-year-old Sitka spruce were removed carefully from their pots and washed. Full details of the tree material are given in Appendix 2. They were then allocated randomly to nine replicates (each with eight plants), of two treatments, and transferred to nutrient solution. They were left for 3½ weeks before being checked for root damage, after which the experiment began.

Each replicate consisted of a polythene bowl with two rows of four seedlings. The roots were inserted through 3 cm diameter holes in a black polypropylene sheet (12 mm thick) and held in place by securing the stem with a closed pore polyurethane foam bung. The black polypropylene covered the basins and excluded light from the solution thus maintaining an algae-free environment.

The nutrient solution was based on that recommended by Ingestad (1979) for spruce and on the recommendations of H. Miller (pers. comm) and I. Rorison (pers. comm.). The concentrated solution shown in Table 6.1 was diluted x 100 with deionised water. Samples from the nutrient solution were taken from all replicates twice weekly. Provisional work at the Unit of Comparative Plant Ecology, Sheffield with the help of Professor Rorison, and a pilot run had shown that nutrient concentrations were unlikely to be drawn down by large amounts during such periods. As the experiment progressed, it became evident that longer periods of sampling would reduce disturbance to the root systems, which had to be lifted out

Table 6.1. Nutrient solution composition to make up 100 dm^{-3}.

Element	Salt	Molecular weight	Amount(g)
Ca	$Ca\ (NO_3)_2 \cdot 4H_2O$	236.15	1.17839
Mg	$Mg\ SO_4 \cdot 7H_2O$	246.48	2.02764
K	KNO_3	101.11	3.15796
	$KH_2\ PO_4$	136.09	1.31811
P	$KH_2\ PO_4$	136.09	1.31811
N	KNO_3	101.11	3.15796
	$Ca\ (NO_3)_2 \cdot 4H_2O$	236.15	1.17839
	$Na\ NO_3$	84.99	11.66684
S	$Mg\ SO_4 \cdot 7H_2O$	246.48	2.02764
B	$H_3\ BO_3$	61.83	0.02859
Cu	$Cu\ Cl_2 \cdot 2H_2O$	170.48	0.00201
Mn	$Mn\ Cl_2 \cdot 4H_2O$	197.91	0.03602
Zn	$Zn\ Cl_2$	136.28	0.00156
Mo	$Na_2\ MoO_4 \cdot 2H_2O$	241.96	0.00076
Fe	$Fe\ Cl_3 \cdot 6H_2O$	270.32	0.08713

of the solutions to give access, and would also allow differences in uptake between the two treatments to be more evident. The interval between sampling was therefore increased to 14 days later in the experiment. pH rose slowly because of the consumption of NO_3^- but remained within the range 4.5-5.5 without the need for added acid.

The 15½" (38 cm) x 12½" (32 cm) x 6" (15 cm) polypropylene bowls (G101, Greeco products, New Milltown, Hampshire) used for holding the nutrient solution were free from toxic phalates (Hardwick and Cole 1986). The bowls were also tested for leakage of ions by filling four bowls with deionised water, covering to eliminate contamination, leaving for four days and then analysing samples of the water. These were found to be free from leached ions.

Aeration was provided using a vacuum pump and compressor (E.B.3. Edwards High Vacuum Ltd., Crowley, Sussex). Eighteen lengths of polyethylene tubing, cleaned in "Decon", hot water and deionised water were led off in parallel from the main tubing. Two holes 13 mm in diameter were drilled in either end of the containers. The tubing was passed through these holes and held taut by a cable gland. The tubing was pierced with a series of small holes through which the air was released both aerating and mixing the solution, and thus ensuring no local depletion or build-up of nutrients. Each bowl was filled with 8 dm^{-3} of nutrient solution. Nutrient distribution was checked by multiple sampling and found to be homogenous. The flow of air was controlled by a "Thumbscrew" controller and "T" piece and the air purified by passing through a filter tube (grade Bx, Balston Ltd., Maidstone).

Two wooden frames were built to house the equipment for spraying the simulated acid cloudwater (SAC), one frame for each set of replicates. Three sprayers for each frame were mounted on cross members. The two treatments were pH 5.6 and pH 3.0. Details of SAC ion content and spraying technique are given in Appendix 1.

Seedlings were sprayed with SAC on Monday, Wednesday and Friday for 14 weeks giving a total of 52 applications (166.4 mm). The positional order of containers was rotated before every spraying and the position of the two complete sets of replicates changed when the nutrient solutions were being renewed. Full details of glasshouse conditions are given in Appendix 3.

The nutrient solution was isolated from the SAC by sealing the seedlings with closed pore polyurethene foam bungs, PTFE tape, parafilm and autoclave tape where they passed through the black polythene sheet. As well as monitoring nutrient uptake, cuticular conductance and shoot nutrient contents were both assessed and the results presented in previous chapters.

6.3 Results.

After three weeks needles began to show brown spots and necrotic tips. The damage was almost exclusively limited to the pH 3.0

treatment. Although very similar to the acid damage seen in the previous experiment, this damage was in fact caused by aphids. It spread rapidly leading to considerable browning before being brought under control by Piramor. Later some of the damaged needles were shed.

Prior to this damage Ca, Mg, NO_3^--N and PO_4^{3-}-P had all shown a tendency to be taken up to a greater extent in the pH 3.0 treatment (Figure 6 a,b,d,e). There was, however, a slight tendency for potassium to be taken up to a greater extent in the pH 5.6 treatment (Figure 6c).

After the aphid damage, the three base metal cations Ca, Mg and K showed a steady lower rate of uptake in the pH 3.0 treatments. NO_3^--N also showed decreased uptake from the pH 3.0 treatment but recovered towards the end of the experimental period. PO_4^{3-}-P showed little sign of difference in uptake rates between the two treatments.

The cumulative mean uptake for the two treatments and the difference between them as a percentage of uptake by the control is given in Table 6.2 and shows that there was more cation uptake in treatment pH 5.6 compared with pH 3.0.

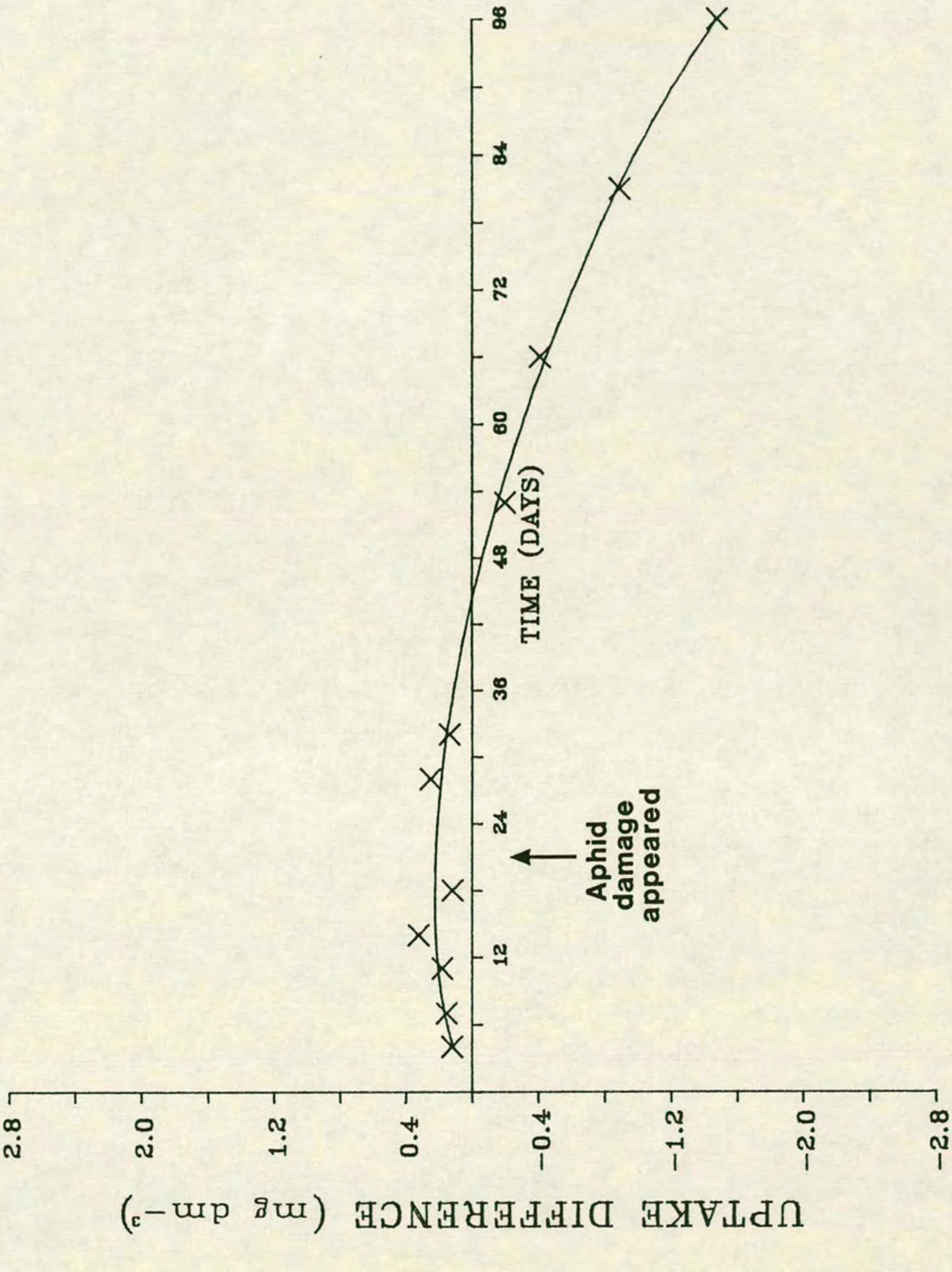


Figure 6a The accumulated mean difference between the uptake of calcium by seedlings exposed to SAC at pH 3.0 minus that taken up by seedlings exposed to SAC at pH 5.6.

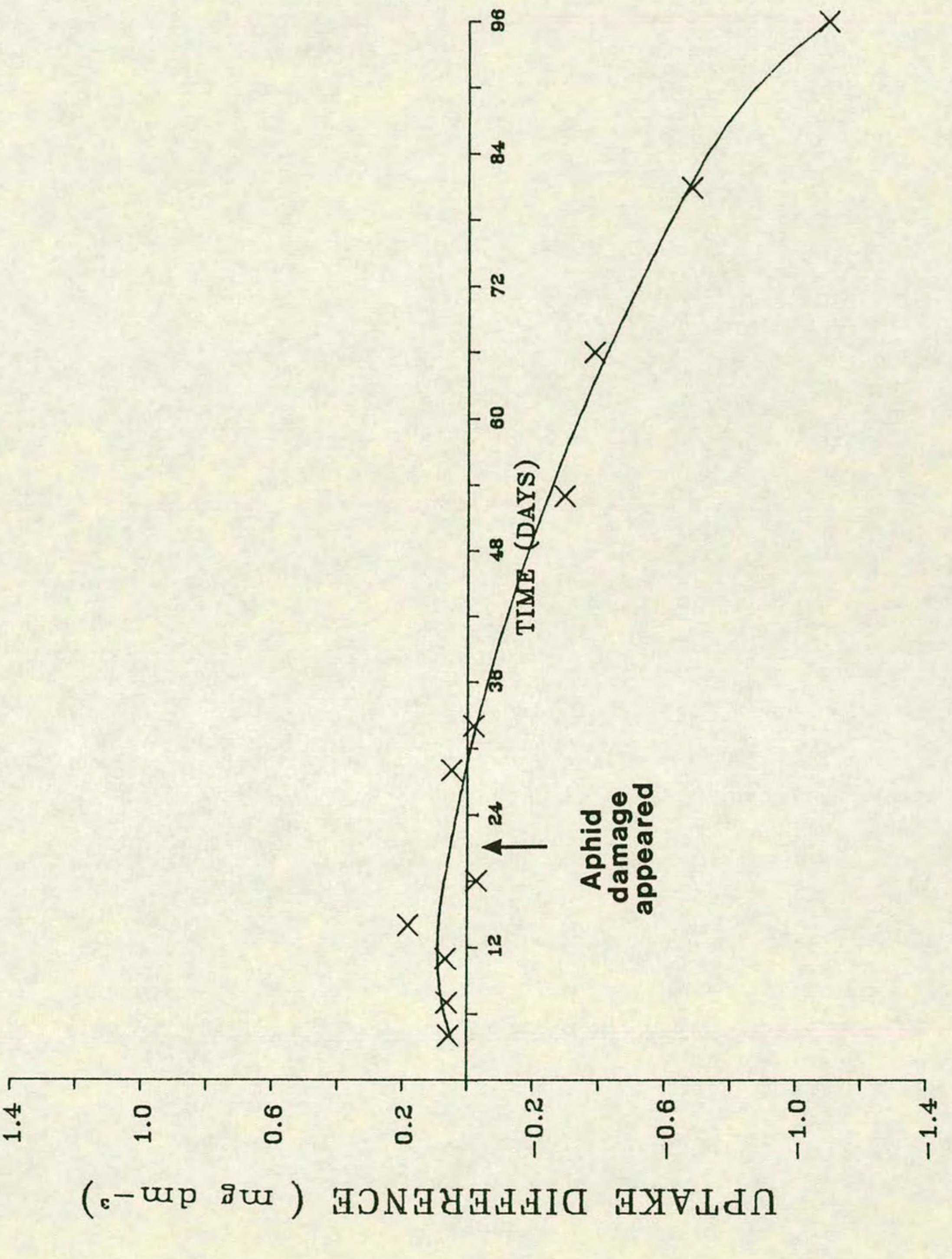


Figure 6b The accumulated mean difference between the uptake of magnesium by seedlings exposed to SAC at pH 3.0 minus that taken up by seedlings exposed to SAC at pH 5.6

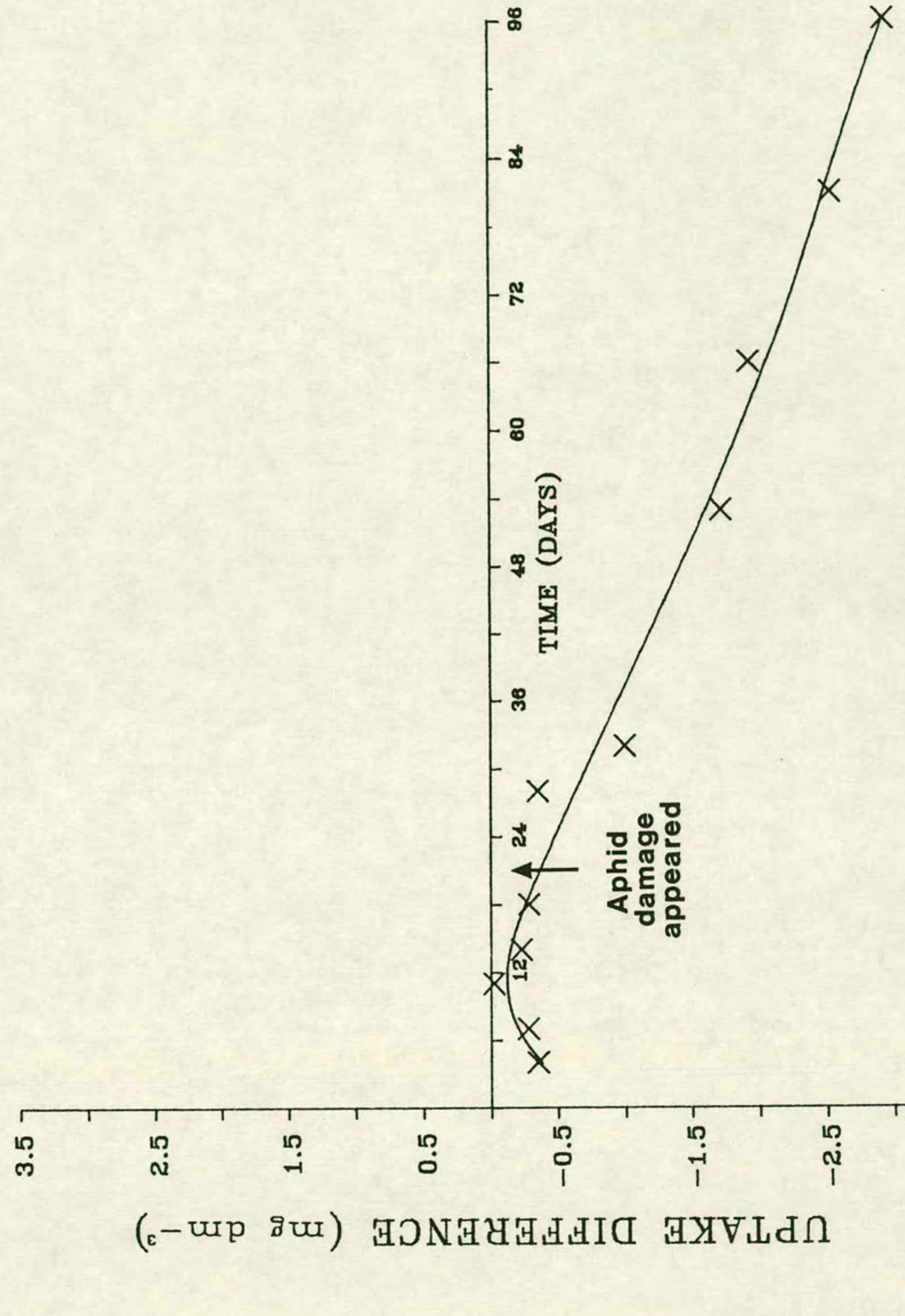


Figure 6c The accumulated mean difference between the uptake of potassium by seedlings exposed to SAC at pH 3.0 minus that taken up by seedlings exposed to SAC at pH 5.6.

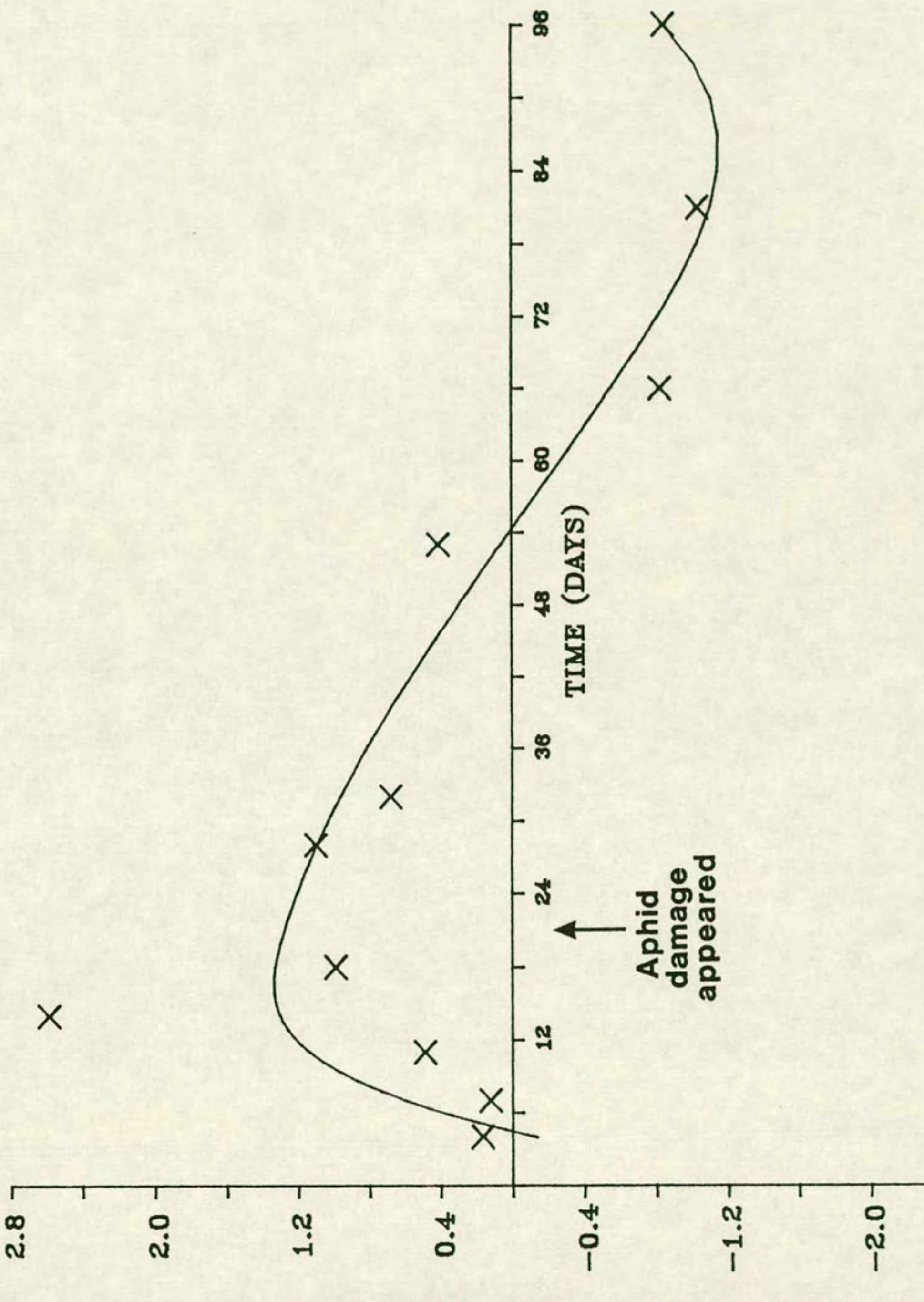


Figure 6d The accumulated mean difference between the uptake of nitrate-n by seedlings exposed to SAC at pH 3.0 minus that taken up by seedlings exposed to SAC at pH 5.6.

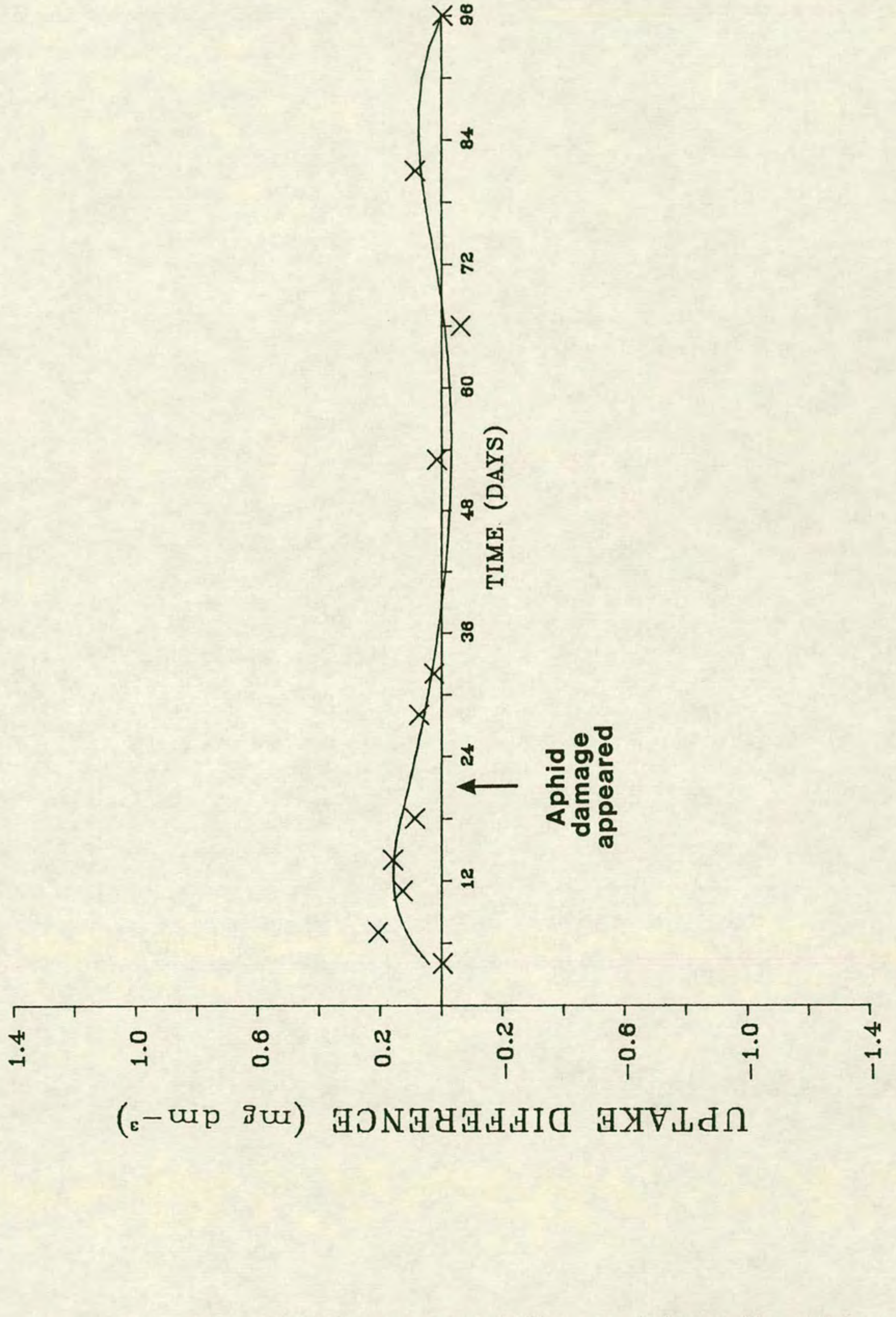


Figure 6e The accumulated mean difference between the uptake of phosphate-p by seedlings exposed to SAC at pH 3.0 minus that taken up by seedlings exposed to SAC at pH 3.0.

Table 6.2 Total uptake of ions (mg dm^{-3}) over experimental period

Ion	pH 5.6	pH 3.0	Differences as % of control
NO_3^--N	21.66	20.28	6.0
PO_4^{3-}-P	1.55	1.42	8.3
Ca^{2+}	3.08	1.61	47.7
Mg^{2+}	3.46	2.38	31.2
K^+	8.57	5.70	33.5

6.4 Discussion.

The aphid damage precluded drawing any firm conclusions from the results as the period of uptake prior to damage was relatively short. It appears that the damaged seedlings, which were almost entirely limited to the pH 3.0 treatment reduced their nutrient uptake in response to the infestation. Needles on shoots which flushed after many of the damaged needles had been shed did have similar, if not larger, nutrient concentrations than in needles from the control (Chapter 4.3.2), despite the lower uptake rates.

The results prior to aphid damage indicated that the seedlings in treatment pH 3.0 took up more nutrients from the solution than those in treatment pH 5.6. The increased uptake, high foliar nutrient concentrations and increased leaching found throughout the series of experiments indicate that compensatory increased uptake in response to acid leaching had occurred. There is little comparable work on Scots pine and Sitka spruce but support for this hypothesis is strong from work on other species.

Mecklenburg and Tukey (1964) showed clearly that Ca uptake increased dramatically in Phaseolus vulgaris to compensate for increased leaching. Circumstantial evidence comes mainly from observations on foliar nutrient concentrations after exposure to acidic precipitation. Abouguendia and Baschak (1987) suggested that replenishment of leached ions was responsible for a lack of difference in nutrient concentrations of Pinus banksiana and Pinus glauca seedlings after exposure to simulated acid rain. Stronger evidence has been found from experiments where leaching was monitored and found to increase in response to simulated rain acidity without leading to a decrease in the Ca, Mg and K content of needles of Norway spruce and Pinus contorta (Horntvedt et al. 1980). Kelly and Strickland (1986) found that despite increased leaching of Ca by simulated rain of low pH, foliar ion concentrations in several species including Pinus virginiana were not affected. They concluded that either the increased leaching of Ca was an artefact and could be attributed to dry deposition (a scenario which is not applicable to the increases in ion concentrations seen between treatments in this thesis) or that rapid replenishment of leached ions was occurring. D. Turner and D. Tingey (pers. comm.) have recently found greater leaching of Douglas fir (Pseudotsuga menziesii) by simulated "fog" of low pH but no change in foliar nutrient levels.

It appears that the response of plants to increased leaching is to compensate by increasing uptake and this may even lead to over-compensation. Skeffington and Roberts (1985b) found no significant effect of acid misting on the nutrient content of Scots pine needles despite an increase in Mg leaching but further experiments (Roberts et al. 1989b) showed that, despite increased leaching, acid misting of Norway spruce and Scots pine seedlings led to higher needle contents of Mg, Mn and Zn in current year's needles and higher Mg, Mn and K in current +1 needles. Similar over-compensation was found for K in 5-year-old Norway spruce after acid misting (Mengel et al. 1988).

Roberts et al. (1989b) suggested that such compensation may not apply to field situations where soil nutrient concentrations are generally lower than in experimental soils but the experiments by Horntvedt et al. (op cit), and Kelly and Strickland (op cit) were in field plots and restructured forest soil respectively.

The role of soil is crucial. Mg, K and Mn may be leached out of Norway spruce differentially from young trees on different soils (Pfirrman et al. 1988). Nutrients may be resupplied to Fagus sylvatica after leaching by acid precipitation where the supply is adequate but not when the supply is limited (Leonardi and Fluckiger 1987, 1989).

It is possible that in certain conditions uptake may not be large enough to compensate for increased leaching because of low soil availability of nutrients. This may be exacerbated by a general reduction of microbial activity if the soil is acidified (Francis et al. 1980, VanLoon 1984, Hern et al. 1985, Ulrich 1985). Acidic precipitation may increase soil leaching by dissolving primary minerals such as $CaCO_3$ or, as is more likely in acid soils, displacing nutrients from the cation exchange complex (VanLoon 1984). Increased cation leaching from soils because of increased rain acidity has been observed by many authors (Wood and Bormann 1977, Abrahamson 1980, Bjor and Teigen 1980, Rutherford *et al*. 1985, Freiesleben and Rasmussen 1986, Skeffington and Brown 1986). In addition, a decrease in base saturation and cation exchange capacity of acid leached soils suggests that weathering and mineralisation are not great enough to compensate for leaching and uptake by plants (Stuanes 1980, Rutherford *et al*. 1985, Kelly and Strickland 1987).

Even where ions have not been leached out of the soil, the actual availability of Ca, Mg, K, N, P and S falls as soil pH drops (VanLoon 1984, Anderson 1986). In addition increased solubility of toxic metals, particularly aluminium, can inhibit uptake of nutrients such as calcium and magnesium, either through aluminium saturating the apoplast or through root damage (Matzner and Ulrich 1985, Hutchinson *et al*. 1986, Blank and Roberts 1987, Matzner *et al*. 1989).

This experiment illustrates the need to view the crown and root system as part of a whole rather than in isolation. No matter how strong crown leaching is, if uptake compensates the trees are unlikely to suffer nutritionally although an energetic cost may be paid. In fact, redistribution of nutrients may be aided by the increased leaching and uptake. Elements such as Ca and Mg whose distribution in the plant tissue is controlled by polarity and do not tend to move basipetally (from apex to base) could be leached, reabsorbed and translocated acropetally, thus aiding redistribution. In addition, increased mobility within leaves has been found for mobile ions such as Rb in several crop plants as a response to increasing acidity (Percy and Baker 1988).

The cost to be paid energetically for such increased uptake is poorly understood but the indications are that it may be significant (Mecklenburg et al. 1966). Amthor (1984) suggests that the energy needed to reabsorb or synthesize leached substances may be diverted away from growth processes. Energy is needed for the uptake of some cations and for the active uptake of co-transported anions (Richardson and Dowding 1988). Experimental support of an energetic cost would be difficult to obtain but an attempt at estimating its importance, possibly through careful measurement of maintenance respiration could be made.

CHAPTER 7

EVAPORATION AND ACIDITY ENHANCEMENT

7.1 Introduction

Chapters 4 and 5 dealt with the interaction between droplets and needles and the influence of dry deposition. Droplets are also substantially modified in their ionic concentration as water is evaporated. Surprisingly little attention had been paid to this phenomenon, although a few authors have addressed the problem, notably Klemm and his co-workers (Klemm *et al*. 1987, Klemm 1989, Adams and Hutchinson (Adams and Hutchinson 1987, Hutchinson *et al*. 1986, Hutchinson and Adams 1987) and workers at the Institute of Terrestrial Ecology, Edinburgh (Unsworth 1984, Crossley *et al*. 1986, Unsworth and Crossley 1987b, Milne *et al*. 1988). There are no published studies of *in situ* evaporation of droplets on Scots pine or Sitka spruce, nor do there appear to have been any studies in which evaporation has been monitored under controlled and measured environmental conditions for these species. Having established that buffering of incoming acidity is inadequate to raise pH, the consequences of increasing acidity as a result of evaporation must be carefully examined.

7.2 Theory

For many years, research on evaporation from forest canopies has focussed on the effect of 'interception loss' on the hydrological cycle (see Stewart 1977), or on the effect of 'wetness duration' on disease development (Brain and Butler 1985, Leclerc et al. 1985). However, when a droplet dries, the non-volatile constituents remain on the leaf/needle and are then concentrated. The main acids contributing to acidity within acid precipitation, H_2SO_4, HNO_3 and HCl remain on the needle surface during evaporation, resulting in a drop in pH. This reduction in pH (increase in acidity) is dependent upon the initial concentration of the acids in the droplet (Klemm et al. 1987). Because the main acids contributing to cloudwater acidity are strong (H_2CO_3 normally contributes less than 1% of rainwater acidity (Klemm and Frevert 1985)) a 90% evaporative reduction in volume of the original cloudwater droplets results in a drop in pH of 0.95 units (Klemm et al. 1987, Klemm 1989).

Droplet evaporation may also occur during cloudwater deposition itself. The evaporation rate from a wet canopy enshrouded with cloud depends on the available energy for heat transfer, the saturation deficit of the air and the efficiency of vapour transfer from the surface through the boundary layer to the free atmosphere (Penman 1948, Unsworth 1984). Although the air in clouds is generally accepted as being saturated or slightly supersaturated (Hudson 1980, Nicholls and Leighton 1986), subsaturated regions can exist near cloud boundaries or even deep within cloud layers, where there is a relatively low liquid water content, (Nicholls 1984, Nicholls and Leighton 1986). When the saturation deficit is close to but just above zero, the evaporation rate, being a linear function of saturation deficit, is strongly influenced by very small changes in that deficit (Lovett 1984, Unsworth and Crossley 1987a). Thus, both deposition and evaporation may take place at the same time, leading to situations where evaporation can reduce visible cloud droplet accumulation to zero (Lovett 1984).

However, far greater evaporation takes place after the cloud-base has risen above the vegetation (Figure 7.1). Available energy increases, saturation deficit increases and acidity enhancement by evaporation increases. Although there is a drop of about 0.95 pH unit for a 90% reduction in droplet volume, there are limits to increases in acidity. In some situations cation exchange will consume hydrogen ions, or weak acids already on the needle surface may buffer strong acids. pH is also slightly buffered around pH 2 because of protonation of sulphate (dissociation of H_2SO_4). Evaporation of hydrochloric acid will also occur at low pH although this is unimportant in solutions with low or negligible HCl concentrations. Eventually at very low pH, the water holding capacity of the concentrated droplet/crystal will be in equilibrium with the humid air and a stable pH is reached. The equilibrium relative humidity over sulphuric acid at pH 2.0 is 99-97%, a value much higher than to be expected after clouds have lifted, allowing pH to fall considerably below pH 2.0. The relevance of "final pH" is discussed later.

7.3 Methods

7.3.1 Rational

There were three main lines of research. Firstly, the pH of

Figure 7.1a 11.00 a.m.

Figure 7.1b 12.15 p.m.

Figure 7.1 Photographic sequence of evaporating droplets on Sitka spruce shoot at Dunslair Heights field site. Photographs were taken just after cloud lifted at 11.00 a.m. until the shoots were almost dry at 14.36 p.m..Conditions were: net radiation rising from 240-505 W m^{-2}, temperature rising from 10.5-16.5 °C, windspeed rising from 1.3-2.0 m s^{-1} and saturation deficit rising from 0.025-0.330 kPa.

Figure 7.1c 13.05 p.m.

Figure 7.1d 13.51 p.m.

Figure 7.1e 14.16 p.m.

Figure 7.1f 14.36 p.m.

droplets of simulated acid cloud (SAC) was measured in one of the four controlled experiments undertaken. Eighteen trees of both seven-year-old Scots pine and Sitka spruce were allocated to three treatments, each with two replicated blocks. Treatments were SAC at pH 5.6, 3.0 and 2.5 with a total of 27 episodes given. Droplets of SAC were collected from current year Sitka spruce and Scots pine shoots and also from sheets of parafilm placed in the upper branches of the trees, using a vacuum-pipette system. Sampling began immediately after spraying stopped and continued for up to 120 minutes. Thereafter, samples were measured for K then pH was measured using a combination microelectrode with a diameter tip of 4.5 mm (Russell Electrodes, Auchtermuchty, Fife). Further details of the experiment are given in Chapter 4.2.1. Secondly, in a controlled-environment wind tunnel the drop in pH of droplets placed on an inert artificial shoot and on live shoots was monitored. Thirdly, direct measurements of the pH of droplets evaporating in the field were taken.

7.3.2 Evaporation of individual droplets. (Pilot study (method and results)).

Young Scots pine trees (7-year-old) were placed in a laboratory without a controlled environment at a temperature around 22 °C, with variable humidity and no disturbance by wind. Using sterile gloves ten pairs of needles on each tree were covered with parafilm. A 0.03 μm^3 (30 μl) droplet of

SAC was dispensed onto these parafilm-covered needles using a finn-pipette (H4027E Labsystems Oy, Helsinki). Ten droplets were also dispensed onto needle pairs not covered with parafilm. Droplet pH was measured at 30 minute intervals in the order of dispensing (to avoid time differentials) using a pH micro-combination electrode with a tip diameter of 1.2 mm (Ml-410, Microelectrodes Inc., Londonderry, New Hampshire, U.S.A.) (Figure 7.2). A micro-manipulator was used to hold and position the electrode. The electrode had a response time of 5 to 15 seconds and was calibrated using dilute acid solution at pH 4.0 and pH 3.0.

The initial droplet pH of 3.0 was only very slightly altered by contact with Scots pine needle (Figure 7.3). The decrease in pH over time was very similar for both Scots pine and parafilm with a slightly smaller slope for Scots pine. The decrease over 120 minutes was from pH 2.995 (1011.6 µeq dm^{-3}) to 2.761 (1733.8 µeq dm^{-3}) for parafilm, a 71.4% increase in acidity, and for Scots pine from pH 2.986 (1032.7 µeq dm^{-3}) to 2.767 (1710.0 µeq dm^{-3}) a 65.6% increase in acidity.

7.3.3 Windtunnel experiment

Five 3-year-old Scots pine plants were placed in the working area of a windtunnel along with an artificial shoot. Both the artificial shoots and shoots from each pine were clamped to hold them steady. The experiments were repeated using

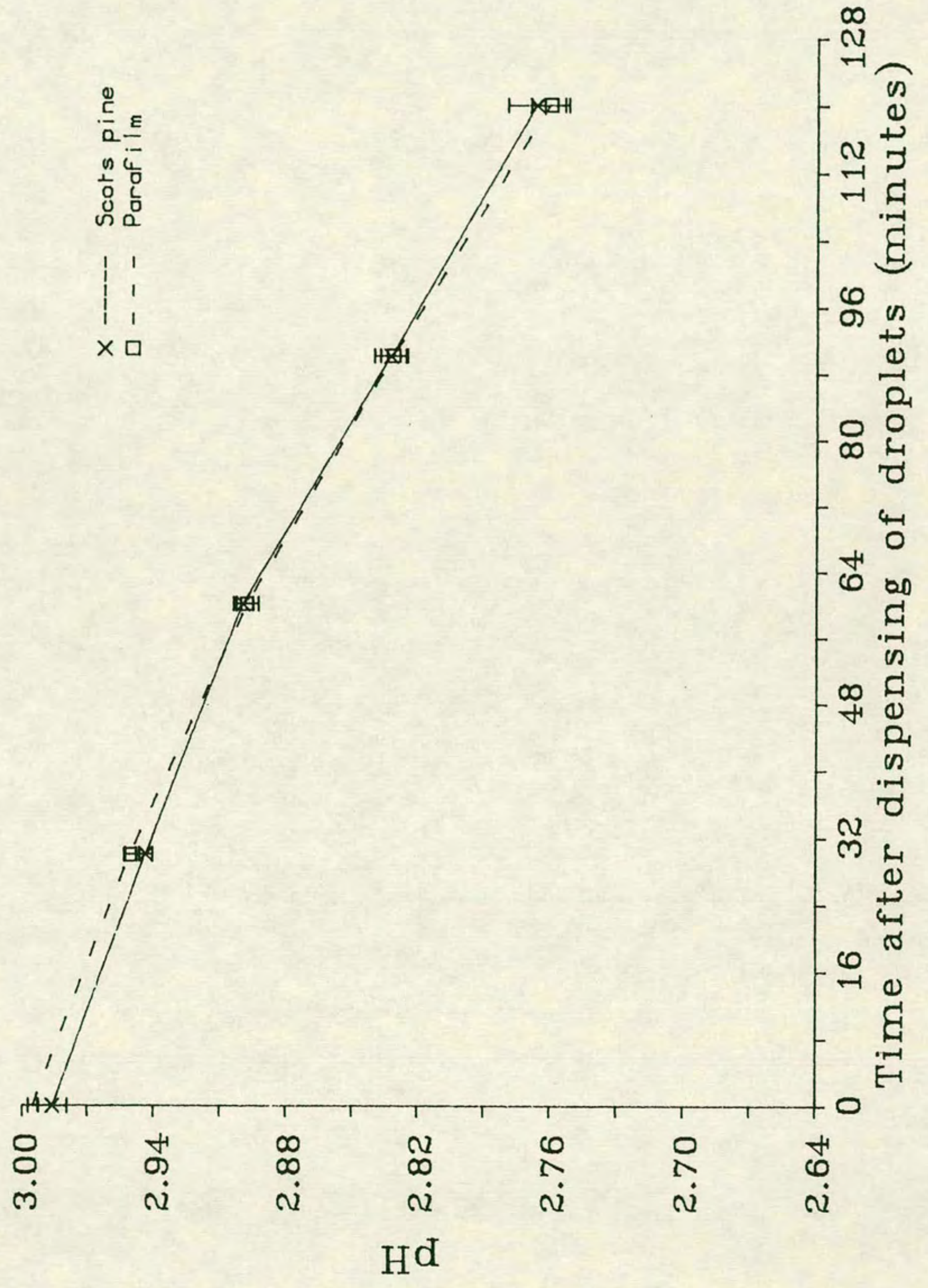
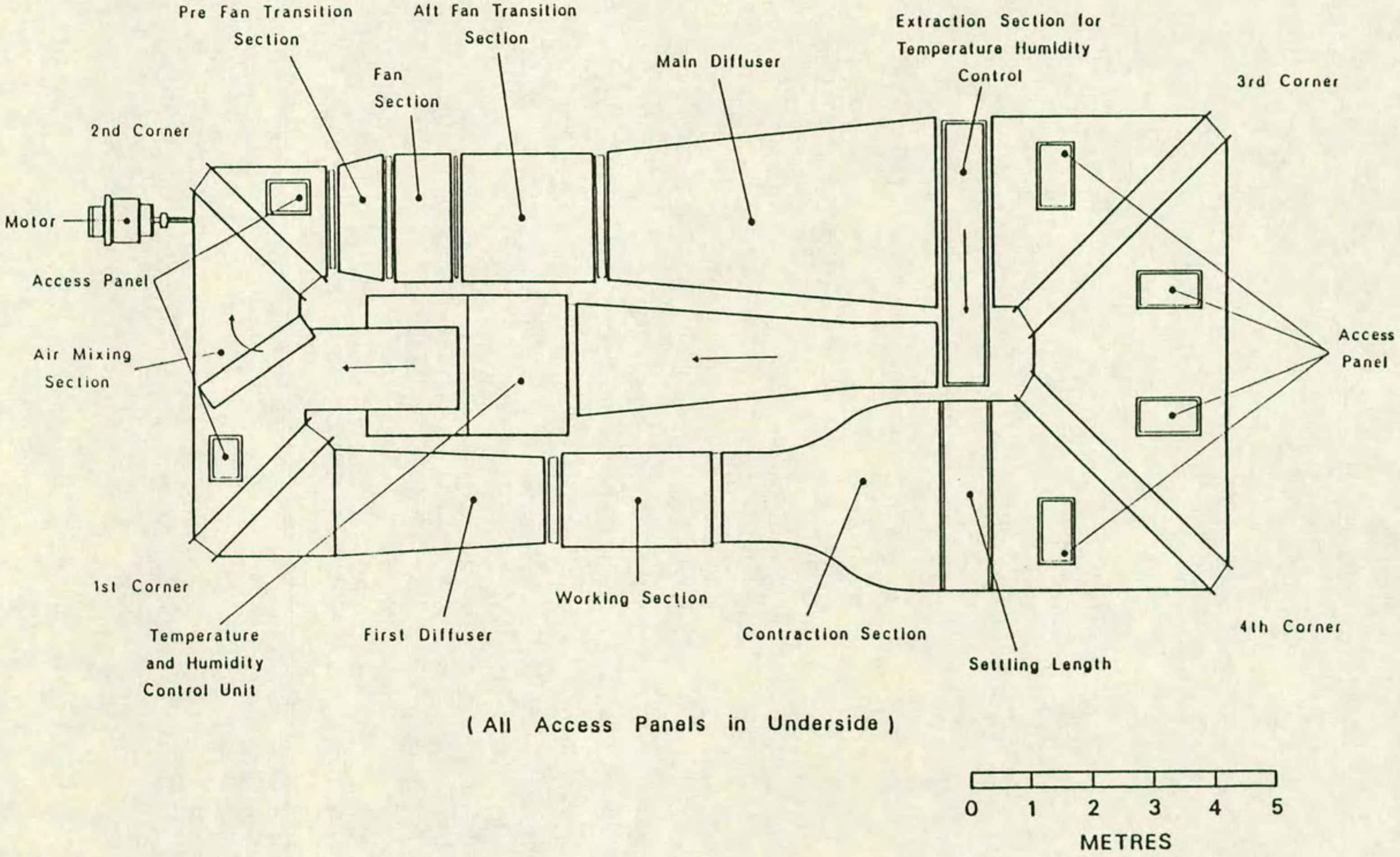


Figure 7.3 Mean pH (±S.E.) of ten droplets of simulated acid cloud placed on parafilm and non-parafilm covered Scots pine needles.

3-year-old Sitka spruce. The windtunnel was of the closed circuit low speed type with near laminar airflow. The working section was 2.5 m long 1.5 m wide and 1 m high (Figure 7.4). Temperature was controlled by four compressors which could be switched on in different combinations. The temperature sensor was calibrated using an electronic thermometer. Humidity was controlled by one humidifier and the relative humidity sensor was calibrated using a water bath and dew point hygrometer. Air circulation was generated by fan units and the resultant windspeed calibrated with a small cup anemometer placed close to the shoots of the trees. The working section was irradiated by 9 fluorescent metal-halide lamps and 6 tungsten bulbs(60 W). Irradiance at working height was 100 W m^{-2}. Lighting and windspeed were set by hand controls, and the temperature and vapour pressure were controlled by a microcomputer. During the experiment, temperature and humidity were measured at ten-minute intervals and means calculated for the duration of the experiment. Conditions in the windtunnel were allowed to stabilise for several hours before each experiment began.

Two 0.03 μm^3 (30 μl) droplets of SAC of pH 3.72 were dispensed onto the needle of one shoot from each of the five trees using the finn-pipette, giving a total of ten droplets. Ten droplets were also dispensed onto the artificial shoot needles. The time and position of each droplet was noted. pH was measured using the micro-combination electrode coupled to the micro-manipulator which allowed the tip of the electrode



(All Access Panels in Underside)

Figure 7.4 Schematic diagram of the windtunnel to be held in a precise and stable position. pH was measured for each droplet as soon as it was placed and thereafter at 20-30 minute intervals. The experiment was repeated nine times using a series of combinations of radiation, windspeed, humidity and temperature. Five of the repetitions were for Scots pine along with the artificial shoot, and four were for Sitka spruce along with the artificial shoot. The environmental values for the variables were chosen after scrutiny of the records of environmental conditions prevailing at the field site after clouds lifted, and involved windspeed at approximately 2 or 4 m s^{-1}, temperature at approximately 6 or 10 °C, vapour pressure deficit between 0.05 and 0.26 k Pa and irradiance on (100 W m^{-2}) or off (0 W m^{-2}). The actual final combinations ranged from an evaporative driving force of no lighting, windspeed 2 m s^{-1}, temperature 6 °C and saturation deficit 0.0525 k Pa to lights on, windspeed 4 m s^{-1}, temperature 10 °C and saturation deficit 0.2631 k Pa. The full range of conditions employed is shown in Table 7.1.

Table 7.1 Conditions in windtunnel during evaporation of droplets.

Date	Species	($100\ W\ m^{-2}$) On or Off	Windspeed ($m\ s^{-1}$)	Air Temperature (°C)	V.p.d. (k Pa)
7/3/88	Scots pine	On	3.9	9.8	0.2631
8/3/88	Scots pine	Off	2.0	6.1	0.0525
9/3/88	Scots pine	On	2.0	10.2	0.0859
10/3/88	Scots pine	Off	4.0	6.0	0.0556
11/3/88	Scots pine	Off	2.0	5.9	0.1639
6/4/88	Sitka spruce	Off	2.0	9.8	0.0728
7/4/88	Sitka spruce	On	2.0	10.9	0.1169
11/4/88	Sitka spruce	Off	4.0	6.4	0.0581
14/4/88	Sitka spruce	On	4.0	10.0	0.2441

V.p.d. = Vapour pressure (saturation) deficit

To establish the relationship between water loss and pH change two experimental runs were made in which droplets were weighed in a time sequence after dispensing, immediately after pH measurements had been taken. To establish accurately the initial weight of the droplets, ten droplets were weighed on an electric balance which was thereafter blotted dry with filter paper. Droplets were weighed both directly from the finn-pipette set at 0.03 μm^3 (30 µl) and also taken by micropipetting from shoots onto which droplets at the same setting had been pipetted.

The difference in weight between droplets placed straight onto the balance (36.68 µg) and those put onto shoots and then finn-pipetted from the shoots to the balance (34.91 µg) was 1.76 µg or less than 5%. It can safely be assumed that finn-pipetting from the shoots onto the balance does not result in the loss of significant amounts of liquid from the droplets.

After the initial weight had been established, ten droplets each were pipetted onto four artificial shoots and the time noted. At time intervals ranging from 0 to 174 minutes, droplets were measured for pH and then taken by the finn-pipette from the shoots and weighed. Windtunnel conditions were: windspeed 2.0 m s^{-1}, air temperature 10.0 °C and v.p.d. 0.2271 k Pa.

On the second run the same procedure was followed except that forty droplets were placed on live Sitka spruce shoots. Conditions were: windspeed 2.0 m s^{-1}, temperature 6.1 °C and saturation deficit 0.1733 k Pa. Since in these experiments it was not the rate of pH decrease that was being measured but its relationship to droplet evaporation, the differences in conditions were irrelevant.

7.3.4 Field experiment

The field site was situated at Dunslair Heights, Glentress Forest in the Moorfoot Hills at a height of 602 m a.s.l. Fifteen 8-year-old Scots pine were transferred from Bush Estate to the field site and sunk into the ground in close proximity to the 23-year-old Sitka spruce that were growing there. Full details of the field site are given in Chapter 5.2.1.

On five occasions when windspeeds were low enough not to dislodge droplets, the pH of evaporating droplets on Sitka spruce in the field was measured after the cloud event had lifted. The micro-electrode and micro-manipulator assembly was set up on an adjustable table adjacent to the trees. A current year shoot was clamped and ten droplets from the shoot measured for pH. After an interval of around 15-30 minutes the process was repeated with the same tree so as to avoid complications resulting from differing tree responses. This was repeated until almost all the droplets on the tree had dried. Different Sitka spruce trees were used on the five different occasions. Environmental conditions during the evaporation were obtained from the five minute meteorological records of the site conditions.

7.4 RESULTS

7.4.1 Controlled experiment

At pH 5.6, Figure 4.12 shows no evidence of pH decreasing with time. At pH 3.0 and pH 2.5, however, a downward trend of pH with time is evident.

When results are tabulated separately for each day of spraying samples taken at two or more different times on the same day can be compared (Appendix 6). This reduces the amount of noise resulting from different drying conditions between days. From these tables it can be seen that for parafilm at pH 5.6 there is little evidence of decreasing pH with time and that the picture at pH 3.0 is unclear. At pH 2.5 however, pH decreases steadily with time, reaching as low as pH 2.0 after 90 minutes.

At pH 5.6 both Scots pine and Sitka spruce samples vary in their response: on some days there was a decrease, whereas on other days many samples were still at or above pH 5.6 60-120 minutes after spraying ceased. At pH 3.0 a steady downward trend becomes apparent. Values reached pH 2.53 (2951.2 μeq dm^{-3}) after 110 minutes for Sitka spruce (an acidity increase of 195%) and pH 2.42 (3801.9 μeq dm^{-3}) for Scots pine (an acidity increase of 280%).

At pH 2.5, there was again a clear decrease in pH although for Sitka spruce initial low values sometimes cut across this. With Scots pine the daily values provide strong evidence for a decreasing pH with time.

7.4.2 Windtunnel experiment: weight loss and acidity increase.

Twenty-seven out of the forty droplets placed on artificial shoots were reweighed. As the droplets dried the increase in acidity was dramatic. The results followed the predicted line closely, with a slight departure towards lower acidity at high weight loss (Figure 7.5). The results for Sitka spruce show a similar pattern, with a slightly higher initial pH (Figure 7.6).

7.4.3 Acidity increase over time

The initial pH of droplets evaporating on artificial shoots was identical to the solution pH . This steadily decreased to a pH dependent on how long the droplets remained on the needles before falling off as a result of movement caused by the wind. Final pH values were as low as pH 3.21 - an increase in acidity of 231% (Figure 7.7). The rate of increase in acidity increased as vapour saturation deficit and windspeed increased and also depended to a lesser extent on irradiation. Differences between the fall in pH on 9/3/88 (v.p.d. 0.089 k Pa, wind 2.01 m s^{-1}, lights on) and the fall on 7/4/88 (v.p.d. 0.117 k Pa, wind 2.01 m s^{-1}, lights on) illustrate the increasing rate of acidity enhancement as v.p.d. is increased. This is also seen in the difference between 14/4/88 (v.p.d. 0.224 k Pa, wind 3.95 m s^{-1}, lights on) and 7/3/88 (v.p.d. 0.263 k Pa, wind 3.94 m s^{-1}, lights on). The difference in the rate of pH decrease between 6/4/88 (v.p.d, 0.073 k Pa, wind 2.02 m s^{-1}, lights off) and 11/4/88 (v.p.d. 0.058 k Pa, wind 4.02 m s^{-1}, lights off) shows how an increase in windspeed from 2.02 m s^{-1} to 4.02 m s^{-1} significantly increased acidity enhancement despite a decrease in v.p.d. Irradiation did not have such a strong effect on the rate of pH decrease as v.p.d. and windspeed.

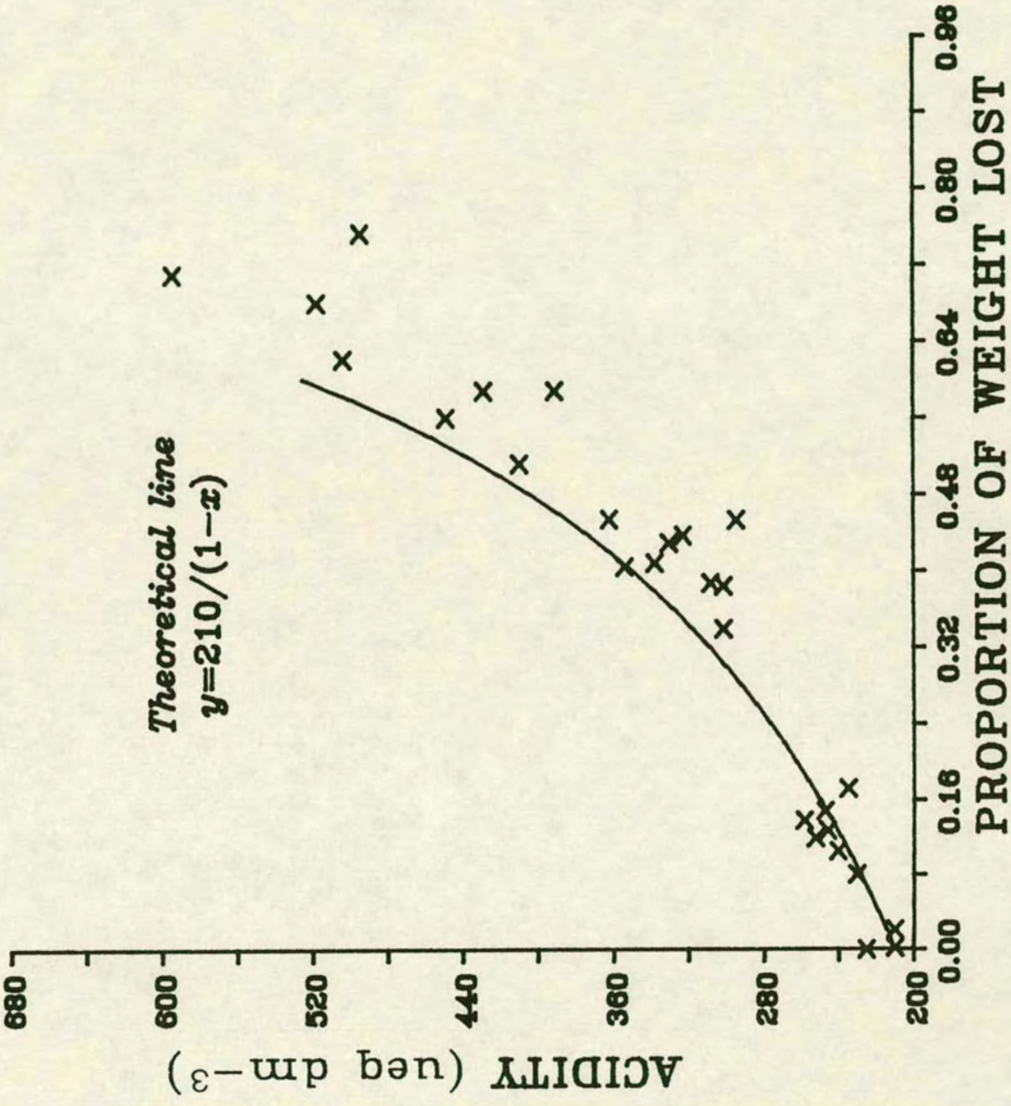


Figure 7.5 Increasing acidity with evaporation of droplets on artificial shoots.

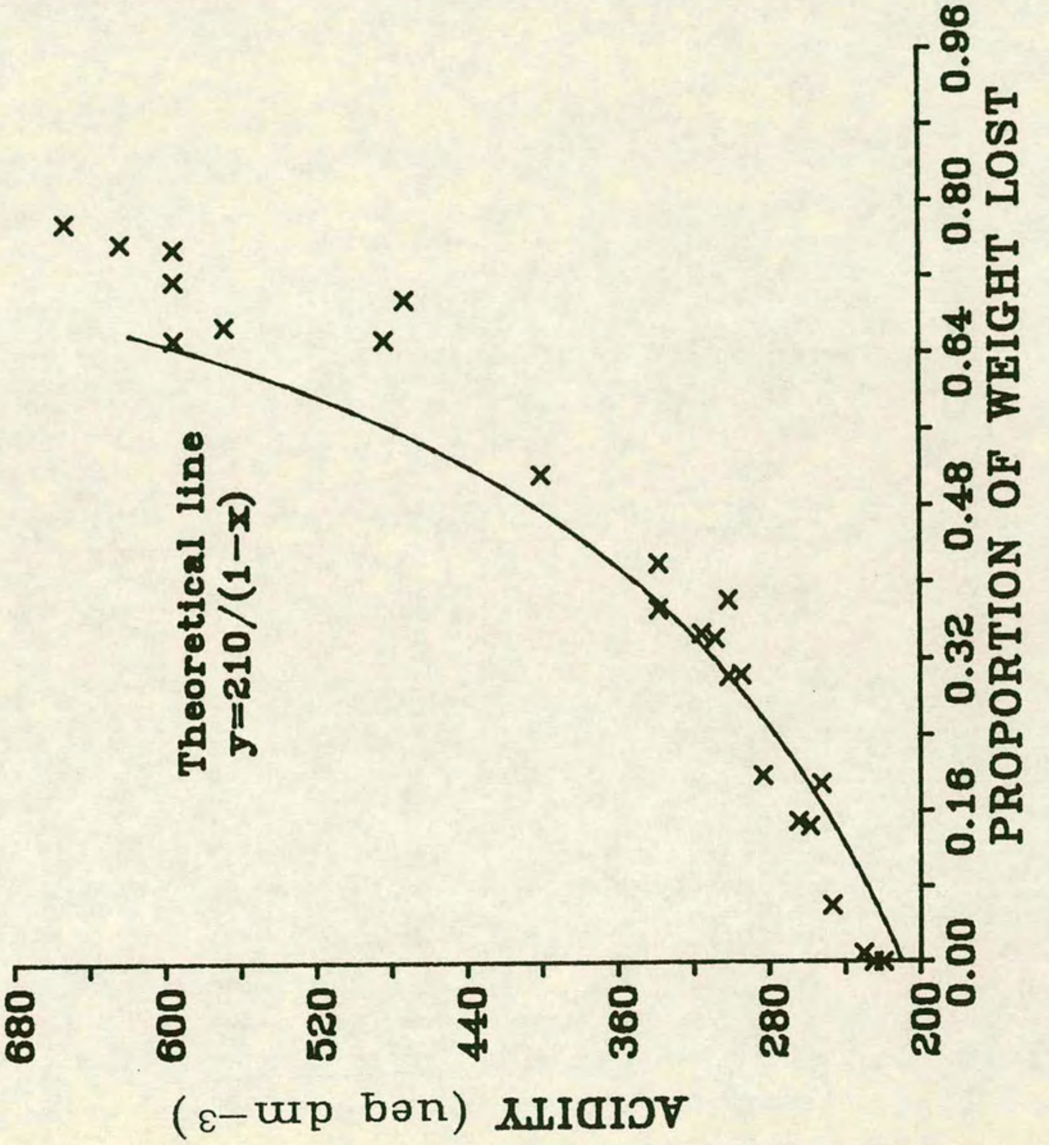


Figure 7.6 Increasing acidity with evaporation of droplets on Sitka spruce.

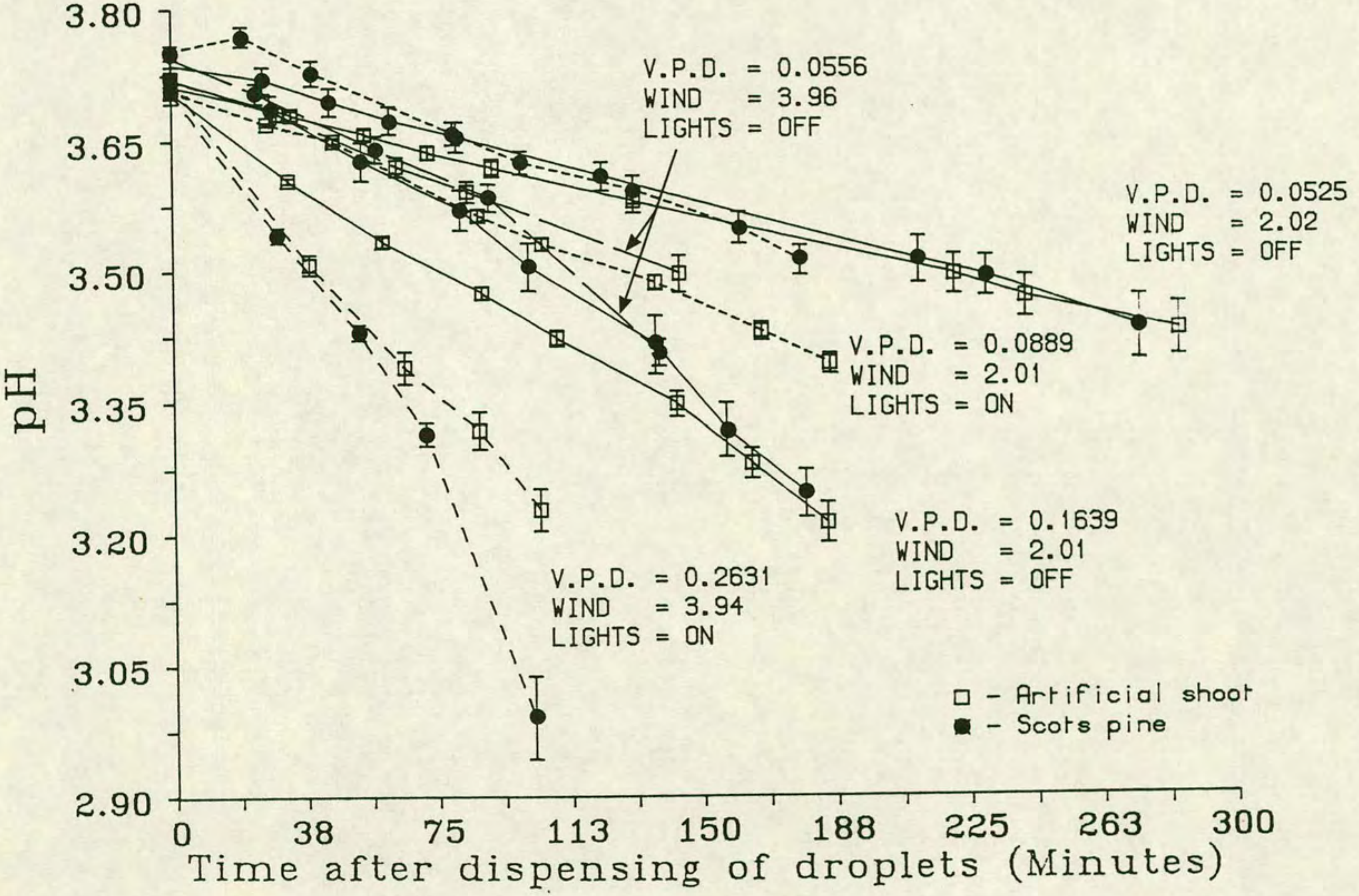


Figure 7.7 Mean pH (±S.E.) of ten droplets of SAC on artificial shoots and Scots pine shoots.

The rate of evaporation, and thus the decrease in droplet size and increase in acidity, can be described by the Penman equation. If the term for radiation is regarded as negligible and left out, pH is seen to decrease steadily with increasing cumulative evaporative flux calculated from

$$\lambda E = \frac{C_p \rho D g_a}{s + \gamma} \qquad \text{with} \quad g_a = f(u) \simeq u$$

where λE is evaporative flux, C_p is specific heat of air, ρ is density of air, D is vapour pressure deficit, u is windspeed, s is change of water vapour saturation pressure per °C and γ is the psychrometric constant (Figure 7.8). When the lights were off, the drop in pH was linear and clearly governed by the evaporation rate. Two patterns for the pH drop when lights were on appear. At low evaporation rates the irradiation had a small effect slightly increasing the rate at which pH dropped (data points to the left of the crosses) but at high evaporation rates the drop in pH appears to be much slower than expected (data points to the right of the crosses).

In comparison with those on artificial shoots, droplets on Scots pine shoots followed a similar pattern with some notable deviations. On the pine shoots at low windspeeds pH either started slightly higher or quickly increased relative to the artificial shoots (Figure 7.7). Thereafter acidity enhancement followed similar lines. However, when windspeed was high pH levels started similarly but then fell much more

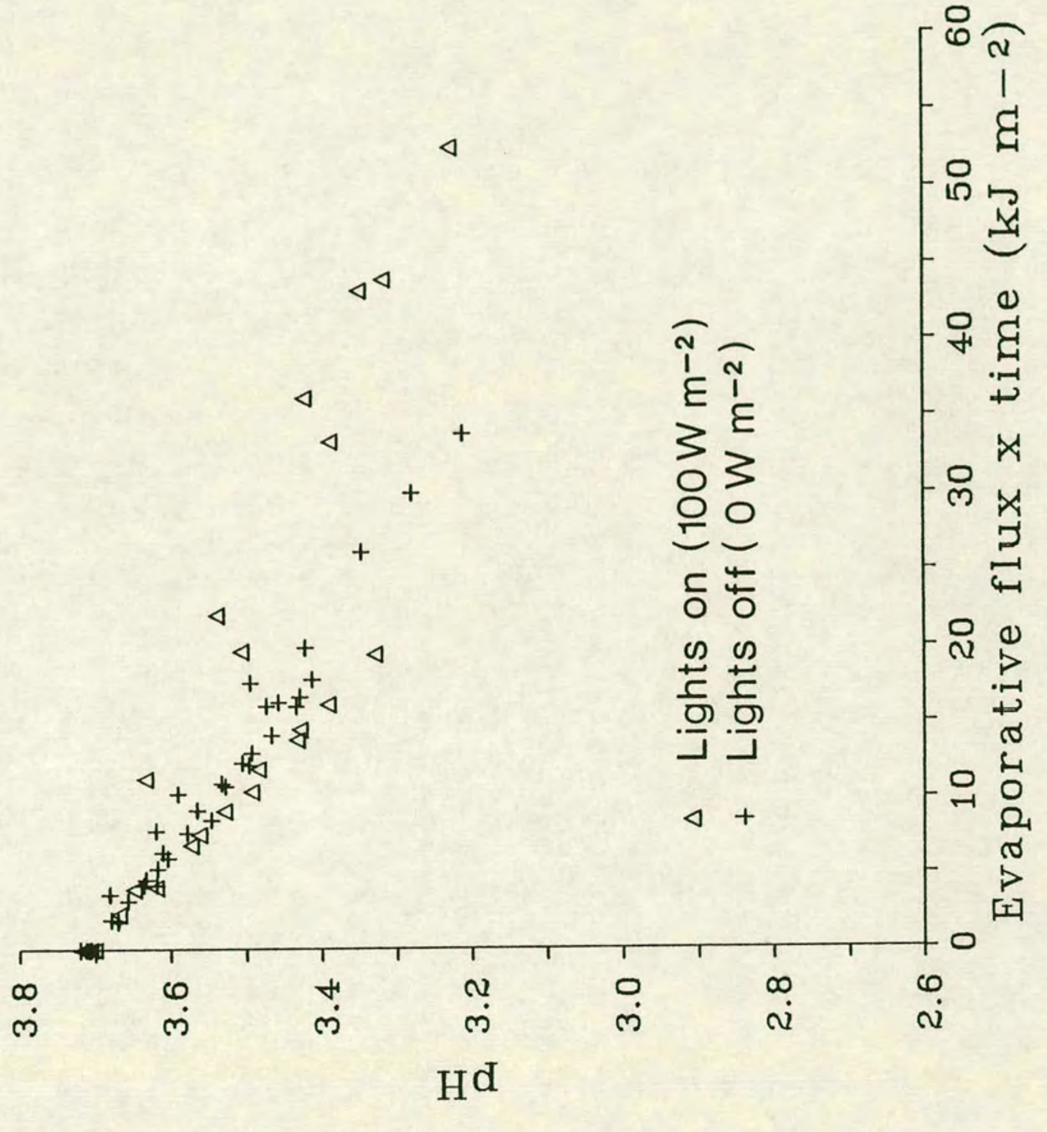


Figure 7.8 Cumulative effect of evaporation on the decrease of pH of droplets on artificial shoots.

quickly on the Scots pine shoots. In some cases this led to extremely rapid acidity enhancement and an increase in acidity of 437% (190.5 μeq dm^{-3} to 1023.3 μeq dm^{-3}) in just over 100 minutes.

On Sitka spruce the opposite happened, droplets on live shoots started at a lower pH but then acidity enhancement proceeded parallel to that in artificial shoots (Figure 7.9). There was no difference in enhancement patterns due to windspeed. Acidity enhancement was again rapid with an increase (204.2 μeq dm^{-3} to 501.2 μeq dm^{-3}) of 145% in 96 minutes. An example of each pattern (Scots pine with low windspeed, Scots pine with high windspeed and Sitka spruce) are shown together in Figure 7.10 to give a clear picture of the three different responses.

7.4.4 Field experiment

There were strong clear increases in acidity after clouds lifted (Figure 7.11). These were directly related to environmental conditions. Because of the necessity for low windspeeds in this experiment, net radiation as well as saturation deficit (v.p.d.) particularly affected enhancement rates. On two occasions, net radiation and saturation deficit were low and similar low enhancement rates were observed. When net radiation and saturation deficit were moderate to high, the rate of enhancement increased, and when both net

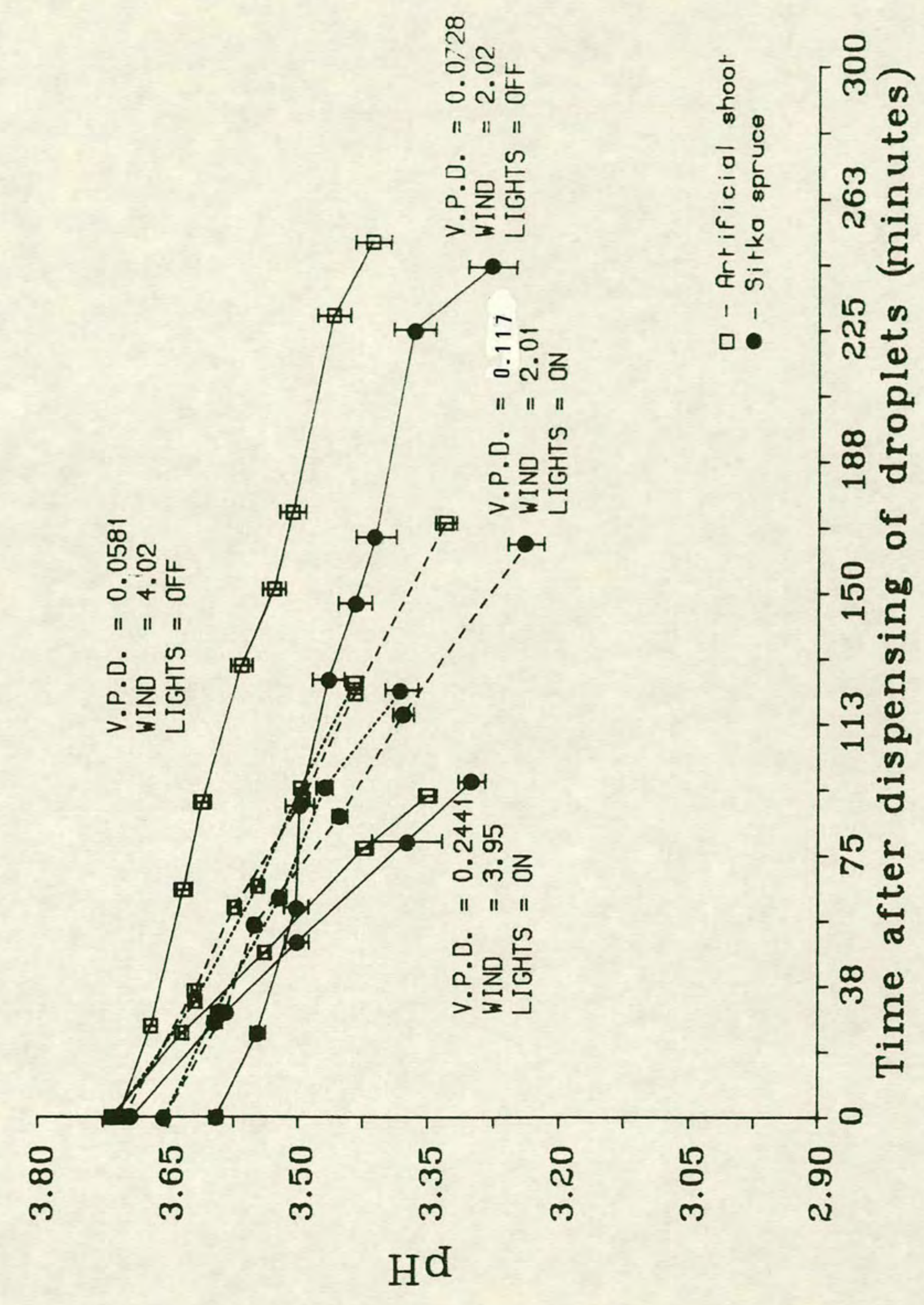


Figure 7.9 Mean pH (±S.E.) of ten droplets of SAC on artificial shoots and Sitka spruce shoots. There were three pairs of shoots, identified by full, broken or dotted lines.

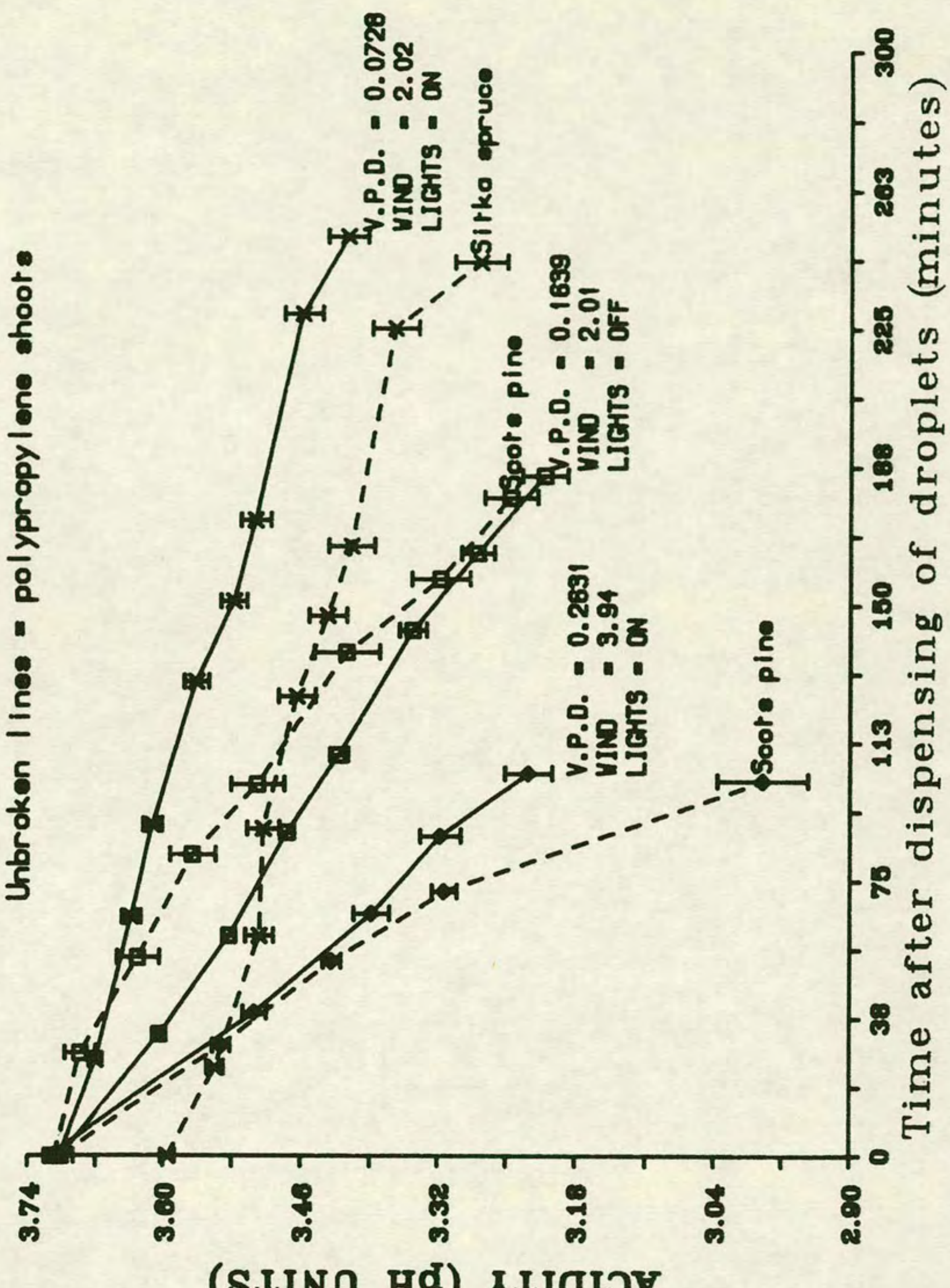


Figure 7.10 Comparison of the increase in acidity (fall in pH) of ten droplets on Scots pine shoots at 2 m s-2 and 4 m s-2 and on Sitka spruce shoots (2 m s-2).

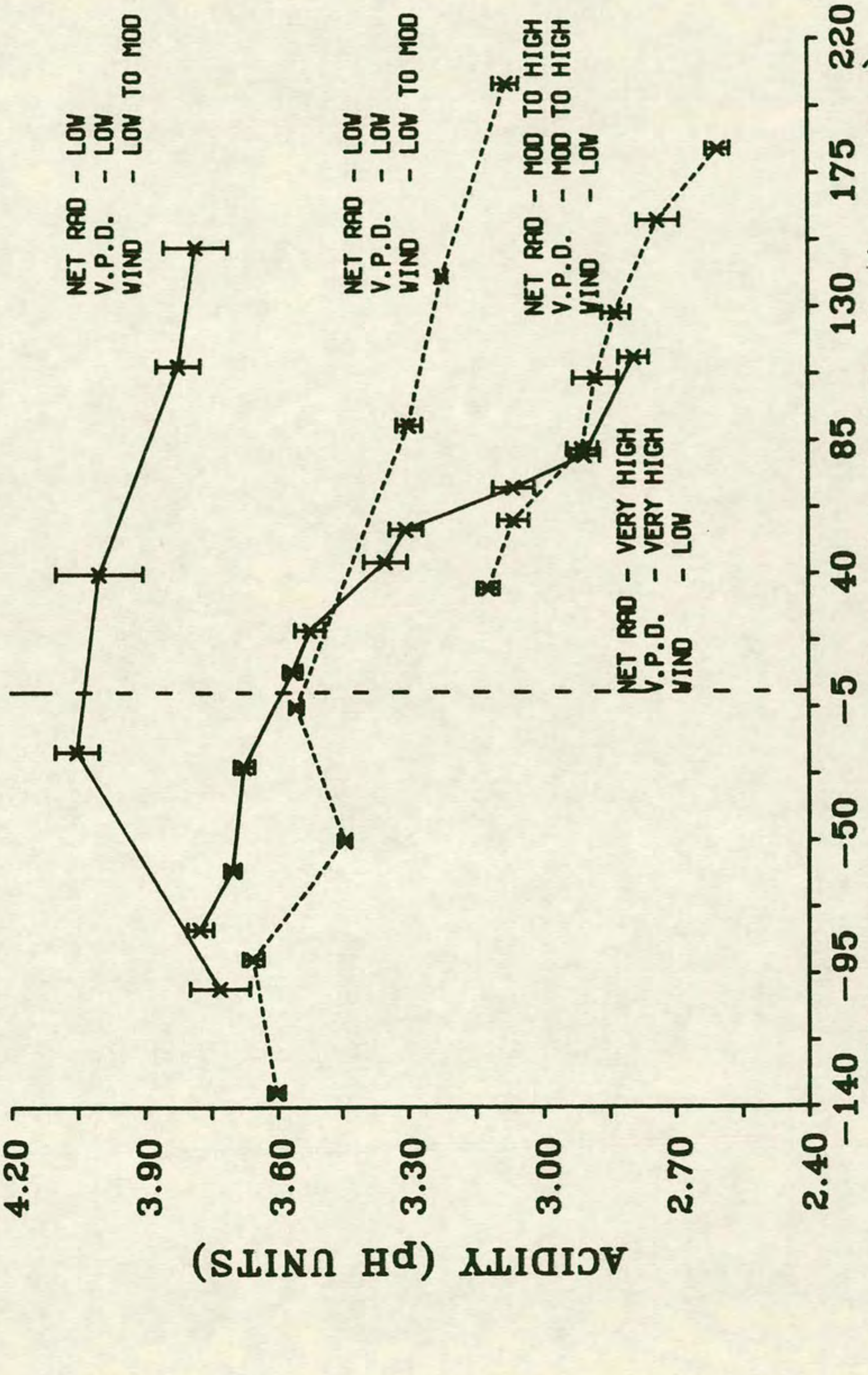


Figure 7.11 Fall in pH of impacted cloudwater on Sitka spruce shoots at Dunslair Heights field site. Error bars are ±S.E. of ten droplets.

radiation and saturation deficit were very high, even though windspeed was low, there was an extremely high rate of acidity enhancement, with an increase of more than 600% in acidity in 106 minutes (pH 3.564 to 2.792).

7.5 Discussion

In the controlled glasshouse experiment 4, because of different drying regimes on different days, is was necessary to look at results for individual days to observe trends. These results indicated that pH decreased over time after an SAC episode.

Acidity increase is governed by water loss and therefore the faster the water loss the faster the acidity increase. The rate of water loss is described by the Penman equation. If net radiation is low, a high saturation deficit and moderate wind speed can still drive evaporation, allowing acidity enhancement to take place at night. However, during the day, when net radiation and saturation deficit are both high, evaporation is more rapid and the fall in pH quicker. The increase of more than 600% in acidity in 106 minutes in the field experiment is a clear indication that evaporating droplets on conifer needles are not buffered by leached cations to an extent that acidity enhancement is neutralized.

It was seen that acidity increased as the droplet lost water on artificial shoots (Figure 7.5) in a manner very close to that predicted by an experimental curve describing acidity increase as directly proportional to water loss. Thus, as the droplet decreased in size, the rate of increase in acidity increases, so that droplets which were too small to be measured by the micro-electrode may have had extremely low pH values. The same is true for Sitka spruce (Figure 7.6). Droplets in the latter stages of drying are therefore likely to be exposing conifer needles to extremely high acidity levels.

Figure 7.8 shows that radiation affected the increase in acidity (drop in pH) only very slightly. It was also seen that where saturation deficit and windspeed were both high and the lights were on the fall in pH appeared more gradual. This is likely to be the result of an overestimate of the evaporative flux from rapidly evaporating droplets as the ratio $\frac{S}{C}$ (where S is the actual amount of water on the shoot and C is the steady state canopy storage capacity) is reduced quickly. As this ratio decreases, the evaporation rate decreases and thus at extremely high evaporation rates the fast removal of liquid from the needles is likely to reduce S and subsequently the ratio $\frac{S}{C}$ and ultimately the evaporation rate.

It could be argued that all droplets will eventually dry and reach extremely low pH levels and consequently the <u>rate</u> of evaporation would be insignificant and final pH would be determined by sulphate protonation, HCl volatalization and droplet/humid air equilibrium. However, a number of factors could affect the inevitability of such a final pH. Neutralization by weak acids or cation exchange would consume hydrogen ions and work against a fall in pH over time. Results summarised in Figure 7.10 showed that there was no evidence of neutralization by Sitka spruce: on the contrary, droplet pH was initially depressed and remained substantially lower than for artificial shoots.

There was some evidence for neutralization by Scots pine. This is in accordance with the results from the previous chapter which pointed to some cation exchange, possibly $K^+ - H^+$ as a neutralizing factor. At the higher windspeed the droplet shape was easily distorted because of the movement of Scots pine needles on the shoots. This increased the surface/volume ratios and evaporation rates compared with shoots in calmer conditions or with the less flexible artificial shoots. The decrease in pH of droplets on live shoots at low windspeeds to levels similar to those on artificial shoots towards the end of the run may arise from droplet spreading resulting in increased evaporation rates or by lack of sustained neutralization of H^+ ions. Droplets evaporating on Scots pine shoots in calm conditions with low evaporative driving force may therefore be buffered to some extent against increasing acidity, although such buffering is still not strong enough to halt a sharp decline in pH.

Such a lack of buffering is contrary to that found in many herbaceous and broadleaved species such as Helianthus annuus, Raphanus sativus, Brassica oleracea (cabbage, broccoli and brussel sprouts), Beta vulgaris, Phaseolus vulgaris, Lycopersicum escultanum, Cornus canadensis, Betula papyrifera, Gossypium hirsutum and Coffea arabica all of which considerably raised the pH of drying droplets (Oertli et al. 1977, Hutchinson et al. 1986). Hutchinson et al (op cit) did however find that leaves of the brassicas; cabbage, broccoli, kohlrabi and brussel sprout had a poorer neutralizing ability than most other species and attributed this to high contact angles and low retention of droplets caused by the extremely hydrophobic waxy surfaces. Oertli et al. (op cit) found that droplets drying on citrus fruit leaves exhibited substantially reduced pH values. Klemm et al. (1987) found no buffering action of Hedera helix (ivy) on evaporating droplets. Klemm (1989) also found no evidence for buffering of evaporating droplets by green Norway spruce needles, although brown needles did display some degree of buffering.

Although internal pH may have some bearing on the neutralizing ability of citrus fruit leaves, a hydrophobic waxy surface is often effective in prohibiting the neutralization of evaporating droplets. Thus Sitka spruce and Scots pine are unable to prevent a sharp increase in acidity through enhancement by evaporation. Field results proved conclusively that acidity enhancement by evaporation occurs under natural conditions and both these results and those from glasshouse experiments pose questions about the thresholds actually responsible for the observed damage. Conversely, if plants are susceptible to damage at a particular pH, cloud or rain pH will not in itself indicate how often the plant will experience potentially damaging acidity levels. The possibility of damage due to lowered pH through evaporation must be taken into account in assessing damage potential to non-neutralizing species such as Scots pine and Sitka spruce. As over 35% of bulk cloud pH measurements over a year (1987-1988) at Dunslair heights were less than pH 3.72, similar pHs to those measured in the windtunnel experiments are likely to occur frequently. The previous chapter also indicated that certain cloud episodes have much lower pH values than bulk cloud collections and acidity enhancement after such events would increase the probability of threshold values being reached.

CHAPTER 8

The release of ions in snowmelt from fresh snow and from snowpack on spruce shoots

8.1 Introduction

Some ions, including hydrogen ions, are preferentially eluted from snowpack during the early stages of snow melt because of their non-homogenous distribution in snow flakes (Overrein et al. 1980, Davies 1989). The sudden release of acids fron snowpack, particularly during the early stage of snow melt, may lead to the acidification of waterways (Johannessen and Henriksen 1977, Overrein et al. 1980, Cadle et al. 1984, Galloway et al. 1987). Little or no work has been done to discover whether melting snow from snowpacks on conifer shoots also exhibits a "flush" of ions at the start of snow melt. As H^+ is one of the ions preferentially eluted (Tranter et al. 1987), there is a possibility that in the early stages of snow melt high levels of acidity are in contact with conifer shoots.

An experiment was therefore set up in which droplets were collected chronologically from snow melting on Sitka spruce shoots. Time series samples were also collected from fresh snow melting in a funnel. Both sets of samples were then analysed for a variety of ions to ascertain whether such a "flush" of ions, particularly H^+, occurred.

8.2 Method

8.2.1 Collection of snowmelt from shoots.

Snowmelt was collected from the current year shoots of 25-year-old Sitka spruce at Dunslair Heights (602m asl). Full details of the field site are given in Chapter 5.2.1.

Snowmelt from conifer shoots drips by two different pathways. Firstly, melting snow may pass through the snow pack on the shoot and drip from the tip of the snow pack. Secondly, meltwater may take a shorter pathway and drip from needles projecting out from underneath the snow pack (Figure 8.1).

Snowmelt droplets from snowpack on Sitka spruce were collected on two occasions. On the first occasion (25/11/87) droplets from both pathways were not discriminated. Melting droplets from a large number of Sitka spruce were collected in a hand held test-tube. This process was repeated nine times over a period of four hours (11.12 - 15.05 h) during which time samples from the earliest drops and the last drops of snowmelt were collected. Approximately 4 cm^3 were collected for each sample. On the second occasion droplets were collected separately from the two pathways. Again, hand held test-tubes were used and meltwater droplets from a large number of shoots collected to make a sample of approximately 4 cm^3. Sampling began when the first droplets of snowmelt appeared at 11.40 h

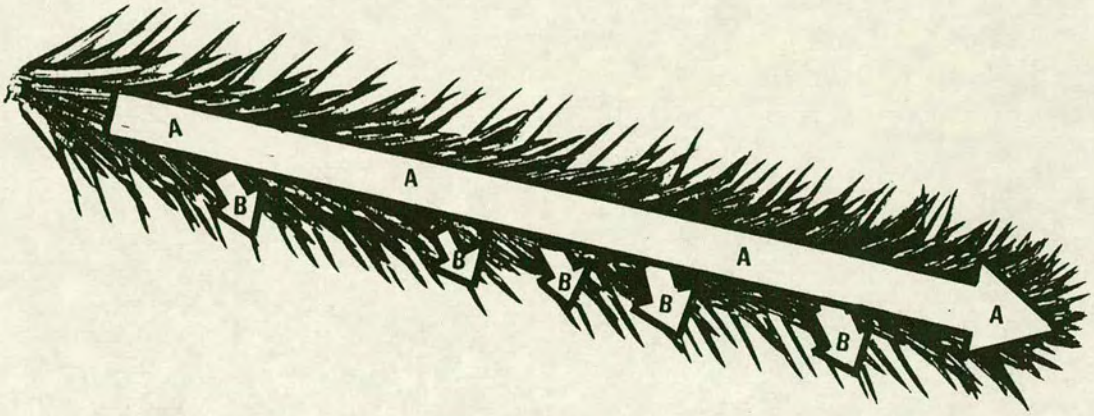


Figure 8.1 The two principal pathways of snowmelt on Sitka spruce shoots. a). Droplets travel through snowpack on shoots and drip from shoot tip. b). Droplets run down and drip from individual needles.

on the 29th January 1988. Sixteen samples of snowmelt dripping from needles (pathway B - Fig. 8.1) and seven samples of snowmelt dripping from the end of the snowpack on the spruce shoot (pathway A - Fig. 8.1) were collected over a period of almost four hours. Samples are thus not replicated over time because of the length of time needed to collect each sample but represent the pooled snowmelt from a large number of shoots on different trees.

8.2.2 Snowmelt in funnels.

Pyrex funnels were washed in "Decon", rinsed thoroughly in hot water then deionized water, and stored in sealed polythene bags. Freshly fallen snow from the ground adjacent to spruce trees at the field site was collected in these funnels. Funnels were then clamped above a rack of test tubes and melt droplets were collected chronologically in 5 cm^3 portions. Melting took place in a small radio station near to the site and took several hours. Three funnels were used on 6/1/88 and 14/1/88 with an average of 22 samples collected from each funnel. On 21/1/88 five funnels were used with an average of 11 samples from each funnel and on 29/1/88 two funnels were used with an average of 22 samples each. Once again, because samples were in a time series, they could not be replicated; but the large number of samples and short time space between each sample gave a good series of results from which to draw conclusions.

It was later felt that snowmelt from shoots would be more effectively simulated by using a small low voltage heating element placed in the centre of the snow to mimic the dark body of a shoot. Therefore, on two occasions a perspex cylinder 21cm long and 7.5cm diameter with a polypropylene mesh base, cleaned in the same way as the funnels, was filled with freshly fallen snow. The heating element which consisted of a small electric coil inside a test tube (which had been similarly cleaned), was connected to a power source in the hut and inserted into the centre of the snow in the cylinder. These experiments were conducted outside and melting again took several hours. Three cylinders were used on both these occasions (14/3/88 and 17/3/88) and, on average, 33 samples were collected from each cylinder.

Samples from spruce shoots were measured for pH only and samples from all funnel/cylinder experiments were analysed for K by flame emission, pH using a microelectrode (Russell Electrodes Ltd., Auchtermuchty), Mg and Ca by atomic absorption, and NO_3^- - N using automated calometric procedures. Full details of these analytical techniques are given in Appendix 4.

8.3 Results

8.3.1 Release of ions in snowmelt from funnels.

Snowmelt collected from funnels without a heating element showed an initial flush of ions followed by a steep decline to very low concentrations (Figure 8.2). Often the initial flush was preceded by a small amount of meltwater of lower ion concentrations. This general pattern was true for all ions studied (H^+, Ca^{2+}, Mg^{2+}, K^+ and NO_3^--N) although NO_3^--N in particular and also H^+, K^+ and Mg^{2+} showed the least scatter and smoothest decline in concentration.

Very similar results were obtained when the heating element was used. Initially, samples had slightly lower ion concentrations rising quickly to a peak and then declining to very low concentrations (Figure 8.3). It can be seen from Figure 8.3 that peak concentrations were up to one hundred times higher than those in the last samples taken.

8.3.2 Release of ions in snowmelt from shoots

Meltwater ions from the Sitka spruce shoots did not follow an obvious flush and decline (Figure 8.4). Meltwater dripping from the end of snowpacks on shoots (pathway A) was much more acid than that dripping from individual needles protruding from the snowpack (pathway B). Meltwater from pathway A also showed some signs of a decrease in H^+ concentration with time although this was neither clear nor strong.

Samples from both pathways had extremely high H^+ concentrations compared with the H^+ concentration in snow collected on the ground at the same time (18.4 μeq dm^{-3}).

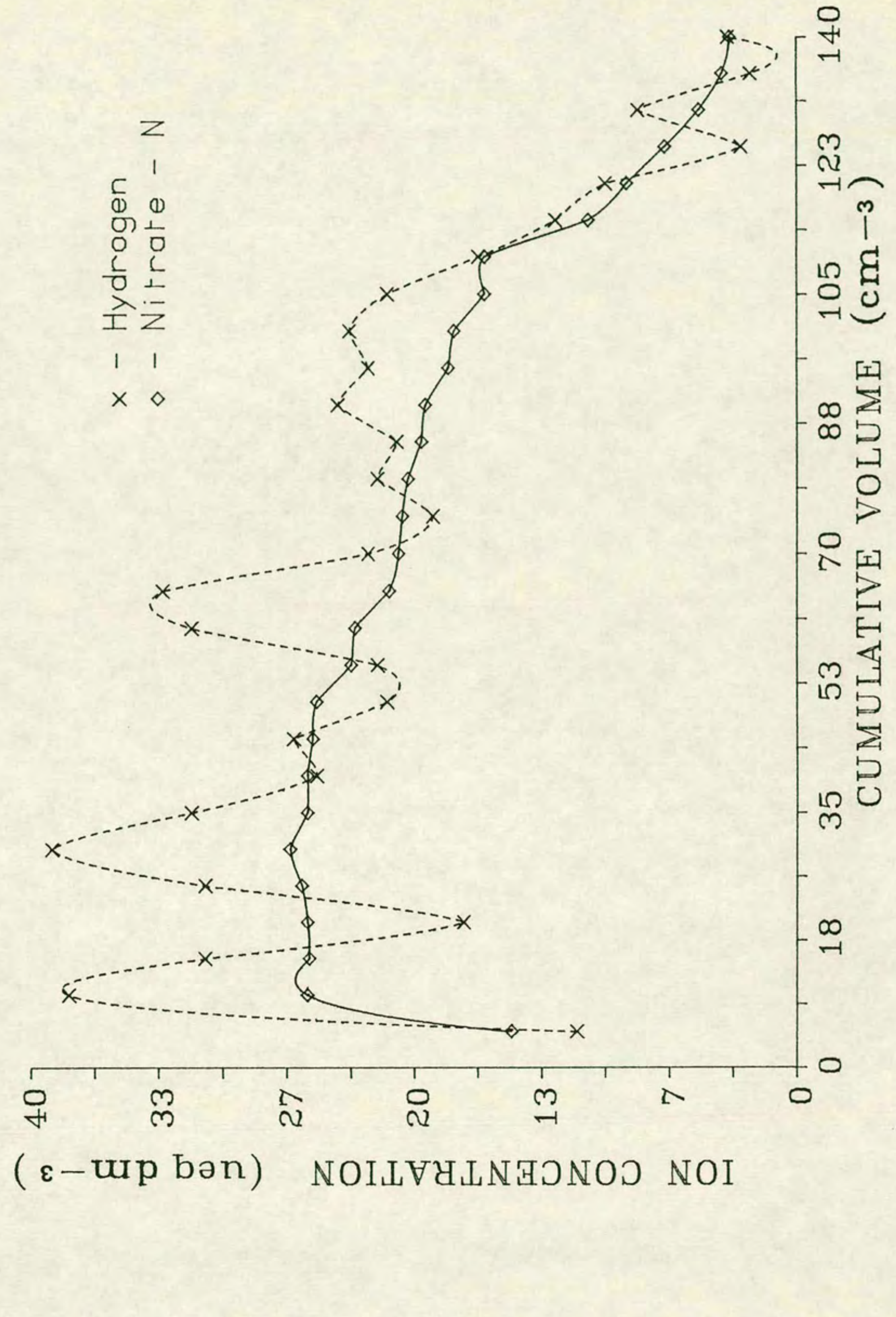


Figure 8.2 Release of hydrogen and nitrate-N ions in snowmelt from a funnel.

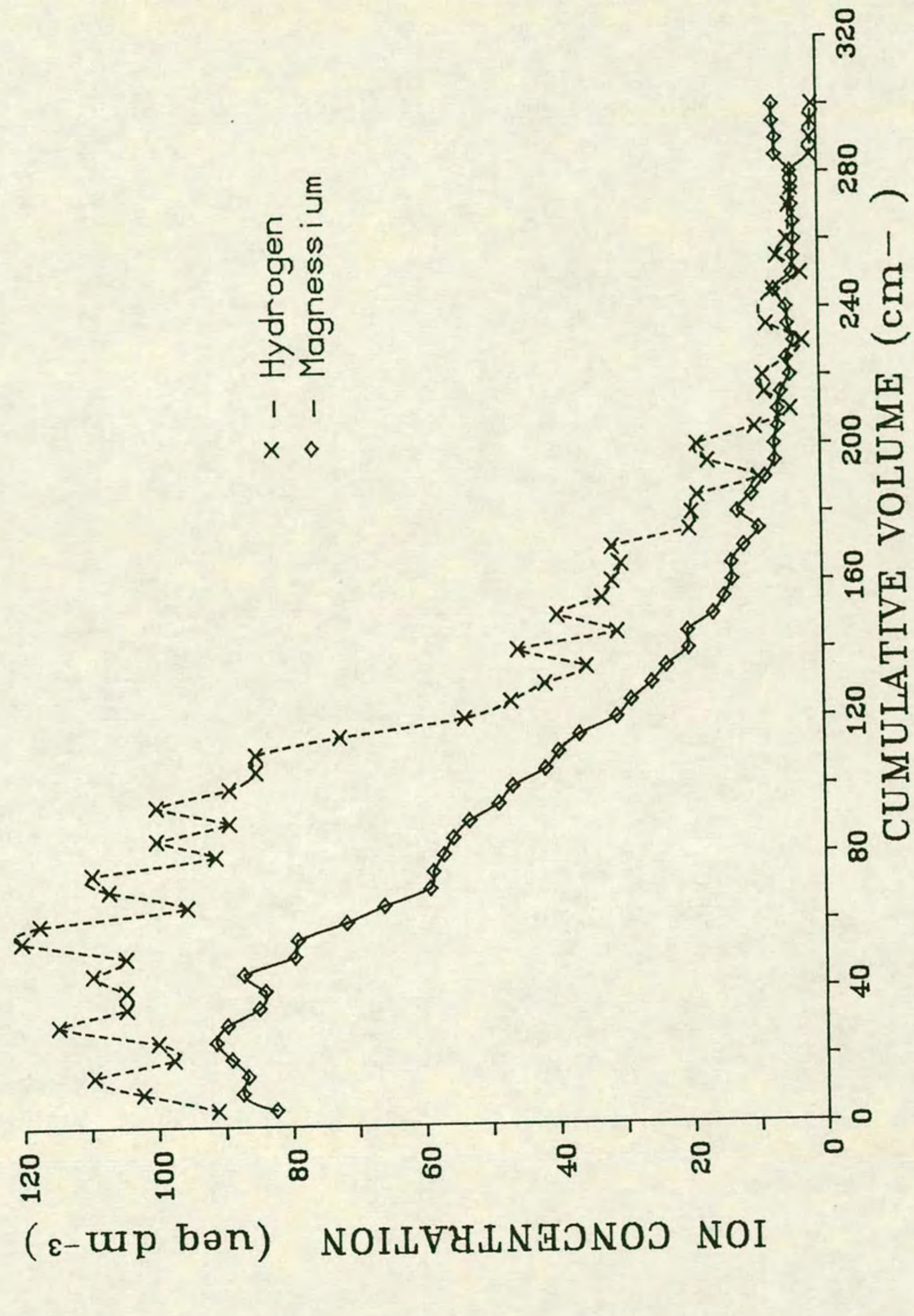


Figure 8.3 Release of hydrogen and magnesium ions in snowmelt from a cylinder with heated element inserted into snow to simulate the dark body of the shoot.

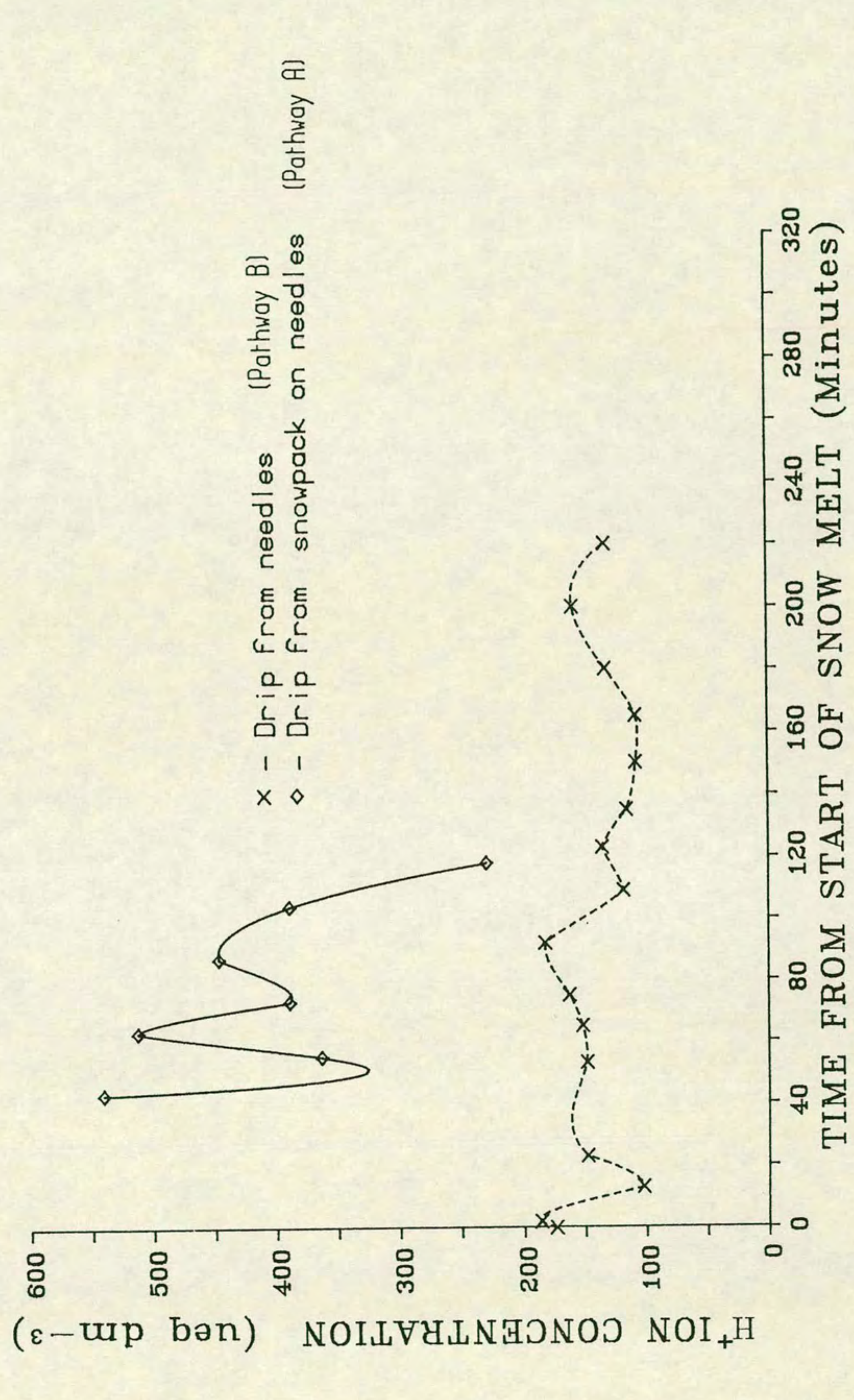


Figure 8.4 The difference in hydrogen ion concentrations in samples of snowmelt dripping from different pathways on Sitka spruce shoots.

This represents up to a 10-fold increase for some samples in pathway B and a 30-fold increase at peak concentrations for pathway A.

8.4 Discussion

Snowflakes are efficient scavengers, particularly of gaseous nitric acid (Hahn et al. 1982, Slinn 1982, Topol 1986). If the snow is partially melted these deposits will dissolve into the snowflake but if not they will remain located at the surface of the snowflake (Johannessen and Henriksen 1977, Slinn 1982). Other solutes distributed throughout the snowflake tend to migrate to the surface so that by the time the snow is beginning to melt a rich concentration of solutes forms a thin film at the junction of snowflakes (Seip 1980, Davies 1989). This leads to a freezing point depression so that solutes drain out in high concentrations from the edge of snowflakes at these junctions leading to high levels of ions, including H^+, in the first stages of snowmelt, often causing an "acid shock" in nearby water systems (Seip 1980, Cadle et al. 1984, Tranter et al. 1986).

This process was observed in the samples of snowmelt from the funnels and cylinders. When a heating element was inserted the flush/decline of ions tended to be smoother as meltwater percolated through the snow rather than down the sides of the cylinder. The ion concentration released was extremely high and, if occurring in contact with vegetation, could be potentially damaging.

However, results from live shoots indicated considerable deviation from this flush/decline pattern. Whether this occurs because the individual snow crystals or the complete snow pack is melting in a different way or whether this is the result of modification by ion exchange with live shoots is not known. The problem was further compounded by the overall higher levels of H^+ in melt from snowpack on shoots compared with that in fresh snow. Differences in snow crystal and snow pack melting are not able to account for an elevated ion concentration, although they may affect the flush/decline pattern. The elevated ion concentration must be attributed either to a further source of H^+ outside that of fresh snow or to a concentrating effect. Dry deposition to snow is low (Hahn et al. 1982, Slinn 1982) and both that and the short residence time before the melting experiments began preclude dry deposition to snow on shoots as a major enrichment pathway. Mobilisation of previous deposits may increase concentration. The two days prior to the 29th January, 1988, were dry and would have allowed dry deposits to build up on the spruce shoots.

Evaporation of snow (sublimination) is often not thought of as being important because of the low energy input in cold high latitude areas and the poor conductance of heat from ground through snow (Ward 1975, Miller 1977). In addition the energy required for sublimination is approximately 8½ times greater than that needed for melting so that at temperatures below 0 °C the volume of snow sublimed is likely to be low: at temperatures above 0 °C melting will dominate sublimination. Sublimination will have some effect on concentrating the H^+ ions in snow on shoots compared with ground snow, because of the lower boundary layer resistance of snow on shoots compared with snow on the ground, but could not account for a large proportion of the increase measured.

Mobilization of dry deposits already on the shoot was found to contribute significantly to acidity in cloudwater samples from shoots and is likely to be an important source of acidity to samples of melting snow from shoots.

The significance of the higher concentration of snow melt ions from shoots than from snow on the ground is that once again general measurements of precipitation acidity do not give a true picture of the acidic stress to which shoots are subjected and close attention must be paid to the meltwater acidity on the shoots when the damage potential of snow is being investigated.

The difference between the two melt pathways indicates that droplets from the longer pathway have much higher H^+ concentrations than those from the shorter pathway. As meltwater percolates through the snowpack, pure water re-freezes as it comes into contact with cold snow crystals. Ionic impurities are thus concentrated (Johannessen and Henriksen, 1977). The longer the pathway the greater the concentration that can take place. Snow melt which proceeds by a shorter pathway is not subject to the process and is more dilute.

The damage potential of melting snow is therefore far greater than would be estimated by a bulk snow pH value. Bulk snow pH values of down to pH 3.0 have been reported in rural areas of Scotland (Tranter _et al_. 1986, Davies _et al_. 1988) and frequently reach values around pH 4.0 at the field site. The results of this study indicate that the actual pH experienced by tree shoots can be substantially lower than bulk pH values, with a consequent increase in damage potential.

CHAPTER 9

GENERAL SUMMARY AND CONCLUSIONS

The aim of the study was to investigate the nature of high altitude precipitation and its effect on Scots pine and Sitka spruce. Throughout Central Europe since the mid-seventies, a decline in the health of forests (known as "Waldsterben") has been attributed to air pollutants. More recently it has been apparent that because of the high acidity of occult precipitation, i.e. precipitation not measured by normal rain collectors and consisting in the main of low clouds impacting upon vegetation, and the general worsening of damage at high altitude, that impacted cloudwater may be a major contributor to the forest decline.

Although much attention has focussed on the effect of acid rain on vegetation, less work has been undertaken on acid clouds. In addition, those studies which have attempted to measure the pH of (collected) cloudwater have often not taken account of the strong temporal and spatial imhomogeniety in cloud (event) pH. Again, although there is no scarcity of work on acid effects in general, there has been little work done on effects of acid precipitation or clouds on Scots pine and Sitka spruce under controlled conditions and even less on trees past the seedling stage. As there has been a deterioration in the health of British forests over the last few years, it is vital to assess the impact of acid clouds on these two important species.

The objectives of the study were therefore:

a) to assess the effect of simulated acid cloud (SAC) on the needles of these species, its effect on growth, the extent of cation loss through accelerated leaching by H^+ as well as the effect of ion exchange on the impacted cloudwater acidity itself;

b) to examine the variability and levels of acidity in cloud events and,

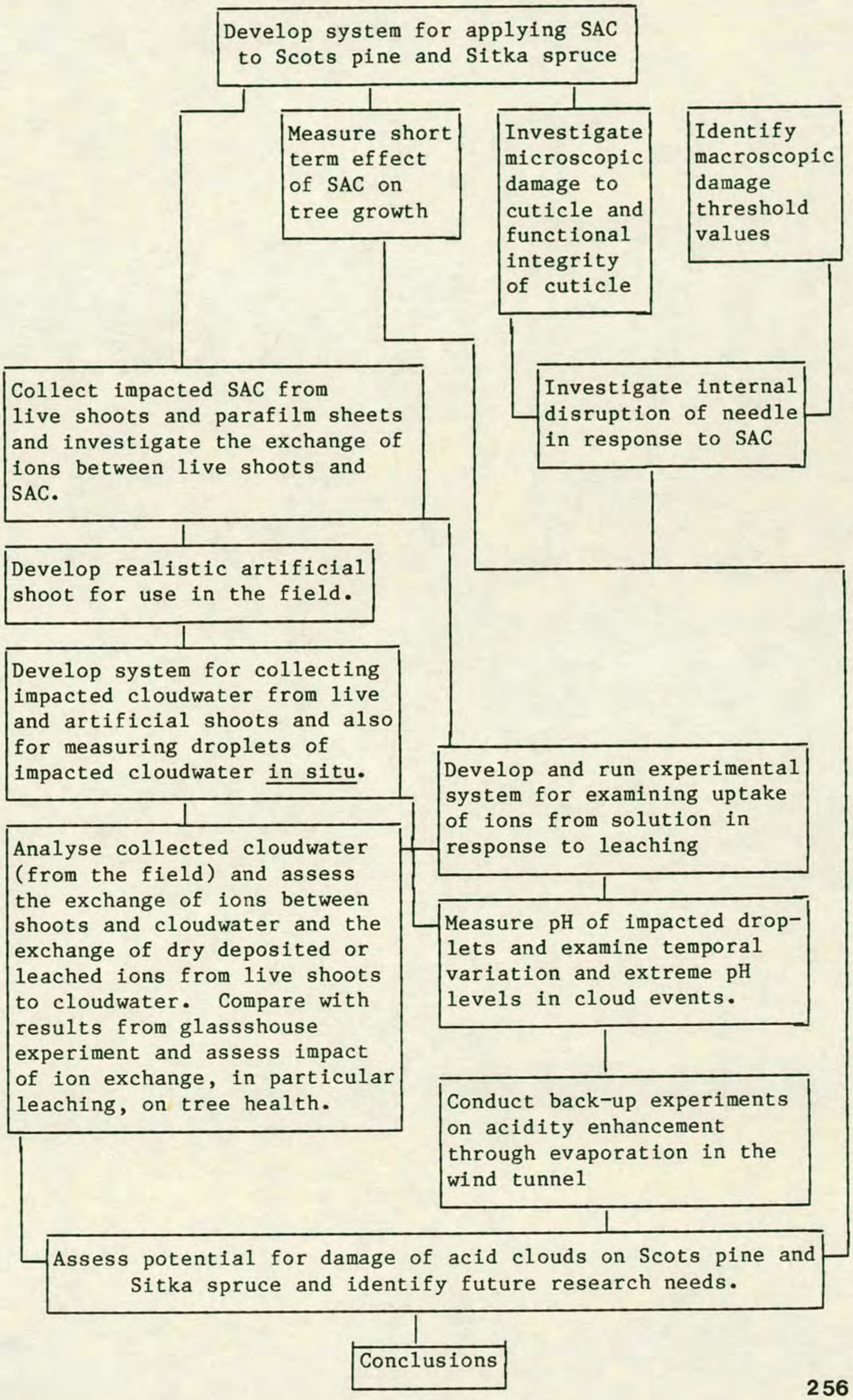
c) to go further than this and investigate the effect of evaporation and snowmelt on short term pH values.

These objectives were achieved both by field work and glasshouse experiments (Figure 9.1).

Without large scale funding and resources, replicated experiments on mature trees are not possible. However, whilst problems of establishment and susceptibility in the seedling stage are important in understanding the effect of acid precipitation on regeneration, work on seedlings cannot be easily extrapolated to forest decline. Thus, using trees of six to eight-years-old allows a closer extrapolation of results to forests than for seedlings.

Four glasshouse experiments were undertaken in which a system for applying SAC was developed using mini-ULVA sprayers. This system proved effective in supplying realistic simulations of cloud both with respect to deposition rates and droplet sizes.

Figure 9.1 Diagrammatic representation of investigative progression in the thesis.



SAC acidity did not significantly affect tree growth, as measured by extension growth and needle area, in line with hypothesis (a) of the introduction. There are several avenues by which SAC could affect growth. Increased growth could occur via stimulation of cell wall extensibility by H^+ or by enhanced nutrient status through nitrogen fertilization. The former was shown to be unlikely because of the need for cuticle removal before such acid growth occurred and the latter is unlikely to be seen in a short-term experiment on conifers. The picture is further complicated by the overwintering of stem units. There is a need for research to investigate the effect of SAC not on this year's growth but on next year's. The means by which acidity inhibits growth are still understood very poorly. Once again, because necrosis leads to a reduction in photosynthetic area there may be an effect on future years' growth. Other effects on carbon allocation, photosynthesis and biochemistry, which could all affect growth, are possible. Unfortunately, because of the large amount of variation in growth parameters of trees, successful experiments in identifying growth responses are likely to be restricted to those with large and long term budgets, allowing large replication over several years. What the results of the experiments in this thesis show is that, as far as Scots pine and Sitka spruce are concerned, growth reductions as a result of high altitude acid precipitation are not likely to be mediated by a direct effect of H^+ on the extension of shoots and needles. This work tends to focus attention away from growth effects towards chronic weakening and in particular to nutrient-disturbance mediated effects as the most likely means of acidity mediated forest decline.

Both species were also seen to be resistant to direct effects of SAC on needle necrosis. Threshold values were between pH 2.0 and pH 2.5 for Scots pine and minor damage occurred at pH 2.5 for Sitka spruce, with significant damage at pH 2.0. In the Scottish Southern Uplands the lowest weekly bulk cloudwater pH collected over recent years has been 2.69. However the intensive sampling of cloudwater (events) showed that temporal variation of pH was high, such that the minimum pH experienced by trees during that week would be less than 2.69. In several mountain ranges including the Pennines, pH values have been in the range 2.2-2.4 (Falconer and Falconer 1980, Gervatt 1985, Schmitt 1988). Some fine-tuning in pinpointing the acidity level between pH 2.0 and pH 2.5 that causes damage and the number and length of events needed is necessary. The experiments showed that both species are very resistant to acid precipitation, in accordance with hypothesis (b), specifically occult precipitation, but that the possibility remains that threshold values may occasionally be reached.

An often overlooked but vital factor is the effect of evaporation on droplet pH of impacted cloudwater. The hydrogen ion concentration in an impacted droplet of cloudwater on a live shoot is not static but dynamic, with ion exchange, mobilisation of dry deposition and evaporation all leading to a changing H^+ concentration. As hypothesis (j) predicted, drying droplets on needles increased in acidity through evaporation and exposed the needles to extremely low pH values. This appears to be the first time that such an important, though predictable, phenomonon has been demonstrated in situ. The rate of enhancement of acidity was seen to be that expected through a direct inverse relationship to droplet volume and was also predictable according to the rate of evaporation, using the Penman equation. Although the windtunnel showed windspeed and saturation deficit to be more important than irradiation, in the field high windspeeds are likely to dislodge drying droplets. Conditions of low wind speed combined with rapidly dispersing cloud, leading to high radiation and saturation deficit are thus most likely to cause a rapid increase in pH of drying droplets. This effect of acidity enhancement raises two important issues. Firstly, needles are exposed to much higher levels of acidity than generally realised. Secondly, at what pH does damage in controlled experiments actually take place? Was the damage to Scots pine at pH 2.0 the result of the H^+ concentration of 10,000 μeq dm^{-3} in the SAC or a much higher concentration sometime after spraying ceased? The answer to such a question is hard to establish but experiments designed to compare the effect of droplets at a constant pH with those at a declining pH should be attempted.

Trees at high altitude are also subject to considerably more snow than those at low altitude and the possibility of this extra exposure to snow contributing to forest decline is generally overlooked. The collection of droplets from snowmelt showed a strong initial flush of ions including H^+ at the start of snowmelt, with concentrations up to one hundred times higher than those in the last samples. This was likely to be the result of a high concentration of dry deposits and solutes at the snowflake surfaces. Although none of the snow events investigated exhibited particularly high H^+ concentrations, such a flush of H^+ at the start of a more acidic snow event could lead to very strong acidic pulses in the run-off on leaves. With bulk snow pH values reaching down to pH 3.0 in Scotland (Tranter et al. 1986, Davies et al. 1988), there is a possibility of acid flushes reaching potentially damaging levels (pH 2.5).

There was, however, a considerable difference in the way snow melted on live shoots. The initial pH of the snow on the shoots was considerably lower than that of snow collected from the ground, partly as a result of sublimation but more probably because of previous dry deposition to the shoots. The flush/decline pattern of H^+ ion concentration was absent from snow melting on live shoots and there was no evidence of any clear chronological patterns of H^+ ion concentration. Instead a distinction was seen between the two main pathways of melt, with the longer pathway, involving the meltwater running down through the snowpack on the shoot, producing higher H^+ ion concentrations than the shorter pathway involving meltwater dripping from needles protruding from the snowpack. Snowmelt from the longer pathway is subjected to considerably higher concentration by the refreezing of pure water in contact with cold crystals, allowing the solutes to concentrate. Once again, levels of acidity which would not be expected from bulk sampling of precipitation (in this case snow) were evident. Trees at high elevations are thus likely to be exposed to both extreme and chronic acidity levels to a much greater extent than trees at low altitude in accordance with hypothesis (k). The acidity of impacted droplets is further lowered both by the solubilisation and mobilisation of dry deposits on the needles and apparently by the leaching of acidic substances from inside the needle. This latter aspect needs more attention and the identity of the acidic substances which led to lower pH of droplets on live shoots, particularly of Sitka spruce, than on DD artificial shoots is necessary.

In general then, direct damage was seen to occur between pH 2.0 and pH 2.5 and temporal inhomogeneity and general high acidity of cloud events, dry deposition, leaching of acidic substances, evaporation and snowmelt all have the potential to make such pH levels possible for trees at high altitudes.

Because in heavily polluted areas epicuticular wax is often degraded at a faster than normal rate, and because most gaseous pollutants and some acid precipitation studies have shown fusion and melting of epicuticular wax tubules in response to increasing pollution load, it is sometimes maintained that epicuticular wax degradation in response to acid precipitation is an established fact (Freer-Smith 1988). The inspecificity of field work studies on cuticle degradation and the lack of a clear distinction between the effects of particulate, gaseous and aqueous pollutants on cuticles in both field and controlled experiments has led to a rather "woolly" idea that pollutants en masse destroy the epicuticular wax structure. In addition, the lack of objectivity in many studies using controlled applications of acid precipitation, whereby there is no unbiased and statistical assessment is a serious shortcoming. However, strong acid solutions are often used to isolate plant cuticles which, on isolation, still appear structurally and functionally intact. Several experiments have shown no effect of precipitation acidity on the epicuticular wax structure of conifer needles (Horntvedt et al. 1980, Skeffington and Roberts 1985a, Turunen and Huttunen 1989, Turner et al. (in press)). There is not yet a satisfactory theory as to why acid precipitation should cause epicuticular wax degradation. There are also differences in the susceptibility of clones within species (Percy 1987). The results of the experiment in this thesis which have an advantage over the results from many other "subjective" experiments in being unbiased and statistically tenable, clearly show that Scots pine, contrary to the expectation of hypothesis (d) can be subjected to extremely acidic SAC and show no degradation of epicuticular wax. It appears that, because wax chemistry and structure vary between species, a general precept is not possible. The experiments here further show that the epicuticular wax structure of Scots pine is totally resistant even to levels of acidity that lead to necrosis. This integrity of the cuticular waxes has important consequences with respect to the functioning of the cuticle. Cuticular conductance and contact angles were also unaffected by SAC acidity. Thus, contrary to hypothesis (e) water loss through the cuticle, pathogenic infection, increased leaching or necrosis as a result of an increase in wettability were all unaffected. Future work must avoid the subjectivity of wax degradation assessment and field work should be careful not to uncritically ascribe cuticular damage to acid precipitation, especially when gaseous pollutants and particulate matter such as pulverised fuel ash are also present.

The external appearance of damage was much more indicative of internal disruption than it was of cuticle disruption (hypothesis (c). The integrity of the cuticle was certainly not a guarantee of the integrity of the needle, with total cell collapse occurring under brown areas of the needles. If we assume that the damage is caused by hydrogen ions (a fair but not flawless assumption), there must be a mode of entry into the needles which allows a high enough concentration of the ion to penetrate. The two obvious modes of entry are through epicuticular pores or by ion exchange. However the amount of H^+ exchanged for basic cations was seen to be fairly small and the concentration of H^+ remaining in impacted droplets (i.e. the lack of neutralization or consumption of H^+) indicates that only a small percentage of the H^+ ions are actually penetrating the needle. It is however unclear whether H^+ penetration and cuticular defence or internal cell resistance is responsible for the resistance to acid damage. It might have been thought that the low wettability and high resilience of the cuticle was mainly responsible, but the lack of any predissposition to injury by even severe abrasion of needle cuticles needs further investigation. There is a real need to study the sensitivity of cells themselves to acidity .

It also appears from the experiments of this thesis that cells have a way of responding to acid stress and forming a barrier against the further spread of damage. In this case the logical extension of this work is to use X-ray analysis to identify the substance that fills the intercellular spaces and also to do a chronological series of frozen-hydrated freeze-fractured SEM studies before, during and after the formation of the barrier, rather than a static time study of damaged, undamaged and transitional tissue.

The method and importance of nutrient imbalance or deficiency resulting from acidic precipitation as a cause of forest decline continues to be the subject of debate, but there is little doubt that nutrient deficiency is at least a symptom if not the only cause of forest decline. The difficulties in assessing the importance and method of the impact of nutrient leaching lie in assessing its relative importance to, or dependence upon other factors. These include membrane damage by ozone, the effect of increasing H^+ concentrations and immersion times within precipitation events and the mode of attack, whether through foliar leaching or soil/root disturbances.

The field and glasshouse experiments were generally in agreement in principle although differing in degrees and amounts. In addition the field experiments had the advantage of allowing more realistic conclusions concerning forest decline to be drawn whilst the glasshouse experiments allowed, in particular, comparison between SAC of different pH levels.

The increase in leaching of cations with increasing acidity was not as clear as had been expected with a high degree of scatter. However, careful breakdown of the data showed a tendency towards increased leaching with acidity. This increase was not entirely apparent for samples taken within the first ten minutes of the end of spraying (when the effect of evaporation would still be low) for Sitka spruce, but Ca, Mg and K all showed increased excess values at pH 3.0 and pH 2.5, compared with pH 5.6 for Scots pine. For both field and glasshouse experiments there was little difference in the amounts of Ca and Mg in samples from Sitka spruce and Scots pine but K was higher in samples from Scots pine. Both sets of experiments tended to point towards K as being susceptible to leaching by acid clouds, possibly because it binds less strongly to carboxylic acid binding sites and can move through cuticular pores more easily than a divalent ion. The relative importance of leaching for K compared with Ca and Mg, is also increased because of the higher dry deposition of Ca and Mg through dust or sea-salts, whereas K is dry deposited in much smaller amounts.

There was little evidence for any strong H^+ - cation exchange mechanism, except possibly for H^+ and K^+ in Scots pine. The leaching of nutrients in general did not neutralize the acidity of the impacted cloudwater as only a small proportion of the incoming H^+ ions could have been consumed by exchange with leached cations. Hypothesis (f) was therefore upheld whilst hypothesis (g) was not. It was not surprising, therefore, that the nutrient status of the trees had no effect on the acidity of the impacted cloudwater or the actual amount of nutrient leached. Resistance to damage in the two species does not appear to be primarily conferred by neutralization of droplets but is mediated either by cuticular resistance to the entry of H^+, or by internal cell resistance to H^+ ions once they have penetrated.

Because of the problems caused by the aphid infestation, it was difficult to assess the uptake of ions in response to exposure to SAC and increased leaching (hypothesis (h)). The initial trend did appear to be towards an increased uptake of nutrients at pH 3.0 compared with pH 5.6, and foliar concentrations certainly did not fall in the pH 3.0 treatment. The experimental approach should be followed up and used in seeking to establish a link between increased leaching in response to increased H^+, and a compensatory increase in nutrient uptake.

In Central Europe, it is primarily Mg and Ca that are deficient in trees affected by "montane yellowing". K and Mn deficiency, generally occurring together, is less frequent but does occur. A seaboard country or island, such as the U.K., receives a plentiful supply of Mg from sea-salts. However the amount of cations leached out of the trees in the experiments was seen to be large compared with the amount in the overstorey. In particular K was easily leached and was also slightly deficient in the shoots. It is felt strongly, that with respect to the downturn in the health of British forests, it would be wise to monitor nutrient concentrations in high elevation forests subject to severe leaching by acid clouds.

The leaching of ions from foliage should not be isolated from other mechanisms of damage, or other pathways to nutrient deficiency. Forest decline must be approached on a broad base, examining not just isolated responses but source-sink relationships between crown and root systems. There has been too much polarisation of opinions on the cause of forest decline and, in particular, too much bias towards the specialist field of the researchers. Increased leaching by acidic clouds is unlikely to be the only factor causing forest decline. It should be remembered that predisposing, inciting and contributing stresses are all likely to be involved. It is not the author's intention to add to the multiplicity of theories concerning forest decline but the following mechanism is proposed to take account of the results from the experiments here and to integrate them into existing frameworks. Because different species respond in different ways to acid clouds, the proposed mechanism is relevant only to Scots pine and Sitka spruce.

Direct damage to needles is likely to be rare and not a serious threat to forests because of the high resistance of the needles to acid cloudwater. The high acidity of clouds and the effect of evaporation and snowmelt mean that it cannot be ruled out as a possibility, and though producing different symptoms to classic forest decline, strongly acidic cloud events followed by "good" evaporative conditions could lead to occasional direct damage. Direct effects on growth also seem unlikely as both extension growth and needle area appear to be unaffected by SAC. Scots pine (and possibly Sitka spruce) is also resistant to effects on the cuticle. Epicuticular waxes are unaffected by very strong acid cloud events and the functioning of the cuticle with respect to transpiration and wettability remains intact. A large amount of nutrients is leached by acid clouds, as a result of both the long immersion times of cloud events and the high acidity of cloudwater. Compared with the nutrient pool in the overstorey foliage the loss of these nutrients is high and nutrient-mediated effects are more likely to be causal rather than direct damage. In Central Europe the problem may be exacerbated by ozone rendering cell membranes more permeable. If nutrient resupply is sufficient no adverse effects occur but if the nutrient supply rate is low or uptake inhibited by acid effects on the soil a lowered nutrient concentration in the shoots may occur. This may lead to a reduction in photosynthetic apparatus, a shortage of assimilates and reduced root growth. Nutrient uptake is thus further disturbed and a cycle of steadily decreasing vitality results which can be reversed by the appropriate fertilizer.

REFERENCES

Abouguendia, Z.M. and Baschak, L.A. 1987. Response of two western Canadian conifers to simulated acidic precipitation. Water, Air and Soil Pollution 33: 15-22.

Abrahamsen, G. 1980. Effects of acid precipitation on soil and forest. 4. Leaching of plant nutrients. Proc. Int. Conf. Ecol. Impact Acid Prec. Norway, SNSF Project, Oslo. 196.

Abrahamsen, G., Bjor, K., Horntvedt, R. and Tveite, B. 1976. Effects of acid precipitation on coniferous forest. In: Impact of acid precipitation on forest and freshwater ecosystems in Norway. Ed. F.H. Braekke. Sur Nedbors Virkning pa skog og. Fisk Fragrapport FR 6/76 Oslo. 37-63.

Adams, C.M. and Hutchinson, T.C. 1984. A comparison of the ability of leaf surfaces of three species to neutralize acidic rain drops. New Phytologist 97: 463-478.

Adams, C.M. and Hutchinson, T.C. 1987. Comparative abilities of leaf surfaces to neutralize acidic raindrops. II. The influence of leaf wettability, leaf age and rain duration on changes in droplet pH and chemistry on leaf surfaces. New Phytologist 106: 437-456.

Adams, C.M., Caporn, S.J.M. and Hutchinson, T.C. 1990. Crystal occurrance and wax disruption on leaf surfaces of cabbage treated with simulated acid rain. New Phytologist 114: 147-158.

Alcock, M.R. and Morton, J. 1981. The sulphur content and pH of rainfall and of throughfalls under pine and birch. Jnl. Appl. Ecol. 18: 835-839.

Alenäs, I. and Skärky, L. 1988. Throughfall and needle content of plant nutrients in relation to crown thinning in a Swedish spruce forest. In: Scientific basis of forest decline symptomatology. Rep. No. 15. Air Poll. Report Series. Environ. Res. Prog. C.E.C. Eds. J.N. Cape and P. Mathy. 227-237.

Amthor, J.S. 1984. Does acid rain directly influence plant growth? Some comments and observations. Environ. Poll. Series A. 36: 1-6.

Amthor, J.S. 1986. An estimate of the 'cost' of nutrient leaching from forest canopies by rain. New Phytologist 102: 359-364.

Andersson, F. 1986. Acidic deposition and its effects on the forests of Nordic Europe. Water, Air and Soil Pollution 30: 17-29.

Appel, B.R., Wall, J.M., Tokiwa, Y. and Haik, M. 1980. Simultaneous nitric acid, particulate nitrate and acidity measurements in ambient air. Atmos. Environ. 14: 549-554.

Ashenden, T.W. and Bell, S.A. 1988. Growth responses of birch and Sitka spruce exposed to acidified rain. Environ. Poll. 51: 153-162.

Ashmore, M.R., McHugh, F.M., Mepsted, R. and Garretty, C. 1989. Effects of ozone and acid mist on saplings of *Fagus sylvatica*. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.G. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 55-60.

Atkinson, D. and Wilson, S.A. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake. In: Mineral nutrition of fruit trees. Eds. D. Atkinson, J.E. Jackson, R.O. Sharples and W.M. Waller. Butterworths, London 137-150.

Azevedo, J. and Morgan, D.L. 1974. Fog precipitation in coastal Californian forests. Ecology 55: 1135-1141.

Bache, D.H. 1977. Sulphur dioxide uptake and the leaching of sulphate from a pine forest. Jnl. Appl. Ecol. 14: 881-895.

Baig, M.N. and Tranquillini, W. 1980. The effects of wind and temperature on cuticular transpiration of Picea abies and Pinus cembra and their significance in desiccation at the alpine treeline. Oecologia 47: 252-256.

Baker, E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In: The Plant Cuticle. Eds. D.J. Cutler, K.L. Alvin and C.E. Price. Academic Press, London. 139-165.

Baker, E.A. and Hunt, G.M. 1986. Erosion of waxes from leaf surfaces by simulated rain. New Phytologist 102: 161-173.

Balloch, H.J. and Brandt, C.J. 1984. BML-Forest decline inventory 1983 - analysis of causes without differential diagnosis? Staub-Reinhalt Luft Vol. 43. 10pp.

Bals, E.J. 1969. Design of rotary atomizers. Proc. 4th Int. Agric. Aviat. Congr. 156-165.

Bamber, D.J., Clark, P.A., Glover, G.M., Healey, P.G.W., Kallend, A.S., Marsh, A.R.W., Tuck, A.I. and Vaughan, G. 1984. Air sampling flights around the British Isles at Low Altitudes: SO_2 oxidation and removal rates. Atmos. Environ. 18: 1777-1790.

Banaszak, K.T. 1975. Relative throughfall enrichment by biological and aerosol derived material in loblolly pines. Miss. Water Res. Inst., Mississippi State Univ., PB-245-258/9ST Nat. Tech. Info. Ctr., Springfield, Virginia. 32pp.

Barrett, C.F., Fowler, D., Irwin, J.G., Kallend, A.S., Martin, A., Scriven, R.A. and Tuck, A.F. 1983. Acidity of rainfall in the UK. Warren Spring Laboratory, Stevenage, Herts.

Barret, C.F., Atkins, D.H.F., Cape, J.N., Crabtree, J., Davies, T.D., Derwent, R.G., Fisher, B.E.A., Fowler, D., Kallend, A.S., Martin, A., Scriven, R.A. and Irwin, J.G. 1987. Acid deposition in the United Kingdom 1981-1985. A Second Report of the United Kingdom Review Group on Acid Rain. Warren Spring Laboratory, Stevenage, Herts.

Barrett, C.F., Hall, D.J., Ralph, M.O. and Upton, S.L. 1988. The microthread method for the sizing of aerosols. Atmos. Environ. 22: 189-191.

Baumgartner, A. 1958. Nebel und Nebelbiederschlag als Stamdortfaktoren am Großen Falkenstein (Bayerischer Wald). Forstwiss. Centralblatt 77: 257-272.

Bennett, J.P., Andersen, R.L., Mielke, M.L. and Ebersole, J.J. 1989. An historical perspective on forest decline of Eastern White pine. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin, SFIFR, Birmensdorf. 69-75.

Berg, V.S. 1987. Plant cuticle as a barrier to acid rain penetration. In: Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Series Vol. G16. Eds. T.C. Hutchinson and K.M. Memma. Springer-Verlag, Berlin, Heidelberg. 145-154.

Binns, W.O. 1987. Surveys of forest health in the UK. In: The Effects of Acid Rain and Air Pollution in Northern Europe. Proc. Scientific Seminar Friends of the Earth, London. Eds. A, Markham and L. Blundell. Friends of the Earth Ltd., London.

Binns, W.O., Mayhead, G.J. and Mackenzie, J.M. 1980. Nutrient deficiencies of conifers in British forests. Forestry Commission Leaflet 76. HMSO.

Binns, W.O., Redfern, D.B. and Rennolls, K. 1987. Forest Decline - the view from Britain. In: Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. NATO ASI, Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin. 69-81.

Bjor, K. and Teigen, O. 1980. Effects of acid precipitation on soil and forest. 6. Lysimeter experiment in greenhouse. Proc. Int. Conf. Ecol. Impact Acid Prec., Norway, SNSF Project, Oslo. 200-201.

Blank, L.W. and Roberts, T.M. 1987. Notes on the 1986 German forest inventory and the 2nd report of the Advisory Council on forest damage/air pollution. CEGB memorandum TPRD/L/LIS/105/M86.

Bosch, C., Pfannkuch, E., Baum, U. and Rehfuess, K.E. 1983. Über die Erkankung der Fichte (Picea abies Karst.) in den Hochlagen des Bayerischen Walder. Forstwiss. Centralblatt 102: 167-181.

Brain, P. and Butler, D.R. 1985. A model of drop size distribution for a system with evaporation. Plant, Cell and Environment 8: 247-252.

Bringmark, L. 1977. A bio-element budget of an old Scots pine forest in central Sweden. Silva Fennica 11: 201-209.

Bucher, J.B. 1987. Forest damage in Switzerland, Asutria and adjacent parts of France and Italy in 1984. In: Effects of Air Pollution on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Series: Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin. 37-58.

Bucher, J.B. 1989. Forest decline, air pollution and clean air maintenance in Switzerland. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air. Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 15-19.

Cadle, S.H., Dasch, J.M. and Grossnickle, N.E. 1984. Northern Michigan snowpack - a study of acid stability and release. Atmos. Environ. 18: 807-816.

Cannell, M.G.R. 1979. Components of conifer shoot growth. In: Proc. 5th N. Am. Forest Biol. Workshop. Eds. C.A. Hollis and A.E. Squillance, Univ. of Florida, Gainesville.

Cannell, M.G.R., Thompson, S. and Lines, R. 1976. An analysis of inherent differences in shoot growth within some north temperate conifers. In: Tree Physiology and Yield Improvement. Eds. M.G.R. Cannell and F.T. Last. Academic Press, London. 173-205.

Cape, J.N. 1983. Contact angles of water droplets on needles of Scots pine (Pinus sylvestris) growing in polluted atmospheres. New Phytologist 93: 293-299.

Cape, J.N. 1986. Effects of air pollution on the chemistry of surface waxes of Scots pine. Water, Air and Soil Pollution 31: 393-399.

Cape, J.N. 1988a. Chemical interactions between cloud droplets and trees. In: Acid Deposition at High Elevations. Proc. NATO Workshop Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 639-649.

Cape, J.N. 1988b. Developments in the diagnosis and quantification of forest decline. In: Air Pollution and Ecosystems. C.E.C. Proc. Int. Sym., Grenoble. D. Reidel Publishing Company, Dordrecht. 292-305.

Cape, J.N. 1988c. Air pollutant effects on conifer leaf surfaces. In: Scientific Basis of Forest Decline Symptomatology. Rep. No. 15. Air Poll. Report Series. Environ. Res. prog. Eds. J.N. Cape and P. Mathy C.E.C. Brussels. 149-159.

Cape, J.N. and Fowler, D. 1981. Changes in epicuticular wax of Pinus sylvestris exposed to polluted air. Silva Fennica 15: 457-458.

Cape, J.N., Fowler, D., Kinnaird, J.W., Nicholson, I.A. and Paterson, I.S. 1987. Modification of rainfall chemistry by a forest canopy. In: Pollutant Transport and Fate in Ecosystems. Special Pub. Series Br. Ecol. Soc. No. 6., Eds. P.J. Coughtrey, M.H. Martin and M.H. Unsworth. 155-170.

Cape, J.N. and Unsworth, M.H. 1988. Deposition, uptake and residence of pollutants. In: Air pollution and plant metabolism. Eds. S. Schulze-Hortede, N.M. Darrall, L.W. Blank and A.R. Wellburn. Elsevier Applied Science, London. 1-18.

Cape, J.N., Paterson, I.S., Wellburn, A.R., Wolfenden, L., Melhorn, M., Freer-Smith, P. and Fink, S. 1988. Early diagnosis of forest decline - a pilot study. In: Air Pollution and Ecosystems. C.E.C. Proc. Int. Sym., Grenoble. D. Reidel Publishing Company, Dordrecht. 609-615.

Caporn, S.J.M. and Hutchinson, T.C. 1986. The contrasting response to simulated acid rain of leaves and cotyledons of cabbage (Brassica oleracea L.). New Phytologist 103: 311-324.

Chamberlain, A.C. 1975. The movement of particles in plant communities. In: Vegetation and the Atmosphere. Vol. 1 Principles. Ed. J.L. Monteith. Academic Press, London. 155-201.

Chamberlain, A.C. and Little, P. 1981. Transport and capture of particles by vegetation. In: Plants and their Atmospheric Environment. Eds. J. Grace, E.D. Ford and P.G. Jarvis. Blackwell Scientific Publications, Oxford. 147-173.

Chamel, A. 1986. Foliar absorption of herbicides: study of cuticular penetration. Physiologie vegetole 24: 491-508.

Chester, P.F. 1987. Acid rain - a prognosis. CEGB Research Document 20: 62-64.

Cleland, R.E. and Rayle, D.L. 1978. Auxin, H^+ - excretion and cell elongation. Bot. Mag. Special issue 1: 125-139.

Clement, C.R., Hopper, M.J., Canaway, P.J. and Jones, L.H.P. 1974. A system for measuring the uptake of ions by plant from flowing solutions of controlled composition. Jnl. Exp. Bot. 25: 81-99.

Cocks, A.T., Kallend, A.S. and Marsh, A.R.W. 1983. Dispersion limitations of oxidation in power plant plumes during long range transport. Nature 305: 122-123.

Cogbill, C.V. 1977. The effect of acid precipitation on tree growth in Eastern North America. Water, Air and Soil Pollution 8: 89-93.

Cole, D.W. and Johnson, D.W. 1977. Atmospheric sulfate additions and cation leaching in a Douglas fir ecosystem. Water Resources Research 13: 313-317.

Cole, D.W. and Rapp. M. 1981. Elemental cycling in forest ecosystems. In: Dynamic Properties of Forest Ecosystems. IBP No. 23. Ed. D.E. Reichle. C.U.P. Cambridge. 341-490.

Costin, A.B. and Wimbush, D.J. 1961. Studies in catchment hydrology in the Australian Alps. (IV). Interception by trees of rain, cloud and fog. Division of Plant Industry Technical Paper No 16. Melbourne, 17pp.

Cowling, E.B. 1982. Acid precipitation in historical perspective. Environ. Science and Tech. 16: 110A-123A.

Cronan, C.S. and Reiners, W.A. 1983. Canopy processing of acidic precipitation by coniferous and hardwood forests in New England. Oecologia 59: 216-223.

Cronan, C.S., Reiners, W.A. and Reynolds, R.C. Jr. 1980. The impact of acid precipitation on forest canopies and soils in the northeastern United States. In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Norway 1980. Eds. D. Drablos and A. Tollan. SNSF Oslo. 158-159.

Crossley, A. 1988. Particles in orographic cloud and the implications of their transfer to plant surfaces. In: Acid Deposition at High Elevation Sites. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 517-539.

Crossley, A. and Fowler, D. 1986. The weathering of Scots pine epicuticular wax in polluted and clean air. New Phytologist 103: 207-218.

Crossley, A., Milne, R. and Unsworth, M.H. 1986. Acid mist and tree injury. CEC/NERC Contract Env. 850 UK (H). I.T.E. Bush Project 924. Final Report to CEC. 15pp.

Daum, P.H., Schwartz, S.E. and Newman, L. 1984. Acidic and related constituents in liquid water stratiform clouds. Jnl. Geophy. Res. 89: 1447-1458.

Davies, T.D. 1989. As pure as the driven snow? New Scientist 122: 45-49.

Davies, T.D., Brimblecombe, P., Blackwood, I.L., Tranter, M. and Abrahams, P.W. 1988. Chemical composition of snow in the remote Scottish Highlands. In: Acid Deposition at High Elevation Sites. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 517-539.

Demarty, M., Morvan, C. and Thellier, M. 1984. Calcium and the Cell Wall. Plant, Cell and Environment 7: 441-448.

DOE 1988. Department of the Environment Statistical Bulletin (88) 1. Air Quality. Government Statistical Services. 84pp.

DOE 1989a. Digest of Environmental Protection and Water Statistics. HMSO 1989. 22pp.

DOE 1989b. Department of the Environment Statistical Bulletin (89) 1. Air Quality Supplement to Digest of Environmental Protection and Water Statistics. No 11. Government Statistical Service. 18pp.

Dollard, G.J. and Unsworth, M.H. 1983. Field measurements of turbulent fluxes of wind-driven fog drops to a grass surface. Atmos. Environ. 17: 775-780.

Dollard, G.J., Unsworth, M.H. and Harvey, M.J. 1983. Pollutant transfer in upland regions by occult precipitation. Nature 302: 241-242.

Eaton, J.S., Likens, G.E. and Bormann, F.H. 1973. Throughfall and stemflow chemistry in a northern hardwood forest. Jnl. Ecology 61: 495-508.

Ekern, D.C. 1964. Soil and water management and conservation. Direct interception of cloudwater on Lanaihale, Hawaii. Soil Science Society of America, Proceedings 1964. 419-421.

Evans, L.S 1984. Acidic precipitation effects of terrestrial vegetation. Ann. Rev. Phytopathol. 1984 22: 397-420.

Evans, L.S. 1986. Proposed mechanism of initial injury-causing apical dieback in red spruce at high elevation in eastern North America. Can. Jnl. For. Res. 16: 1113-1116.

Evans, L.S., Gmur, N.F. and DaCosta, F. 1977. Leaf surface and histological perturbations of leaves of Phaseolus vulgaris and Helianthus annuus after exposure to simulated acid rain. Amer. Jnl. Bot. 64: 903-913.

Evans, L.S. and Curry, T.M. 1979. Differential response of plant foliage to simulated acid rain. American Jnl. Bot. 66: 953-962.

Evans, L.S., Curry, T.M. and Lewin, K.F. 1981. Responses of leaves of Phaseolus vulgaris to simulated acidic rain. New Phytologist 88: 403-420.

Everard, J.E. 1973. Foliar analysis, sampling methods, interpretations and application of results. Quart. Jnl. Forestry 67: 51-66.

Fairfax, J.A.W. and Lepp, N.W. 1975. Effect of simulated 'acid rain' on cation loss from leaves. Nature 255: 324-325.

Falconer, R.E. and Falconer, P.D. 1980. Determination of cloudwater acidity at a mountain observatory in the Adirondack Mountains of New York State. Jnl. Geophy. Res. 85: 7465-7470.

Fisher, B.E.A. 1984. Acid rain and where it comes from. The Institute of Mathematics and its Applications (Southend) Vol. 20. 3-7.

Forestry Commission 1985. Forest, health and Air Pollution, 1984 Survey. Forestry Commission, Edinburgh Research and Development paper 142. 17pp.

Forschner, W. and Wild, A. 1988. Comparative investigations on the nutrient composition of healthy and injured spruces of different locations. In: Air Pollution and Ecosystems. Proc, CEC Symposium, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company, Dordrecht. 604-608.

Foster, N.W. 1974. Annual macro-element transfer from Pinus banksiana Lamb. forest to soil. Can. Jnl. For. Res. 4: 470-476.

Foster, N.W. 1985. Acid precipitation and soil solution chemistry within a maple-birch forest in Canada. For. Ecol. and Manage. 12: 215-231.

Fowler, D. 1980. Removal of sulphur and nitrogen compounds from the atmosphere in rain and by dry deposition. In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Norway. Eds. D. Drablos and A. Tollan. SNSF, Oslo. 22-32.

Fowler, D. 1984. Transfer to terrestrial surfaces. Phil. Trans. R. Soc. Lond. B. 305: 281-297.

Fowler, D., Cape, J.N., Nicholson, I.A., Kinnaird, J.W. and Paterson, I.S. 1980. The influence of a polluted atmosphere on cuticle degradation in Scots pine (Pinus sulvestris). In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Sandefjord. Eds. D. Drablos and A. Tollan. SNSF, Oslo. 146-147.

Fowler, D., Cape, J.N., Leith, I.D., Paterson, I.S., Kinnaird, J.W. and Nicholson, I.A. 1982. Rainfall acidity in northern Britain. Nature 297: 383-386.

Francis, A.J., Olson, D. and Bernatsky, R. 1980. Effect of acidity on microbial processes in a forest soil. Proc. Int. Conf. Ecol. Impact Acid Prec. Norway, SNSF Project, Oslo. 166-167.

Freer-Smith, P.H. 1988. Direct effects of dry and wet deposition on forest ecosystems - in particular canopy interactions. In: C.E.C. Proc. Int. Sym. Air Pollution and Ecosystems, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company, Dordrecht. 217-224.

Freiesleben, N.E. and Rasmussen, L. 1986. Effects of acid rain on ion leaching in a Danish forest soil. Water, Air and Soil Pollution. 31: 965-968.

Fröhlich, H. 1984. Report on the stage reached in research on damage to forests. Deutsche Forschungsgemeinschaft, Bonn. 3pp.

Gallagher, M.W., Choularton, T.W., Morse, A.P. and Fowler, D. 1988. Measurements of the size dependance of cloud droplet deposition at a hill site. Quart. Jnl. R. Meteorol. Soc. 114: 1291-1303.

Galloway, J.N. and Parker, G.G. 1980. Difficulties in measuring wet and dry deposition on forest canmopies and soil surfaces. In: Effects of Acid Precipitation on Terrestrial Ecosystems. Eds. T.C. Hutchinson and M. Havas. Plenum Press, New York. 57-68.

Galloway, J.N., Hendrey, G.R., Schofield, C.L., Peters, N.E. and Johannes, A.H. 1987. Processes and causes of lake acidification during spring snowmelt in the west-central Adirondack Mountains, New York. Can. Jnl. Fish Aquat. Sci. 44: 1595-1602.

Garland, J.A. 1971. Some fog droplet size distributions obtained by an impaction method. Quart. Jnl. R. Meteorol. Soc. 97: 483-494.

Garrec, J.P. and Laebens, C. 1988. Effets des "pluies acides" sur les cuticules et les surfaces foliaires. In: Proc. Int. Sym. Air Pollution and Ecosystems, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company, Dordreicht. 704-709.

Garten, C.T. Jnr., Bondietti, E.A. and Lomac, R.D. 1988. Contribution of foliar leaching and dry deposition to sulfate in net throughfall below deciduous trees. Atmos. Environ. 21: 1749-1757.

Gervat, G.P. 1985. Clouds at ground level: samples from the Southern Pennines. CEGB Publication. TPRD/L/2700/N84. 31pp.

Goldsmith, P., Smith, F.B. and Tuck, A.F. 1984. Atmospheric transport and transformation. Phil. Trans. R. Soc. Lond. B 305: 259-279.

Gorham, E. 1958. The influence and importance of daily weather conditions in the supply of chloride, sulphate and other ions to freshwater from atmospheric precipitation. Phil. Trans. R. Soc. London, Ser. B. 241: 147-148.

Goulding, K.W.T., Poulton, P.R., Thomas, V.H. and Williams, R.J.B. 1986. Atmospheric deposition at Rothamstead, Saxmunden and Woburn experimental stations, England 1969-1984. Water, Air and Soil Pollution 29: 27-49.

Grace, J. (1990). Cuticular water loss unlikely to explain tree-line in Scotland. Oecologia 84:64-68.

Granett, A.L. and Musselman, R.C. 1984. Simulated acidic fog injures lettuce. Atmos. Environ. 18: 887-890.

Graustein, W.C. and Armstrong, R.L. 1978. Measurement of dust input to a forested watershed using $^{87}Sr/^{86}Sr$ ratios. Geol. Soc. Am. Abstracts 10: 411.

Greszta, J., Barszcz, J. and Nientur, S. 1989. Evaluation of damage to montane forests in Southern Poland. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on Forest Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR. Birmensdorf. 41-50.

Grill, D., Pfeifhoffer, M., Halbwachs, G. and Waltinger, H. 1987. Investigations on epicuticular waxes of differently damaged spruce needles. European Jnl. Forest. Path. 17: 246-255.

Grunow, J. 1955. Der Niederschlag in Bergwald. Niederschlagszuruckhaltung und Nebel Zuschlag. Forstwiss. Centralblatt 74: 21-36.

Hadley, J.L. and Smith, W.K. 1986. Wind effects needles of timberline conifers: seasonal influence on mortality. Ecology 67: 12-19.

Hahn, J.H., Garrels, R.M., Goldberg, E.D., Hammer, C.U., Lorius, C.,Rudolph, J., Schneider, S.H., Schütz, L., Seiler, W., Stuiver,M., Wagenbach, D., Walker, J.C.G., Wigley, T.M.L., Wright, H.E. Jr. 1982. Changes in atmospheric composition. In: Atmospheric Chemistry: Dahlem Konferenzen, Berlin. Ed. E.D. Goldberg. Springer-Verlag, Berlin. 181-198.

Haines, B.L., Stefani, M. and Hendrix, F. 1980. Acid rain: threshold of leaf damage in eight plant species from a South Appalachian forest succession. Water, Air and Soil Pollution 14: 403-407.

Haines, B.L., Jernstedt, J.A. and Neufeld, H.S. 1985. Direct foliar effects of simulated acid rain. II. Leaf surface characteristics. New Phytologist 99: 407-416.

Hanover, J.W. and Reicosky D.A. 1971. Surface wax deposits on foliage of Picea pungens and other conifers. Amer. Jnl. Bot. 58: 681-687.

Hardwick, R. and Cole, R. 1986. Plastics that kill plants. New Scientist 30: 38-40.

Hart, G.E. and Parent, D.R. 1974. Chemistry of throughfall under Douglas fir and Rocky Mountain juniper. Am. Midland Nat. 92: 191.

Hauhs, M. and Wright, R.F. 1986. Regional pattern of acid deposition and forest decline along a cross section through Europe. Water, Air and Soil Pollution 31: 463-474.

Henderson, G.S., Harris, W.F., Todd, D.E. Jr. and Grizzard, T. 1977. Quantity and chemistry of throughfall as influenced by forest type and season. Jnl. Ecology 65: 365-374.

Hern, J.A., Rutherford, G.K. and Van Loon, G.W. 1985. Chemical and pedogenic effects of simulated acid precipitation on two eastern Canadian forest soils. I. Nonmetals. Can. Jnl. For. Res. 15: 839-847.

Hileman, B. 1983. Acid fog - A new twist in the acid deposition story. Environ. Sci. and Technol. 173: 117A-120A.

Hindawi, I.J. and Ratsch, H.C. 1974. Growth abnormalities of Christmas trees attributed to sulphur dioxide and particulate acid aerosol. For presentation at the 67th Annual Meeting of the Air Pollution Control Association. National Ecological Research Lab. Corvallis, Oregon.

Hindawi,I.J., Rea, J.A. and Griffis, W.L. 1980. Response of bush beans exposed to acid mist. Amer. Jnl. Bot. 67: 168-172.

Hoffman, W.A. Jr., Lindberg, S.E. and Turner, R.R. 1980. Some observations of organic constituents in rain above and below the forest canopy. Environ. Science and Tech. 14: 999-1002.

Höfken, K.D., Meixner, F.X. and Ehhalt, D.H. 1983. Deposition of atmospheric trace constituents onto different natural surfaces. In: Precipitation Scavenging, Dry Deposition and Resuspension. Eds. H.R. Pruppacher, R.G. Semonin and W.G.N. Slinn. Elsevier, New York. 825-835.

Holloway, P.J. 1970. The chemical and physical characteristics of leaf surfaces. In: Ecology of Leaf Surface Micro-organisms. Eds. T.F. Preece and C.H. Dickinson. Academic Press, London. 39-53.

Horntvedt, R. and Joranger, E. 1976. Kjemisk imhold i nedbor under traer og annen vegetasjon: juni-oktober 1974. Technical Report, SNSF Project No 27/76. SNSF, Oslo. 16pp.

Horntvedt, R., Dollard, G.J. and Joranger, E. 1980. Effects of acid precipitation on soil and forest. 2. Atmosphere-vegetation interactions. In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Norway. Eds. D. Drablos and A. Tollan. SNSF Oslo. 192-193.

Hudson, J.G. 1980. Relationship between fog condensation nuclei and fog microstructure. Jnl. Atmos. Sci. 37: 1854-1867.

Hutchinson, T.C. 1987. Workshop Summary. In: Effects of Atmospheric Pollution on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Series Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin XV-XXVII.

Hutchinson, T.C., Adams, C.M. and Gaber, B.A. 1986. Neutralization of acidic raindrops on leaves of agricultural crop and boreal forest species. Water, Air and Soil Pollution 31: 475-485.

Hutchinson, R.C. and Adams, C.M. 1987. Comparative abilities of leaf surfaces to neutralise acidic raindrops. I. The influence of calcium nutrition and charcoal-filtered air. New Phytologist 106: 169-183.

Hütterman, A. 1985. The effects of acid deposition of the physiology of the forest ecosystem. Experientia 41: 584-590.

Huttunen, S. and Laine, K. 1983. Effects of air-borne pollutants on the surface wax structure of Pinus sylvestris needles. Ann. Bot. Fennici 20: 79-86.

Hygen, G. 1951. Studies in plant transpiration I. Physiol. Plant. 4: 57-183.

Hygen, G. and Midgaard, E. 1954. A re-investigation of the influence of varying air humidity on cuticular transpiration in Pinus sylvestris L. Physiol. Plant. 7: 128-140.

Ingersoll, R.C. 1985. Interaction of acid precipitation and tree canopy in four forest ecosystems (Summary). For. Abstracts 46: 647.

Ingestad, T. 1979. Mineral nutrient requirements of Pinus sylvestris and Picea abies seedlings. Physiol. Plant. 45: 373-380.

Innes, J.L. and Boswell, R.C. 1987. Forest health Surveys 1987. Part 1:Results. Forestry Commission Bulletin 74 HMSO. 23pp.

Innes, J.L. and Boswell, R.C. 1988. Forest Health Surveys 1988 - Preliminary Results. Research Information Note 139. Forestry Commission Research Division. 6pp.

Isaac, G.A. and Daum, P.H. 1987. A winter study of air, cloud and precipitation chemistry in Ontario, Canada. Atmos. Environ. 21: 1587-1600.

Jacobsen, J.S. 1984. Effects of acidic aerosol, fog, mist and rain on crops and trees. Phil. Trans. R. Soc. London B. 305: 327-338.

Jacobsen, J.S. and Van Leuken, P. 1977. Effects of acidic precipitation on vegetation. Proc. 4th Int. Clean Air Congress, Tokyo, 1977. 124-127.

Jacobsen, J.S., Troiano, J. and Heller, L. 1985. Stage of development response and recovery of radish plants from episodic exposure to simulated acidic rain. Jnl. Exp. Bot. 36: 159-167.

Jacobsen, J., Irving, P., Kuja, A., Lee, J., Shriner, D., Troiano, Perrigan, S. and Cullinan, V. 1988. A collaborative effort to model plant response to acidic rain. Jnl. Air Poll. Contr. Assoc. 38: 777-783.

Jarvis, P.G., James, G.B. and Landsberg, J.J. 1976. Coniferous forest. In: Vegetation and the Atmosphere. Vol. 2. Ed. J.L. Monteith. Academic Press, London. 171-236.

Jeffree, C.E., Johnson, R.P.C. and Jarvis, P.G. 1971. Epicuticular wax in the stomatal antechamber of Sitka spruce and its effects on the diffusion of water vapour and carbon dioxide. Planta 98: 1-10.

Jeffree, C.E., Baker, E.A. and Holloway, P.I. 1976. Origins of the fine structure of plant epicuticular waxes. In: Microbiology of Aerial Plant Surfaces. Eds. C.F. Dickinson and T.F. Preece. Academic press, London. 119-158.

Jiusto, J.E. 1981. Fog Structure. In: Clouds: Their Formation, Optical Properties and Effects. Eds. P.V. Hobbs and A. Deepak. Academic Press, London. 187-235.

Johannes, A.H., Altwicker, E.R. and Clesceri, N.L. 1985. The integrated lake-watershed acidification study: atmospheric inputs. Water, Air and Soil Pollution 26: 339-353.

Johannessen, M. and Henriksen, A. 1977. Chemistry of snowmelt water: Changes in concentration during melting. SNSF - Project Report FR 11/77, SNSF, Oslo. 11pp.

Johnsen, I., Kielberg, L., Kristiansen, L., Mikkelsen, T.N., Reimer, J. and Ro-poulsen, H. 1987. Effects of ozone, sulphur dioxide, nitrogen dioxide and simulated acid rain on nutrient content and water status on young Picea abies. In: Direct Effects of Dry and Wet Deposition on Forest Ecosystems. In Particular Canopy Interaction. CEC Air Poll. Res. Rep. 4: 71-75.

Johnson, A.H., Siccama, T.G., Wang, D., Turner, R.S. and Barringer, T.M. 1981. Recent changes in patterns of tree growth rate in the New Jersey Pinelands: a possible effect of acid rain. Jnl. Environ. Qual. 10: 427-430.

Johnson, A.H. and Siccama, T.G. 1983. Acid deposition and forest decline. Environ. Science and Tech. 17: 294-305.

Johnson, D.W., Turner, J. and Kelly, J.M. 1982. The effect of acid rain on forest nutrient status. Water Resources Research 18: 449-461.

Johnson, D.W., Richter, D.D., Lovett, G.M. and Lindberg, S.E. 1985. The effects of atmospheric deposition on potassium, calcium and magnesium cycling in two deciduous forests. Can. Jnl. For. Res. 15: 773-782.

Jonsson, B. and Sundberg, R. 1972. Has the acidification by atmospheric pollution caused a growth reduction in Swedish forests? A comparison of growth between regions with different soil properties. Research Note,Institutionen for Skogsproduction, Stockholm, No. 20. 46pp.

Jonsson, B. and Svensson, L.G. 1982. A study of the effects of air pollution on forest yield. A follow-up of the report of Jonsson and Sundberg 1972 and a new study based on forest types. Avdelningen för Skogsuppskattning och Skogsindelning, Sveriges Lantbruksuniversitet. No. 9. 61pp.

Johnstone, K.A. and Johnstone, D.R. 1976. Power requirement and droplet size characteristics of a new ultra-low volume, hand-carried, battery operated insecticide sprayer. Centre for Overseas Pest Research. Misc. Rep. No. 26. College Hse. Wright Lane, London. 14pp.

Juniper, B.E. and Jeffree, C.E. 1983. Plant surfaces. Edward Arnold, London. 93pp.

Kandler, O. 1988. Epidemiologische Bewertung der Waldschadenserhebungen 1983 bis 1987 in der Bundesrepublik Deutschland. Allgemeine Forst-Und Jagdzeitung 159: 179-194.

Kandler, O. 1989. Epidemiological evaluation of the course of "Waldsterben" from 1983 to 1987. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.G. Bucher and I. Bucher-Wallin. SFIFR Birmensdorf. 297-302.

Kandler, O., Miller, W. and Ostner, R. 1987. Dynamik der "akuten Vergilbung" der Fichte: Epidemiologische und Physiologische Befunde. Allgemeine Forst. Zeitschrift 27/28/29. 715-722.

Karhu, M. and Huttunen, S. 1986. Erosion effects of air pollution on needle surfaces. Water, Air and Soil Pollution 31: 417-423.

Kaupenjohann, M., Schneider, B.U., Hantschel, R., Zech, W. and Horn, R. 1988. Sulfuric acid treatment of Picea abies (Karst. L.): Effects on nutrient solution, throughfall chemistry, and tree nutrition. Zeitschrift für Pflanzenernähr und Bodenkunde 151: 123-126.

Ke, J. and Skelly, J.M. 1989. An evaluation of Norway spruce in Northeastern United States. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.G. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 55-60.

Keever, G.J. and Jacobsen, J.S. 1983. Response of Glycine Max (L.) Merrill to simulated acid rain. I. Environmental and morphological influences on the foliar leaching of 86 Rb. Field Crops Research 6: 241-250.

Kelly, J.M. and Strickland, R.L. 1986. Throughfall and plant nutrient concentration response to simulated acid rain treatments. Water, Air and Soil Pollution 29: 219-231.

Kerfoot, O. 1968. Mist precipitation on vegetation. For. Abstracts 29: 8-20.

Kilsby, C.G. and Smith, M.H. 1987. Comparisons of the physical and optical properties of atmospheric aerosol from airborne and surface measurements on the east coast of England. Atmos. Environ. 21: 2233-2246.

Kins, L., Müller, R.P., Ehhalt, D.H. and Meixner, F.X. 1988. Experiment Alsdan (1985): Some preliminary results of height resolved measurements of trace gases aerosol composition, cloud- and precipitation water. In: Acid Deposition at High Elevations. Proc. Nato Conf. Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 375-394.

Klemm, O. and Frevert, T. 1985. Saüre- und redoxchemisches Verhalten von Niederschlagwasser beim Abdampfen von Oberflächen. VDI Berichte 560: 457-463.

Klemm, O., Riederer, M. and Frevert, T. 1987. pH - response on leaves and isolated cuticles of Hedera helix L. after wetting with artificial rainwater. Environ. Exp. Bot. 27: 349-355.

Klemm, O. 1989. "Introduction" and "Can needle surfaces buffer acid rain". In: Forest decline and "Air Pollution". A Study of Spruce (Picea abies) L. on Acid Soils. Eds. E.-D. Schulze, O. Lange and R. Oren. Springer Ecological Studies. Vol. 77. Springer-Verlag, Berlin, Heidelberg. 212-215.

Knollenberg, R.G. 1981. Techniques for probing cloud microstructure. *In*: Clouds: Their Formation, Optical Properties and Effects. Eds. P.V. Hobbs and A. Deepak. Academic Press, London. 15-91.

Krause, G.H.M., Arndt, U., Brandt, C.J., Bucher, J., Kenk, G. and Matzner, E. 1986. Forest decline in Europe: development and possible causes. Water, Air and Soil Pollution *31*: 647-668.

Kreutzer, K. and Bittersohl, J. 1986. Stoffauswaschung aus Fichtenkronen (*Picea abies* L. Karst.) durch saure Beregnung. Forstwiss. Centralblatt *105*: 357-363.

Kuroiwa, D. 1953. The turbulent diffusion of fog water near the ground and the fog-preventing effect of an artificial model forest. *In*: Studies on Fogs in Relation to Fog Preventing Forest. Ed. Hori. Tame Trading Co. Ltd., Sapporo, Japan. 279-302.

Labavitch, J.M. 1981. Cell wall turnover in plant development. Ann. Rev. Plant Physiol. *32*: 385-406.

Lakhani, K.H. and Miller, H.G. 1980. Assessing the contribution of crown leaching to the element content of rainwater beneath trees. *In*: Effects of Acid Precipitation on Terrestrial Ecosystems. NATO Conference. Plenum Press, New York. 161-172.

Lang, G.E., Reiners, W.A. and Shellito, G.A. 1982. Tissue chemistry of *Abies balsamii* and *Betula papyrifera* var. *cordifolia* from subalpine forests of northeastern U.S.A. Can. Jnl. For. Res. *12*: 311-318.

Larsen, B.R. 1988. Chemical and physical processes in acid rain drops on leaf surfaces. In: Acid Deposition at High Elevation. Proc. Nato Conf. Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 651-666.

Läuchli, A. 1976. Apoplasmic transport in tissues. In: Transport in Plants. II. Part B. Tissues and organs. Eds. U. Lüttge and M.G. Pitman. Springer-Verlag. 3-49.

Laurence, J.A., Kohut, R.J. and Amunsden, R.G. 1989. Response of red spruce seedlings exposed to ozone and simulated acidic precipitation in the field. Arch. Environ. Contam. Toxicol. 18: 285-290.

Lauver, T.L. and McCune, D.C. 1984. Kinetics of removal of particulate deposits from foliage in precipitation. In: Second New York Symposium on Atmospheric Deposition. Eds. J.S. Jacobsen and L.S. Raymond Jr. Albany, New York. 83-90.

Leclec, M.Y., Thurtell, G.W. and Gillespie, T.J. 1985. Laboratory simulation of evaporation of water droplets on artificial soybean leaves. Agric. For. Meteorol. 36: 105-111.

Lee, I.J. 1986. Numerical simulation of chemical and physical properties of cumulus clouds. Atmos. Environ. 20: 767-771.

Lee, I.J. and Weber, D.E. 1979. The effect of simulated acid rain on seedling emergence and growth of eleven woody species. Forest Sci. 25: 393-398.

Lefohn, A.S. and Krupa, S.A. 1988. Acidic precipitation. A technical amplification of NAPAP's findings. Jnl. Air Poll. Contr. Assoc. 38: 766-776.

Leith, I.D., Murray, M.B., Sheppard, L.J., Cape, J.N., Deans, J.D., Smith, R.I. and Fowler, D. 1989. Visible foliar injury of red spruce seedlings subjected to simulated acid mist. New Phytologist 113: 313-320.

Ledzian, K.J. 1984. Permeability of plant cuticles to gaseous air pollutants. In: Gaseous Air Pollutants and Plant Metabolism. Eds. M.J. Koziol and F.R. Whatley. Butterworths, London. 77-81.

Leonardi, S. and Flückiger, W. 1987. Short term canopy interactions of beech trees: mineral ion leaching and absorption during rainfall. Tree Physiology 3: 137-145.

Leonardi, S. and Flückiger, W. 1989. Effects of cation leaching on mineral cycling and transpiration: investigations with beech seedlings, Fagus sylvatica L. New Phytologist 111: 173-179.

Lindberg, S.E. and Harriss, R.C. 1981. The role of atmospheric deposition in an eastern U.S. deciduous forest. Water, Air and Soil Pollution 16: 13-31.

Lindberg, S.E. and Garten, C.T. Jr. 1988. Sources of sulphur in forest canpopy throughfall. Nature 336: 148-151.

Linzon, S.N. 1983. Trip report - U.S. - Canadian visit to northeast U.S.A. to view red spruce mortality. Aug. 16-19 1983. Ontario Ministry of Environment Memorandum. Air Resources Branch. 10pp.

Linzon, S.N. 1984. Summary report of visit to West Germany to observe forest damage, Sept 1 - Sept 13, 1984. Ontario Ministry of Environment. Air Resources Branch memorandum. 7pp.

Lockhart, J.A. 1965. An analysis of irreversible plant cell elongation. Jnl. Theoret. Biol. 8: 264-275.

Lovett, G.M. 1984. Rates and mechanisms of cloudwater deposition to a subalpine balsam fir forest. Atmos. Environ. 18: 361-371.

Lovett, G.M., Reiners, W.A. and Olson, R.K. 1982. Cloud droplet deposition in subalpine balsam fir forests: hydrological and chemical inputs. Science 218: 1303-1304.

Lovett, G.M. and Lindberg, S.E. 1984. Dry deposition and canopy exchange in a mixed oak forest as determined by analysis of throughfall. Jnl. Appl. Ecol. 21: 1013-1027.

Lovett, G.M., Lindberg, S.E., Richter, D.D. and Johnson, D.W. 1985. The effects of acidic deposition on cation leaching from three deciduous forest canopies. Can. Jnl. For. Res. 15: 1055-1060.

McColl, J.G. and Johnson, R. 1983. Effects of simulated acid rain on germination and early growth of Douglas fir and Ponderosa pine. Plant and Soil 74: 125-129.

MacDonald, N.W., Hart, J.B. Jr. and Nguyen, P.V. 1986. Simulated acid rain effects on Jack pine seedling establishment and nutrition. Soil Science Society of America Journal 50: 219-225.

McFarlane, J.C. and Berry, W.L. 1974. Cation penetration through isolated leaf cuticles. Plant Physiol. 53: 723-727.

McLaughlin, S.B. 1985. Effects of air pollution on forests. A critical review. Jnl. Air Poll. Contr. Assoc. 35: 512-534.

Mahendrappa, M.K. 1983. Chemical characteristics of precipitation and hydrogen input in throughfall and stemflow under some eastern Canadian forest stands. Can. Jnl. For. Res. 13: 948-955.

Mallant, R.K.A.M. 1988. A fog chamber and wind tunnel facility for calibration of cloudwater collectors. In: Acid Deposition at High Elevations. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 479-490.

Mansfield, T.A., Lucas, P.W. and Wright, E.A. 1988. Interactions between air pollutants and other limiting factors. In: Proc. Int. Sym. Air Poll. and Ecosystems, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company, Dordrecht. 123-141.

Marloth, R. 1905. Results of further experiments on Table Mountain for ascertaining the amount of moisture deposited from the south-east clouds. Trans. S. Afr. Phil. Soc. Cape Town 16: 97-105.

Martin, J.T. and Juniper, B.E. 1970. The Cuticles of Plants. Edward Arnold, London. 347pp.

Matziris, D.I. and Nakos, G. 1978. Effect of simulated "acid rain" on juvenile characteristics of Aleppo pine (Pinus halepensis Mill). For. Ecol. and Manage. 1: 267-272.

Matzner, E. and Ulrich, B. 1985. 'Waldsterben': Our dying forests - Part II. Implications of the chemical soil conditions for forest decline. Experiertia 41: 578-584.

Matzner, E. and Ulrich, B. 1987. Results of studies on forest decline in Northwest Germany. In: Effects of Atmospheric Pollution on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Series. Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin. 25-42.

Matzner, E., Blank, K. and Hartmann, G. 1989. Needle chlorosis pattern in relation to soil chemical properties in two Norway spruce forests of the German Harz Mountains. In: Air Pollution and Forest Deckline. Proc. 14th Int. Meet. Spec. Air. Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 195-200.

May, K.R. 1945. The cascade impactor: An instrument for sampling course aerosols. Jnl. Sci. Instr. 22: 187-195.

Mayer, R. and Ulrich, B. 1978. Input of atmospheric sulphur by dry and wet deposition to two central European forest ecosystems. Atmos. Environ. 12: 375-377.

Mecklenburg, R.A. and Tukey, H.B. Jr. 1964. Influence of foliar leaching on root uptake and translocation of calcium - 45 to the stems and foliage of Phaseolus vulgaris. Plant Physiol. 39: 533-536.

Mecklenburg, R.A., Tukey, H.B. Jr. and Morgan, J.V. 1966. A mechanism for the leaching of calcium from foliage. Plant Physiol. 41: 610-613.

Mengel, K., Breininger, M. Th. and Lutz, H.J. 1988. Effect of acidic mist on nutrient leaching, carbohydrate status and damage symptoms of Picea abies. In: Proc. Int. Sym. Air Poll. and Ecosystems, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company, Dordrecht. 312-320.

Mengel, K., Hogreke, M.R. and Esch, A. 1989. Effect of acidic fog on needle surfaces and water retention of Picea abies. Physiol. Plant. 75: 201-207.

Miller, D.H. 1977. Water at the Surface of the Earth: An Introduction to Ecosystem Hydrodynamics. Academic Press, London. 557pp.

Miller, H.G. 1987. Effects of dry and wet deposition on tree canopies - in particular mineral and nutritional status. In: Direct Effects of Dry and Wet Deposition on Forest Ecosystems - In particular Canopy Interactions. CEC Air Poll. Res. Rep. 4. EUR 11264. 10-24.

Miller, H.G., Miller, J.D. and Cooper, J.M. 1981. Optimum foliar nitrogen copncentration in pine and its change with stand age. Can. Jnl. For. Res. 11: 563-572.

Miller, H.G. and Miller, J.D. 1987. Nutritional requirements of Sitka spruce. In: Sitka spruce. Proc. Royal Soc. Edinburgh, 93B. Eds. D.M. Henderson and R. Faulkner. 75-83.

Miller, H.G., Miller, J.D. and Cooper, J.M. 1987. Transformations in rainwater chemistry on passing through forested ecosystems. In: Pollutant Transport and Fate in Ecosystems. Special Pub. Br. Ecol. Soc. No. 6. Eds. P.J. Coughtrey, M.H. Martin and M.H. Unsworth. Blackwell Scientific Publications, Oxford. 171-180.

Miller, P.R. and McBride, J.R. 1989. Trends of ozone damage to conifer forests in the Western United States, particularly Southern California. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 61-68.

Milne, R., Crossley, A. and Unsworth, M.H. 1988. Physics of cloudwater deposition and evaporation at Castlelaw South-East Scotland. In: Acid Deposition at High Elevations. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 299-307.

Morrison, I.K. 1984. Acid Rain. A review of literature on acid deposition effects in forest ecosystems. For. Abstracts 45: 483-506.

Mrose, H. 1966. Measurements of pH, and chemical analysis of rain-, snow- and fog-water. Tellus 18: 266-270.

Musselman, R. and Sterrett, J.L. 1988. Sensitivity of plants to acidic fog. Jnl. Environ. Qual. 17: 329-333.

Munzert, K. 1988. Chemistry of cloudwater and precipitation at an alpine mountain station. In: Acid Deposition at High Elevations. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler, Kluwer Academic Publishers, Dordrecht. 395-401.

Nagel, J.F. 1956. Fog precipitation on Table Mountain. Quart. Jnl. R. Meteorol. Soc. 82: 452-460.

Neufeld, H.J., Jernstedt, J.A. and Haines, B.L. 1985. Direct foliar effects of simulated acid rain. I. Damage, growth and gas exchange. New Phytologist 99: 389-405.

Nicholls, S. 1984. The dynamics of stratocumulus: aircraft observations and comparisons with a mixed layer model. Quart. Jnl. R. Meteorol. Soc. 110: 783-820.

Nicholls, S. and Leighton, J. 1986. An observational study of the structure of stratiform cloud sheets. Part 1. Structure. Quart. Jnl. R. Meteorol. Soc. 112: 431-460.

Nihlgärd, B. 1970. Precipitation, its chemical composition and effect on soil water in a beech and a spruce forest in southern Sweden. Oikos 21: 208-217.

Nöjd, P. 1989. The correlation between estimated needle loss and diameter investment - preliminary results. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR. Birmensdorf. 489.

Noonkester, V.R. 1984. Droplet spectrum observed in marine stratus cloud layers. Jnl. Atmos. Sci. 41: 829-845.

Oberlander, G.T. 1956. Summer fog precipitation on the San Francisco peninsula. Ecology 37: 851-852.

Oden, S. 1968. The acidification of air and precipitation and its consequences in the natural environment. Ecology Committee Bulletin No. 1. Swedish National Science Research Council, Stockholm. Translation Consultants Ltd., Arlington, Va.

Oden, S. 1976. The acidity problem - an outline of concepts. Water, Air and Soil Pollution 6: 137-166.

Oertli, J.J., Harr, J. and Goggenheim, R. 1977. The pH value as an indication for the leaf surface microenvironment. Zeirschrift für Pfanzenkrankheiten und Pflanzenschutz 84: 729-737.

Ogren, J.A., Heintzenberg, J., Zuber, A., Hansson, H.-C., Noone, K.J., Covert, D.S. and Charlson, R.J. 1988. Measurements of the short-term variability of aqueous-phase mass concentrations in cloud droplets. In: Acid Deposition at High Elevation. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler, Kluwer Academic Publishers, Dordrecht. 125-136.

Overrein, L., Seip, H.M. and Tollan, A. 1980. Acid precipitation effects on forests and fish. Final report, SNSF Project, Oslo, Norway.

Paparozzi, E.T. and Tukey, J.B. Jr. 1983. Developmental and anatomical changes in leaves of Yellow Birch and Red Kidney Bean exposed to simulated acid precipitation. Jnl. Am. Soc. Hort. Sci. 108: 890-898.

Parsons, J.J. 1960. 'Fogdrip' from coastal stratus, with special reference to California. Weather 15: 58-62.

Pell, E.J. and Puente, M. 1986. Emission of ethylene by oat plants treated with ozone and simulated acid rain. New Phytologist 103: 709-715.

Penman, H.L. 1948. Natural evaporation from open water, bare soil and grass. Proc. Roy. Soc. A 193: 120-145.

Penman, H.L. 1963. Vegetation and Hydrology. Tech. Comm. No 53 Commonwealth Bureau of Soils. 124pp.

Percy, K. 1986. The effects of simulated acid rain on germinative capacity, growth and morphology of forest tree species. New Phytologist 104: 473-684.

Percy, K.E. 1987. Effects of simulated acid rain on leaf cuticular characteristics and surface properties. Ph.D. thesis submitted to University of Bristol. 172pp.

Percy, K.E. and Riding, R.T. 1978. The epicuticular waxes of Pinus strobus subjected to air pollutants. Can. Jnl. For. Res. 8: 474-477.

Percy, K.E. and Baker, E.A. 1987. Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. New Phytologist 107: 577-589.

Percy, K. and Baker, E.A. 1988. Effect of simulated acid rain on leaf wettability, rain retention and uptake of some inorganic ions. New Phytologist 108: 75-82.

Pfirrmann, T., Runkel, K.H., Albrecht, U., Payer, H.D. and Blank, L.W. 1988. Foliar leaching of Norway spruce during 14 months exposure to ozone and acid mist. In: Scientific Basis of Forest Decline Sumptomatology. Rep. No. 15 Air Pollution Report Series, Environ. Res. Prog. C.E.C. Eds. J.N. Cape and P. Mathy. 354-357.

Prinz, B. 1983. Gedanken zum Stand der Diskussion über die Urasche der Waldschäden in der Bundesrepublik Deutschland. Forst-und Holzwirt 38: 460-466.

Prinz, B., Krause, G.H.M. and Jung, K.-D. 1987. Development and causes of novel forest decline in Germany. In: Effects of Atmospheric Pollution on Forests, Wetlands and Ahricultural Ecosystems. NATO ASI Series Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin. 1-24.

Prinz, B. and Krause, G.H.M. 1989. State of scientific discussion about the causes of the novel forest decline in the Federal Republic of Germany and surrounding countries. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 27-34.

Puckett, L.J. 1982. Acid rain, air pollution and tree growth in Southeastern New York. Jnl. Environ. Qual. 11: 376-381.

Rayle, D.L. and Cleland, R. 1970. Enhancement of wall loosening and elongation of acid solution. Plant Physiol. 46: 250-253.

Raynal, D.J., Roman, J.R. and Eichenlaub, W.M. 1982. Response of tree seedlings to acid precipitation - II. Effects of simulated acidified canopy throughfall on sugar maple seedling growth. Environ. Exp. Bot. 22: 385-392.

Rehfuess, K.E. 1987. Perceptions on forest diseases in central Europe. Forestry 60: 1-11.

Reich, P.B., Schoettle, A.W. and Amundson, R.G. 1986. Effects of ozone and acidic rain on photosynthesis and growth in sugar maple and Northern red oak seedlings. Environ. Poll. (Ser. A) 40: 1-15.

Reiners, W.A. and Olson, R.K. 1984. Effect of canopy components on throughfall chemistry. Oecologia 63: 320-330.

Reiners, W.A., Lovett, G.M. and Olsen, R.K. 1987. Chemical interaction of a forest canopy with the atmosphere. In: Proc. Forest-Atmosphere Interaction Workshop, New York. USDE Conf. 8510250. 247 pp.

Rentschler, I. 1973. Die Bedentung der Wachsstruktur auf den Blättern für die Empfindlichkeit der Pflanzen gegenüber Luftverunreinigungen. In: Proc. 3rd Int. Clean Air Congress. Ed. H Schackmann. A139-A142.

Richardson, D.H.S. and Dowding, P. 1988. The processes involved in the leaching of leaves under non-polluted conditions and the influence of leachates on leaf-surface micro-organisms. In: Scientific basis of forest decline symptomatology. Rep. No. 15. Air. Poll. Report Series. Environ. Res. Prog. CEC. Eds. J.N. Cape and P. Mathy. 133-148.

Rinallo, C., Raddi, P., Gellini, R. and DiLonardo, V. 1986. Effects of simulated acid deposition on the surface structure of Norway spruce and Silver fir needles. European Jnl. For. Path. 16: 440-446.

Roberts, T.M. 1987. Effects of air pollutants on agriculture and forestry. CEGB Research Note 20: 39-52.

Roberts, T.M. and Blank, L.W. 1985. The 1985 German Forest Decline Inventory and a report on a visit to sites showing forest decline symptoms in Southern Germany. CEGB Memorandum TPRD/BY/084/M85.

Roberts, T.M., Skeffington, R.A. and Blank, L.W. 1989a. Causes of Type 1 spruce decline in Europe. Forestry 62: 179-222.

Roberts, T.M., Skeffington, R.A., Brown, K.A. and Blank, L.W. 1989b. Interactive effects of O_3, acid mist and cold stress on Norway spruce. In: Woody Plant Growth in a Changing Physical and Chemical Environment. Ed. D.P. Lavender. IUFRO Vancouver: University of British Columbia. 109-133.

Rutherford, G.K., VanLoon, G.W., Mortensen, S.F. and Hern, J.A. 1985. Chemical and pedogenic effects of simulated acid precipitation on two eastern Canadian forest soils. II. Metals. Can. Jnl. For. Res. 15: 848-854.

Scherbatskoy, T. and Klein, R. 1983. Response of spruce and birch foliage to leaching by acidic mist. Jnl. Environ. Qual. 12: 189-195.

Schier, G.A. 1986. Seedling growth and nutrient relationships in a New Jersey pine Barrens soil treated with "acid rain". Can. Jnl. For. Res. 16: 136-142.

Schier, G.A. 1987. Germination and early growth of four pine species on soil treated with simulated acid rain. Can. Jnl. For. Res. 17: 1190-1196.

Schlesinger, W.H. and Reiners, W.A. 1974. Deposition of water and cations on artificial foliar collectors in fir krummholz of New England mountains. Ecology 55: 378-386.

Schmid-Haas, P. 1989. Do the observed needle losses reduce increments? In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on Forest Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 271-275.

Schmitt, G. 1988. Measurements of the chemical composition in cloud and fogwater. In: Acid Deposition at High Elevation Sites. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 517-539.

Schmitt, U., Ruetze, M. and Liese, W. 1987. Scanning electron microscopical investigations on stomatal wax plugs of fir and spruce needles after fumigation and acid rain treatment. European Journal of Forest Pathology 17: 118-124.

Scholz, F. and Reck, S. 1977. Effects of acids on forest trees as measured by titration *in vitro*, inheritance of buffering capacity in *Picea abies*. Water, Air and Soil Pollution 8: 41-45.

Schönherr, J. 1976. Water permeability of isolated cuticular membranes. The effect of pH and cations on diffusion, hydrodynamic permeability and size of polar pores in the cutin matrix. Planta 128: 113-126.

Schönerr, J. 1982. Resistance of plant surfaces to water loss: transport properties of cutin, suberin and associated lipids. In: Physiological Plant Ecology II Vol 12B. Water Relations and Carbon Assimilation. Eds. O. Lange, P.S. Novel, C.B. Osmond and H. Ziegler. 154-179.

Seip, H.M. 1980. Acid snow - snowpack chemistry and snowmelt. In: Effects of Acid Deposition on Terrestrial Ecosystems. Eds. T.C. Hutchinson and M.J. Havas. Plenum, London. 77-94.

Semb, A. and Dovland, H. 1986. Atmospheric deposition in Fenno-Scandia. Characteristics and trends. Water, Air and Soil Pollution 30: 5-16.

Seufert, G. 1987. Effects of long-term applications of SO_2, O_3 and acid rain on mineral cycling of tree seedlings in model ecosystems. In: Direct Effects of Dry and Wet Deposition on Forest Ecosystems - In Particular Canopy Interactions. C.E.C. Air Pollution Research Report 4 EUR 11264. 51-59.

Seufert, G., Arndt, U., Jäger, H.-J. and Bender, J. 1989. Long-term effects of air pollutants on forest trees in open-top chambers. I: Experimental approach and results on mineral cycling. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 159-166.

Shriner, D.S. 1978. Effects of simulated acid rain on host-parasite interactions in plant diseases. Phytopathology 68: 213-218.

Shriner, D.S., Decot, M.E. and Cowling, E.B. 1975. Simulated acid precipitation causes direct injury to vegetation (Abstract). For. Abstracts 1976 37: 149.

Shuttleworth, W.J. 1977. The exchange of wind driven fog and mist between vegetation and the atmosphere. Boundary-layer Meteorology 12: 463-489.

Skeffington, R. and Roberts, M. 1985a. Effects of ozone and acid mist on Scots pine and Norway spruce - an experimental study. In: VDI Berichte 560 "Waldschäden (1985) VDI - Verlag, Dusseldorf. 747-760.

Skeffington, R. and Roberts, M. 1985b. The effects of ozone and acid mist on Scots pine saplings. Oecologia 65: 201-206.

Skeffington, R.A. and Brown, L.A. 1986. The effect of five years acid treatment on leaching, soil chemistry and weathering of a humo-ferric soil. Water, Air and Soil Pollution 31: 891-900.

Skiba, U., Peirson-Smith, T.J. and Cresser, M.S. 1986. Effects of simulated precipitation acidified with sulphuric and/or nitric acid on the throughfall chemistry of Sitka spruce Pinus sitchensis and heather Calluna vulgaris. Environ. Poll. (Series B) 11: 255-270.

Slingo, A., Nicholls, S. and Schmetz, J. 1982. Aircraft observations of marine stratocumulus during JASIN. Quart. Jnl. R. Meteorol. Soc. 108: 833-838.

Slinn, W.G.N. 1982. Some influences of the atmospheric water cycle on the removal of atmospheric trace constituents. In: Atmospheric Chemistry: Dahlem Workshop on Atmospheric Chemistry, Berlin. Ed. E.D. Goldberg. Springer-Verlag, Berlin: 57-90.

Stephens, G.L. and Platt, C.H.R. 1987. Aircraft observations of the radiative and microphysical properties of stratocumulus and cumulus cloud fields. Jnl. Clim. Appl. Meteor. 26: 1243-1269.

Stewart, J.B. 1977. Evaporation from the wet canopy of a pine forest. Water Resources Research 13: 915-921.

Strand, L. 1980. The effect of acid precipitation on tree growth. In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Sandefjord, Norway. Eds. D. Drablos and A. Tollan. SNSF, Oslo. 64-67.

Stuanes, A.O. 1980. Effects of acid precipitation on soil and forests. 5. Release and loss of nutrients from a Norwegian forest soil due to artificial rain of varying acidity. Proc. Int. Conf. Ecol. Impact Acid Prec. Norway, SNSF Project Oslo. 198-199.

Sud, G. and Bhutani, V.P. 1988. Effects of soil and foliar applications of urea on productivity and the tree nitrogen status of apricot. Jnl. Hort. Sci. 63: 583-585.

Sweden, 1971. Air pollution across national boundaries. The impact on the environment of sulfur in air and precipitation. Sweden's Case Study for the United Nations conference on the human environment. Stockholm 1971.

Temple, P.J. 1988. Injury and growth of Jeffrey pine and Giant sequoia in response to ozone and acidic mist. Environ. Exp. Bot. 28: 323-333.

Thompson, F.B. and Leyton, L. 1971. Method for measuring the leaf surface area of complex shoots. Nature 229: 572.

Tomlinson, G.H. 1987. Acid deposition, nutrient imbalance and tree decline. In: Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. NATO ASI, Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer Verlag, Berlin. 189-199.

Topol, L.E. 1986. Differences in the ionic composition and behaviour in winter rain and snow. Atmos. Environ. 20: 347-360.

Tranter, M., Brimblecombe, P., Davies, T.D., Vincent, C.E., Abrahams, P.W. and Blackwood, I. 1986. The composition of snowfall, snowpack and meltwater in the Scottish Highlands - evidence for preferential elution. Atmos. Environ. 20: 517-525.

Tranter, M., Davies, T.D., Brimblecombe, P. and Vincent, C.E. 1987. The composition of acidic meltwaters during snowmelt in the Scottish Highlands. Water, Air and Soil Pollution. 36: 75-90.

Tukey, H.B. Jr. 1970. The leaching of substances from plants. Ann. Rev. Plant Physiol. 21: 305-324.

Turner, D.P., Tingey, D.T. and Hogsett, W.E. 1989. Acid fog effects on conifer seedlings. In: Air pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. I.B. Bucher and I. Bucher-Wallin. SFIFR. Birmensdorf. 125-130.

Turunen, M. and Huttunen, S. 1989. The pH and structure of the needle surfaces of Scots pine and Norway spruce seedlings after acid precipitation. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on For. Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin, SFIFR, Birmensdorf. 535-537.

Tveite, B. 1980. Effects of acid precipitation on soil and forest. 8. Foliar nutrient concentrations in field experiments. In: Ecological Impact of Acid Precipitation. Proc. Int. Conf. Sandefjord, Norway. Eds. D. Drablos and A. Tollan. SNSF, Oslo. 204-205.

Ulrich, B. 1980. Production and consumption of hydrogen ions in the ecosphere. In: Proc. NATO Conf. Effects of Acid Precipitation on Terrestrial Ecosystems. Eds. T.C. Hutchinson and M. Havas. Plenum Press, New York. 255-282.

Ulrich, B. 1982. Dangers for the forest ecosystem from acid precipitation. Land Institute for Ecology, Landscape Development and Forestry Planning: Pollution Loading on Forest Ecosystems. Special Publication. LOLF - Mitteilungen Sanderheft, Recklinghausen. 9-25.

Ulrich, B. 1985. Interaction of indirect and direct effects of air pollutants in forests. In: Air Pollution and Plants. Proc. 2nd Eur. Conf. Chem. Environ. Ed. C. Troyanowsky. Lindau, VCH, Weinheim. 151-181.

Ulrich, B., Mayer, R. and Khanna, P.K. 1980. Chemical changes due to acid precipitation in a losses-derived soil in Central Europe. Soil Science 130: 193-199.

Ulrich, B., Benecke, P., Harris, W.F., Khanna, P.K. and Mayer, R. 1981. Soil processes. In: Dynamic Properties of Forest Ecosystems. IBP23 C.U.P., Cambridge.

Unsworth, M.H. 1983. Acidic inputs to the natural environment and their effects. All-Party Conservation Committee, 9 Feb. 1983, Summary notes.

Unsworth, M.H. 1984. Evaporation from forests in cloud enhances the effects of acid deposition. Nature 312: 262-264.

Unsworth, M.H. and Crossley, A. 1987a. Consequences of cloud water deposition on vegetation at high elevation. In: Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin. 171-188.

Unsworth, M.H. and Crossley, A. 1987b. The Capture of wind-driven cloud by vegetation. In: Pollutant Transport and Fate in Ecosystems. Special publication series of the Br. Ecol. Soc. No. 6. Eds. P.J. Coughtrey, M.H. Martin and M.H. Unsworth. Blackwell Sc. Pub. 125-138.

Van Elsecker, P. and Impens, I. 1987. Direct effects of simulated acid wet deposition on gas exchange of Norway spruce. In: Direct Effects of Dry and Wet Deposition on Forest Ecosystems. In Particular Canopy Interactions. CEC Air Poll. Res. Rep. 4. Proc. Workshop Lökeburg. 122-131.

Van Elsecker, P., Martens, C. and Impens, I. 1988. Direct effects of acid wet deposition on photosynthesis, stomatal conductance and growth of *Populus* c.v. Beaupre. In: Proc. Int. Sym. Air Poll. and Ecosystems, Grenoble. Ed. P. Mathy. D. Reidel Publishing Company Dordrecht. 728-732.

Van Loon, G.W. 1984. Acid rain and soil. Canadian Jnl. Physiol. Pharmacology 62: 991-997.

Van Volkenburgh, E. and Cleland, R.E. 1980. Proton excretion and cell expansion in bean leaves. Planta 148: 273-278.

Van Volkenburgh, E., Cleland, R.E. and Schmidt, M.G. 1985. The mechanisms of light stimulated leaf cell expansion. In: Control of Leaf Growth. Eds. N.R. Baker, W.J. Davies and C.K. Ong. CUP, Cambridge. 223-237.

Verry, E.S. and Timmons, D.R. 1977. Precipitation nutrients in the open and under two forests in Minnesota. Can. Jnl. For. Res. 7: 112-119.

Vogelmann, H.W., Siccama, T., Leedy, D. and Ovitt, D.C. 1968. Precipitation from fog moisture in the Green Mountains of Vermont. Ecology 49: 1205-1207.

Waldman, J.M., Munger, J.W., Jacob, D.J., Flagan, R.C., Morgan, J.J. and Hoffmann, M.R. 1982. Chemical composition of acid fog. Science 218: 677-680.

Walker, N.A. and Pitman, M.G. 1976. Measurements of fluxes across membranes. In: Transport in Plants II. Part A Cells. Eds. U. Lüttge and M.G. Pitman. Springer-Verlag, Berlin. 93-126.

Ward, R.C. 1975. Principles of hydrology. 2nd edition. McGraw Hill, Maidenhead. 367pp.

Watt (1984). Acid rain. Papers presented at the Fifteenth Consultative Council meeting of the Watt Committee on Energy, London, 1983. The Watt Committee on Energy Report No 14. 58pp.

Wattendorf, J. and Holloway, P.J. 1980. Studies on the ultrastructure and histochemistry of plant cuticles: the cuticular membrane of Agave americana L. in situ. Ann. Botany 46: 13-28.

Weathers, K.C., Likens, G.E., Bormann, F.H., Eaton, J.S., Kimball, K.D., Galloway, J.N., Siccama, T.G. and Smiley, D. 1988. Chemical concentrations in cloud water from four sites in the eastern United States. In: Acid Deposition at High Elevation. Proc. NATO Workshop, Edinburgh. Eds. M.H. Unsworth and D. Fowler. Kluwer Academic Publishers, Dordrecht. 345-357.

Wellburn, A.R. and Wolfenden, J. 1987. Internal pH changes within vegetation in response to acidic precipitation as revealed by high-field ^{31}P-NMR. In: Direct Effects of dry and wet deposition in forest ecosystems - In particular canopy interactions. C.E.C. Air Poll. Res. Report 4. Proc. Workshop Lökeburg 1986. 161-166.

Westmann, W.E. and Temple, P.J. 1989. Acid mist and ozone effects on the leaf chemistry of two western conifer species. Environ. Poll. 57: 9-26.

Wild, A., Woodhouse, P.J. and Hopper, M.J. 1979. A comparison between the uptake of K by plants from solutions of constant K concentration and during depletion. Jnl. Exp. Bot. 30: 697-704.

Wittwer, S.H. and Teubner, F.G. 1969. Foliar absorption of mineral nutrients. Ann. Rev. Plant Physiol. 10: 13-32.

Wolfenden, J. and Wellburn, A.R. 1986. Cellular re-adjustments of barley seedlings to simulated acid rain. New Phytologist 104: 97-109.

Wood, F.A. and Pennypacker,S.P. 1976. Etiological study of the needle disease of pine. Proceedings of the American Phytopathological Society 1: 977-978.

Wood, T. and Bormann, F.H. 1974. The effects of an artificial acid mist upon the growth of Betula alleghaniensis Britt. Environ. Poll. 7: 259-268.

Wood, T. and Bormann, F.H. 1975. Increases in foliar leaching caused by acidification of an artificial mist. Ambio 4: 169-171.

Wood T. and Bormann, F.H. 1977. Short-term effects of a simulated acid rain upon the growth and nutrient relations of Pinus strobus L. Water, Air and Soil Pollution 7: 479-488.

Woodhouse, P.J., Wild, A. and Clement, C.R. 1978. Rate of uptake of potassium by three crop species in relation to growth. Jnl. Exp. Bot. 29: 885-894.

Wright, E.A. and Lucas, P.W. Changes in epidermal conductance of birch and grasses exposed to SO_2 and NO_2. In: Meeting of the Committee for Air Pollution Effects Research (CAPER). Lancaster 1987. Summaries of reports. 12.

WSL 1987. United Kingdom Acid Rain Monitoring. Warren Spring Laboratory 8pp.

Yawney, H.W., Leaf, A.L. and Leanard, R.E. 1978. Nutrient content of throughfall and stemflow in fertilized and irrigated Pinus resinosa AIT. Stands. Plant and Soil 50: 433-445.

Yoshie, F. and Sakai, A. 1985. Types of florin rings, distributional patterns of epicuticular wax and their relationships in the genus Pinus. Can. Jnl. Bot. 63: 2150-2158.

Ziegler, M. 1987. Interactive effects of different air pollutants on structure and function of tree leaves. In: Proc. C.E.C. Workshop Direct Effects of Dry and Wet Deposition on Forest Ecosystems. Lökeburg. C.E.C. Brussels. 102-109.

Zöttl, H.W. 1987. Responses of forests in decline to experimental fertilization. In: Effects of Atmospheric Pollution on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Vol. G16. Eds. T.C. Hutchinson and K.M. Meema. Springer-Verlag, Berlin 255-266.

Zöttl, H.W. and Hüttl, R.F. 1986. Nutrient supply and forest decline in south-west Germany. Water, Air and Soil Pollution. 31: 449-462.

Zöttl, H.W. and Hüttl, R.F. 1989. Nutrient deficiencies and forest decline. In: Air Pollution and Forest Decline. Proc. 14th Int. Meet. Spec. Air Poll. Effects on Forest Ecosystems. IUFRO P2.05, Interlaken. Eds. J.B. Bucher and I. Bucher-Wallin. SFIFR, Birmensdorf. 189-194.

APPENDIX 1

Cloud Simulation

A1.1 Introduction

In the simulation of a cloud episode, the amount of water delivered, the delivery period and droplet size spectrum should all be controlled. In this appendix, research into cloud microphysics and chemistry and cloud capture by vegetation, as well as previous simulations of precipitation are discussed. Conclusions are drawn as to appropriate simulation methodology for the series of experiments undertaken in this study and the experimental system adopted and its performance are described.

A1.2 Cloud droplet size

The choice of droplet size in the application of simulated acid cloud (SAC) is important for several reasons. The capture of cloud droplets by vegetation varies with droplet size. There are two main aspects: impaction efficiency and droplet retention. Impaction efficiency increases with droplet size as smaller droplets tend to follow the air flow round an obstacle and are captured less efficiently than larger droplets, which tend to leave the flow lines and impact with the obstacle, in this case a needle (Chamberlain 1975, Shuttleworth 1977, Chamberlain and Little 1981).

However, the kinetic energy of the droplet must be dissipated on impact and in small droplets this energy can be consumed in heat and plastic deformation, whereas in large droplets the energy is often used in fragmentation and deflection. Thus small droplets tend to be retained at the primary contact sites whereas larger droplets tend to bounce off (Baker and Hunt 1986, Cape 1988a, Larsen 1988). Potential damage will thus be altered by an unrealistic droplet size spectrum affecting the amount of SAC captured.

Surface wetting will also be determined by the spreading of the droplet. The larger droplets that impact and adhere to the needle surfaces will spread and cover a larger area with a smaller contact angle. This will affect the potential area of damage and will also govern the area available for leaching to take place.

Droplet size, in particular the surface/volume ratio, also affects the subsequent concentrating effect as droplets evaporate on the needles. The degredation of wax structure is also affected by droplet size as impacting and deflecting droplets remove significant amounts of epicuticular wax from leaf surfaces (Baker and Hunt 1986). Cloud droplet size must therefore be characterised and then simulated satisfactorily.

Droplet sizes are presented using various definitions. The complete range of droplet diameters, or radii, may be given, with or without the mean. The mode and median and effective diameter are also used, but the predominant term used in cloud microphysics is the mean volume diameter (or radius) which is the diameter (or radius) of a droplet whose volume is equal to the average droplet volume. If there is a large variation in droplet size and particularly if artificial spraying techniques produce secondary droplets of small size, the mean volume diameter may be much larger than the mean diameter (e.g. Evans et al. 1977). When droplet sizes are fairly uniform the various definitions will approximate to the same value.

Early estimations of droplet size were generally made using impaction methods in which either an instrument was passed through cloudwater, or the air itself was drawn into the sampling device (May 1945, Knollenberg 1981). If air is drawn into the instrument anisokinetic sampling and errors in estimating droplet concentration values may occur. Evaporation in the air flow, difficulties in resolution of small droplets and droplet impaction on equipment walls may also lead to serious underestimation of small droplets. Garland (1971) partly overcame these difficulties by mounting the impactor inside a wind tunnel which orientated into the wind, and by using Nomarski interference contrast microscopy to enhance detection of small droplets. His results indicated a large number of very small droplets (<2 µm diamter) in fog, and frequently a bimodal distribution of diameter around 2 µm and 20 µm.

Other droplet sizing methods such as holography, cloud particle cameras, electrostatic methods and spider web microthreads may be used (Garland 1971, Knollenberg 1981, Barrett et al. 1988) but the main recent development is the use of optical particle counters. Optical array probes (OAP) use an illumination source to cast a shadow from the droplets over light-sensitive elements. OAP measure larger cloud droplets and rain droplets (usually by using two probes, covering the ranges 12.5 - 400 µm and 400 - several thousand µm (Knollenberg 1981, Nicholls 1984, Nicholls and Leighton 1986, Isaac and Daum 1987, Stephens and Platt 1987). Scattering spectrometers, the most common of which are the axially scattering spectrometer probe (A.S.S.P.) and the forward scattering spectrometer probe (F.S.S.P.), can be used to measure droplets down to submicron size (Knollenberg 1981, Slingo et al. 1982, Cocks et al. 1983, Noonkester 1984, Nicholls and Leighton 1986, Kilsby and Smith 1987). Using a variety of optical counters mean cloud droplet diameters can be found. Representative cloud droplet diameters are 8-20 µm (effective diameter) for cumulus (Stephens and Platt 1987), 10-20 µm (mode diameter) (Slingo et al. 1982), 14-22 µm (effective diameter) (Stephens and Platt 1987), and 12-20 µm (mean volume diameter) (Nicholls 1984) for stratocumulus; and 6 µm (mean volume diameter) (Cocks et al. 1983), 10-40 µm (mean volume diameter) (Nicholls and Leighton 1986) and 1-12 µm (range) (Noonkester 1984) for stratus. Maritime clouds generally have larger diameters than clouds from continental air (Jiusto 1981, Noonkester 1984, Stephens and Platt 1987). Droplet diameter also tends to increase with height above the cloud base by adiabatic growth and coalescence, although not always as much as theoretical adiabatic values, resulting because of the effect of entrainment of dry air from above (Slingo et al. 1982, Nicholls 1984, Noonkester 1984, Nicholls and Leighton 1986, Stephens and Platt 1987).

Stratocumulus is the most common cloud type in Britain (Davies 1972 in Nicholls 1984) and is the type most frequently experienced at the Dunslair Height field site and from the above discussion a droplet diameter range of 5-25 µm would appear to be representative.

There is a large difference in size between cloud droplet diameter and the diameter of rain droplets the minimum diameter of which is estimated at 50-300 µm (Ekern 1964, Knollenberg 1981, Jacobsen 1984, Nicholls 1984) and the mean diameter being of several hundreds or thousands of microns (Jacobsen 1984, Nicholls 1984).

Most experiments involving simulated acid precipitation have used rain droplets with diameters ranging from 200-2000 µm (e.g. Wood and Bormann 1977, Haines et al. 1985, Jacobsen et al. 1985, Caporn and Hutchinson 1986, Percy 1986, Reich et al. 1986, Jacobsen et al. 1988, Adams et al. 1990). However some experiments have used acid "mist" or "fog" and these have generally used droplets with diameters less than 100 µm by using impingement type fog nozzles (Wood and Bormann 1974, Evans et al. 1977, Scherbatskoy and Klein 1983, and Musselman and Sterrett 1988), sonic atomizing nozzles

(Hindawi et al. 1980), or rotary atomizers (Grannet and Musselman 1984, Westman and Temple 1989). Unfortunately in many papers the droplet size spectrum is omitted.

A1.3 Cloud water deposition

Particles and droplets in the atmosphere may be deposited onto plant surfaces by interception, Brownian motion, impaction and sedimentation (Chamberlain and Little 1981). Interception occurs when a droplet, without deviating from the airflow in which it resides, passes so close to an obstacle as to collide with it. This is unlikely to be a method of droplet capture by bluff bodies such as shoots or leaves (Chamberlain and Little 1981) and is ignored by Lovett (1984) and Unsworth and Crossley (pers. comm.). Brownian motion is also irrelevant for cloud capture, as cloud droplets are larger than particles or droplets subject to Brownian motion (Fowler 1980, Chamberlain and Little 1981). The main methods for droplet transfer to tree surfaces are therefore impaction, whereby the momentum of a droplet causes it to leave the airflow diverging round an obstacle and to collide with it, and sedimentation - the vertical movement of a droplet due to gravity. Impaction by turbulent diffusion is normally dominant (Lovett 1984), but sedimentation becomes increasingly important with reducing wind speeds.

Measurements of the amount of cloud water deposited onto trees are scarce and very variable. Fog or cloud capture by trees was noted as far back as 1789 in Britain and 1764 in the Canary Islands (see reviews by Penman (1963) and Kerfoot (1968)) but very little quantitative work was done until the mid twentieth century. Several methods have been used to quantify cloudwater deposition including micrometeorological estimates, artificial cloud-capturing devices, tracers, direct weighing and throughfall estimates.

Much of the earlier work centred on semi-arid zones which received considerable inputs of moisture from cloud deposition and involved the collection of intercepted cloud or throughfall. Such collections led to underestimates of deposition to trees because of high levels of canopy retention and are also subject to considerable error because of a combination of very variable deposition patterns under trees and small sample sizes. However, results indicated that large amounts of water could be intercepted. Deposition amounts in throughfall in California included 52 mm (*Sequoia sempervirens*) to 1524 mm (*Lithocarpus densiflorus*) over 40 days (Oberlander 1956), 250 mm (*Pinus radiata*) over the summer "fog season" (Parsons 1960) and 217 mm (*Pseudotsuga taxifolia*) and 184 mm (*Pseudotsuga taxidolia/Sequoia sempervirens*) over 46 days (Azevedo and Morgan 1974). This compares with an annual rainfall of around 500 mm in the area.

Other recorded values using throughfall collection include cloud interception by *Araucaria excelsa* on Hawii of 4 mm d^{-1} (Ekern 1964) and 25.4 - 127 mm annually underneath *Eucalyptus niphophila* in the Australian Alps (Costin and Wimbush 1961). Cloudwater interception was also found to contribute large proportions of the annual water input to forests in West Germany (Baumgartner 1958), Austria (Grunow 1955) and Vermont, U.S.A. (Vogelmann et al. 1968).

The use of artificial collectors was pioneered at Table Mountain in South Africa with its orographic "tablecloth". Grass stalks placed between wires were found to collect more water than ordinary rain gauges (Marloth 1905) and a later study using a cylindrical mesh gauze obtained cloudwater deposition 1.7 times more than the rainfall (Nagel 1956).

However although these results point to an important role of cloud deposition there is difficulty in extrapolating quantitatively either throughfall or horizontally intercepted cloudwater on artificial collectors to the cloudwater deposition onto forest trees. Micrometeorological methods and calibrated artificial cloud interceptors have been used in an effort to overcome these difficulties.

Kuroiwa (1953) was the first to devise a workable model for estimating cloud droplet deposition from profiles of eddy diffusivity and liquid water content. This approach was taken up later by Lovett et al. (1982) Dollard and Unsworth (1983) and Lovett (1984). The foundation for their work had been laid when Shuttleworth (1977) showed that the capture of cloud droplets was essentially the same as other vegetation - atmospheric exchange processes. Fog/cloud water fluxes are therefore essentially limited by rates of turbulent transfer and can be modelled from a knowledge of momentum transfer (Unsworth and Crossley 1987b). The turbulent flux density of fog/cloud water to the surface can thus be estimated, as it was by Kuroiwa (op cit), as the product of eddy diffusivity and the vertical gradient of the liquid water content of fog/cloud drops in air. Other factors such as evaporation/condensation terms and canopy structure models can also be added (Shuttleworth 1977, Lovett 1984, Milne et al. 1988). Values of cloudwater deposition fluxes obtained using the micrometeorological method include 5 mg m^{-2} s^{-1} (0.018 mm h^{-1}) to grassland at low elevation and 20 mg m^{-2} s^{-1} (0.072 mm h^{-1}) over grassland at higher elevation (Dollard and Unsworth 1983, Dollard et al. 1983). These values are considerably higher than Shuttleworth's (1977) estimates of 0.036 mm h^{-1} for coniferous forest and 0.0036 mm h^{-1} for grassland. Gallagher et al. (1988) obtained fluxes of 5.8-10.1 µg m^{-2} s^{-1} (0.021-0.036 mm h^{-1}) to moorland vegetation whereas Lovett (1984) found cloudwater fluxes larger than 0.4 mm h^{-1} and averaging several tenths of a millimetre per hour to Abies balsamea. Later results (Lovett 1988) produced a value of 640 mm for the June-October growing season, equivalent to approximately 4.2 mm d^{-1}. Comparisons were made with a simultaneously used throughfall method which yielded deposition of 240-410 mm over the same period. Lovett (op cit) made the point that although micrometeorological methods give high quality short term measurements longer term results would require very expensive monitoring.

Longer term monitoring can be done using artificial passive cloud collectors and, if collection efficiency of the gauge and a conversion factor from gauge capture to forest capture is known, deposition to forests can be estimated. Such gauges are often built as a cone or cylinder of string/ribbon/gauze etc. held together by a plastic frame. Teflon or E.T.F.E. (ethyl-tetra-fluoro-ethylene), are now the preferred materials for stringing as nylon has an affinity for nitric acid vapour (Appel et al. 1980). The collecting surfaces can be shielded from rainfall by using a lid (Crossley et al. 1986, Munzert 1988), or electronically moved in and out of a covering cylinder by using fog sensors (Schmitt 1988). Collection efficiency can be estimated using a wind tunnel (Mallant 1988) or by comparison with cloudwater deposition simultaneously measured by optical particle counters.

The glasshouse experiments in this study were designed to produce misting episodes resembling those in the Scottish southern uplands. Work by Crossley and his co-workers using lidded E.T.F.E. stringed cloud gauges at the site used for my field studies (Dunslair Heights 602 m ASL) allowed cloud deposition rates to be estimated. The collection efficiency of the guage varied from 11% to 46% with a mean of 29%. From Shuttleworth (1977) they obtained the deposition rate (D) as

$$D = W/r_a$$

Where W is liquid water content (L.W.C.) and r_a is aerodynamic resistance. Because r_a depends on type and structure of vegetation and on windspeed the above can be written as

$$D = W\ Cd\ u$$

where u is windspeed and Cd is a low level drag coefficient that is dependent on vegetation type and structure. Where windspeed is in m s^{-1}, Cd is equal to 0.09h for stands of *Pinus sylvestris* and *Picea sitchensis* where h is tree height (Jarvis *et al*. 1976). Measurements using the cloud gauge throughout an overnight cloud event gave a deposition rate of 0.5 g m^{-2} s^{-1} (1.8 mm h^{-1}). A general value for the upland forest at the site can be arrived at by assuming L.W.C. as 0.4 - 0.5 g m^{-3} (Bamber *et al*. 1984, Daum *et al*. 1984, Goldsmith *et al*. 1984, Crossley *et al*. 1986), windspeed during cloud events at Dunslair Heights as 5 m s^{-1} (Crossley pers. comm., Crossley *et al*. 1986) and tree height (for both Dunslair Heights and the glasshouse experiments) as 2 m, giving

$$\begin{aligned} D &= 0.45 \times 0.09 \times 2 \times 5 \\ &= 0.405 \text{ g m}^{-2} \text{ s}^{-1} \\ &= 1.458 \text{ kg m}^{-2} \text{ h}^{-1} \\ &= 1.458 \text{ mm h}^{-1} \end{aligned}$$

Total annual deposition is difficult to estimate accurately but a rough estimate is possible. A tipping bucket cloud collector at the field site records the number of hours over which cloud is deposited. 1.5 mm h^{-1} is equivalent to fairly thick cloud and Crossley (pers. comm) considers 0.75 mm h^{-1} to be a reasonable estimate of cloud deposition during an event at Dunslair heights. Cloud was deposited for a total of 3012 hours during 1988 giving a total annual cloudwater deposition of 2259 mm.

A1.4 Experimental system adopted and its performance.

After estimation of cost, ease of supply and installation and in particular droplet size spectrum, a cloudwater simulation system using mini, ultra-low-volume applicators (ULVAS) (Micron Sprayers Ltd., Hereford) proved acceptable. The applicators spray liquid from an atomiser disc with peripheral teeth. At low rotation speeds droplets are produced directly at the edge of the disc. At higher rotation speeds (>9000 r.p.m.) filaments are formed as surface tension forces the liquid to issue from the 'zero' issuing points at the tapering end of the fine teeth. Droplets which break off from these filaments are much smaller than those formed directly and also maintain a much narrower size band, producing a uniform droplet size (Bals 1969). The water feed rate to the disc is also important, low rates (0.4 cm^3 min^{-1}) produce direct droplet formation and a bimodal distribution with a main peak of larger droplets and a subsidiary peak of smaller "satellite" droplets. At very high flow rates (>40 cm^3 min^{-1}) excessive supply to the disc causes sheets to form with a larger and wider droplet range (Johnstone and Johnstone 1976). The disc is attached to a motor housing, containing the mini ULVA motor and distribution brush. An attached threaded bottle holder normally holds a 500 cm^3 plastic bottle and water is fed by gravity through a graded feed stem to the disc. Power is normally supplied by up to eight 1.5 V batteries contained in an attached plastic holder.

For the experiments in this thesis the 500 cm^3 plastic bottles were replaced by 2500 cm^3 Winchester bottles held in place by encasement in plastic piping on a wooden frame. Each of the sprayer motors (one per replicate) was connected to a 12 V battery, and operated at around 15,000 r.p.m. (Bals 1969, Johnstone and Johnstone 1976). The chosen feed stems were calibrated and found to give 23.5 ± 0.1 cm^3 min^{-1}, giving an application time of around 106 mins. The combination of flow rate and rotation speed produces a narrow band of droplets between 30-50 μm in diameter with a volume mean diameter of 45 μm and a number mean diameter of 33 μm (Bals pers. comm., Johnstone and Johnstone 1976). Although larger than many cloud droplet spectra, these diameters are still well within realistic cloud droplet diameter spectra and compare favourably with values for other simulated acid mist experiments.

The sprayers were fixed approximately 0.7 m above the top whorl of the trees being sprayed and produced a circular spray of approximately 1 m diameter at tree height. Trees were placed in 1 x 1 x 1.85 m wooden cages covered with polythene sheeting. The corners of the cages were not occupied as these were just outside the main spray. Deposition of 2500 cm^3 over 0.785 m^2 during 106 minutes was equivalent to 1.8 mm h^{-1}. This value is similar to those estimated for upland regions in the area (see earlier), although the episode duration is shorter than most of those cloud episodes.

Total deposition for a thrice weekly application (controlled experiments 1, 3 and 4) was 9.6 mm per week, equivalent to 500 mm a^{-1}. In experiment 2 five applications were given weekly, totalling 16 mm per week, equivalent to a rate of 832 mm a^{-1}. At the time the experiments were set up the data from Dunslair Heights on cloud hours were not available. Estimates available indicated deposition rate to forests of several tenths of a millimetre per hour and immersion times of around 600 hours (Dollard et al. 1983) giving a total cloud deposition of around 120-240 mm per annum. Field observations by myself and particularly by Dr Crossley cast doubt on such a low figure and deposition levels for controlled experiments were set considerably higher. Subsequent data from Dunslair Heights indicated that the deposition level could be further increased. However it should be remembered that 2259 mm was an estimate for the top of Dunslair heights and deposition would fall quickly with decreasing elevation. The deposition level applied in the experiments therefore appears to be realistic for large tracts of upland forests in S.E. Scotland.

Generally there was an even distribution of mist during the experiments but as a precaution trees were also rotated every 20 minutes during application to ensure this. The system worked well although the following problems were encountered.

i) Initially many of the motors burnt out. This was because the wrong motors for a 12 V supply were supplied by MICRON Sprayers Ltd. These were replaced free of charge with no further problems.

ii) Occasionally the atomiser heads siezed and needed the retaining nut holding them to the motor housing to be loosened.

iii) Occasionally the atomiser air vent became flooded, disrupting the flow. This was rectified by unscrewing the atomiser, emptying the reservoir of water and replacing.

iv) If the glasshouse vents were open and it was windy outside the even distribution of the mist could be affected. Rotation of the trees minimised this effect.

A1.5 Chemistry of simulated cloud.

For the purpose of experimental design it was decided that SAC should have an ionic content similar to that found in cloudwater in S.E. Scotland. Table A1.1 shows the range and typical values of the major ions in cloudwater collected at Castlelaw Hill, Pentlands, S.E. Scotland.

Table A1.1 Cloud water concentrations from lidded E.T.F.E. gauges at Castlelaw Hill 1986. Data from Cape and Crossley (pers. comm.). Comparative data from several samples in the Pennines are also shown (Gervatt 1985).

Ion	Range		Typical		Pennines	
	mmol m^{-3}	µeq l^{-1}	mmol m^{-3}	µeq l^{-1}	mmol m^{-3}	µeq l^{-1}
H^+	1- 575	1- 575	400	400	300-2600	300-2600
NH_4^+	62- 807	62- 807	400	400	200-1100	200-1100
Na^+	75- 8679	75- 8679	800	800	5- 300	5- 300
K^+	5- 162	5- 162	50	50	5- 50	5- 50
Ca^{2+}	10- 7620	19- 520	125	250	10- 100	20- 200
Mg^{2+}	12- 274	25- 549	85	170	5- 50	10- 100
Cl^+	222-10280	222-10280	850	850	450- 550	450- 550
SO_4^{2+}	95- 790	190- 1581	375	750	125-1250	250-2500
NO_3^-	63- 1074	63- 1074	300	300	100-1700	100-1700

Acidity levels chosen were pH 5.6, 3.0, 2.5 and 2.0. pH 5.6 corresponds to water in equilibrium with CO_2 which dissolves to form carbonic acid. Analysis of the Castlelaw cloud data showed the pH frequently approached 3.0 with 14% of all samples between pH 3.0 and pH 3.5 and a minimum value of pH 3.06. More recent data from the field site at Dunslair Heights showed 16% of weekly bulk samples less than pH 3.5 with a minimum of 2.90 (Figure A1.1). These weekly samples represent volume weighted mean pH over extended periods of cloud deposition and indicate that pH 3.0 will be frequently experienced by upland forests. pH 3.0 was therefore chosen as the value representing non extreme but highly acidic cloud.

It would be expected that short term variations in cloud events would have pH values considerably below 3.0. Event data were not available for S.E. Scotland but results from elsewhere show cloud pH values reaching down to 2.2 in the Pennines (Gervatt 1985), 2.26-2.45 in the Adirondack Mts., New York State (Falconer and Falconer 1980), 2.38 in the Taunus Mountains in West Germany (Schmitt 1988) and 2.4 at a low level site in Maine, U.S.A. (Weathers et al. 1988). In addition urban fogs in Southern California can reach pH levels of 2.06 (Waldman et al. 1985) and even 1.69 (Hileman 1983). pH 2.5 and 2.0 were thus chosen as representing the extreme event end of cloud episodes. Table A1.2 shows the salt and ion concentrations of SAC at these pHs. Acidity levels were adjusted by adding H_2SO_4:HNO_3 in the ratio 2:1.

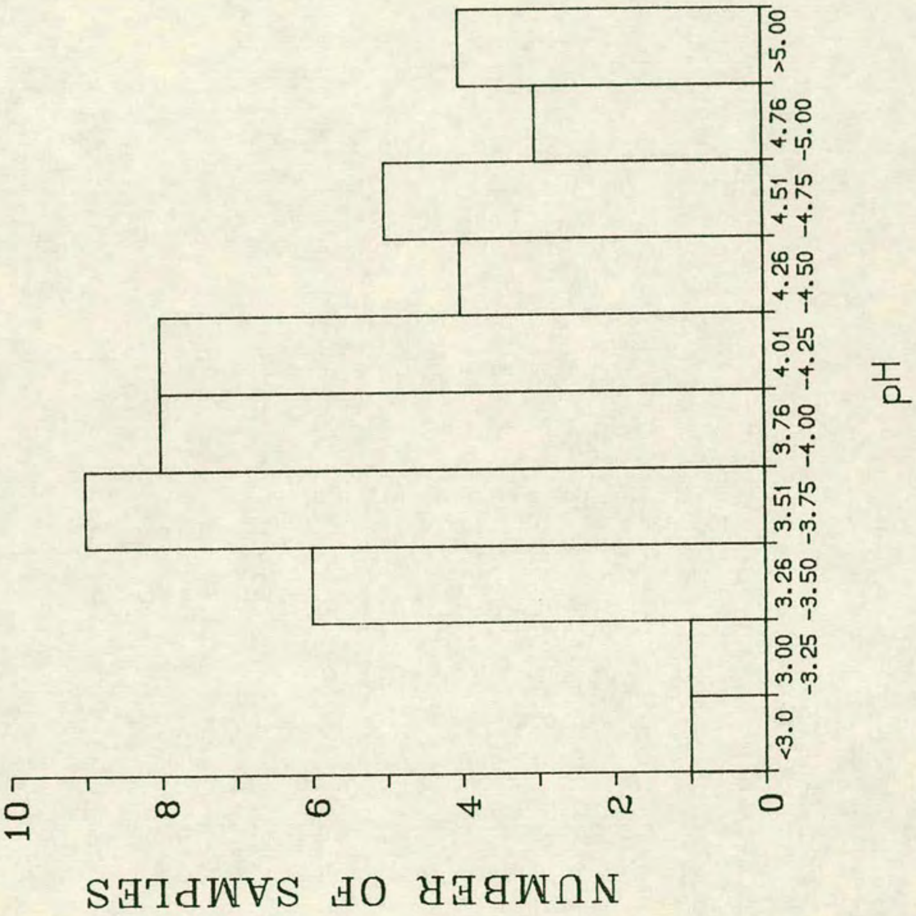


Figure A1.1 Frequency distribution of pH, of weekly bulked cloudwater samples from Dunslair Heights (1988).

Stock solutions were made up at 100 times the required concentration, stored in the dark at 2-3 °C and diluted with deionised water not more than 24 h before spraying. The stock solutions were renewed at around four to six week intervals.

Table A1.2 Salt (a) and Ion (b) concentrations of simulated acid cloud.

(a)

	pH			
Salt (mmol m^{-3})	5.6	3.0	2.5	2.0
$(NH_4) SO_4$	285	285	285	285
Na Cl	730	730	730	730
Ca $(NO_3)_2$	125	125	125	125
Mg $(NO_3)_2$	85	85	85	85
K Cl	50	50	50	50
H Cl	2.512	70	70	70
$H_2 SO_4$	-	465	1185.8	3465
$NH_4 NO_3$	-	430	430	430
HNO_3	-	-	720.8	3000

Table A1.2 (cont'd)

(b)

				pH				
Ion	5.6		3.0		2.5		2.0	
	mmol m^{-3}	µeq dm^{-3}	mmol m^{-3}	µeq dm^{-3}	mmol m^{-3}	µeq dm^{-3}	mmol m^{-3}	µeq dm^{-3}
H^+	2.512	2.512	1000	1000	3162.3	3162.3	10000	10000
NH_4^+	1000	1000	1000	1000	1000	1000	1000	1000
NO_3^-	420	420	850	850	1570.8	1570.8	3850	3850
SO_4^{2-}	285	570	750	1500	1470.8	2941.6	3750	7500
Cl^-	782.5	782.5	850	850	850	850	850	850
Na^+	730	730	730	730	730	730	730	730
Mg^{2+}	85	170	85	170	85	170	85	170
Ca^{2+}	125	250	125	250	125	250	125	250
K^+	50	50	50	50	50	50	50	50

APPENDIX 2

Tree Material

The tree material used in the four glasshouse experiments was as follows:

Experiment 1 - Sixth year Scots pine. Grown from seed collected at Ledmore in Autumn 1979 from Forestry Commission clone number 290. Transferred from 5" square pots to 7½" round pots in Spring, 1983 and into 10" scrubbed and cleaned pots in November, 1985. The compost used was four parts Irish moss, one part quartz grit, one part loam. No fertilizers were added.

Experiment 2 - Scots pine as for experiment 1 except in this experiment clone number 365 was used. Trees were now in their seventh year.

Experiment 3 - Third year Sitka spruce grown from seed from Economic Forestry group at Maelor Nursery. They were in seedbeds for one year and then transferred to troughs kept in glasshouses for their second year.

Experiment 4 - Scots pine as for experiment 1 except clone number 307 was used. The trees were now late on in their seventh year. They were from the British seed lot of Queen Charlotte Island provenance. They were potted up into 10" scrubbed and cleaned pots. Both species were given a small amount of top dressing before the start of the experiment (Vitax Q4, Vitax Ltd., Skelmersdale).

APPENDIX 3

Glasshouse conditions

Glasshouse conditions for the four glasshouse experiments were as follows:

	Temperature range	Light supplement (Mercury vapour lamps)	Daylength
Experiment 1			
Pre-flushing	3-10°C	16 x 400 W	08.15-5.00
Flushing	14-22°C	16 x 400 W	05.00-23.00
Experiment 2	17-30°C	16 x 400 W	05.00-23.00
Experiment 3	18-23°C	16 x 400 W	05.00-23.00
Experiment 4	18-25°C	12 x 400 W	05.00-23.00

APPENDIX 4

Analysis of ions

4.1 Throughfall, snowmelt and cloudwater samples.

The following ions were analysed:

Glasshouse experiment 1 - NH_4^+-N, NO_3^--N, PO_4^{3-}-P, Ca, Mg, K.

Glasshouse experiment 4 - NO_3^--N, PO_4^{3-}-P, Ca, Mg, K.

Field experiment - Ca, Mg, K

Snowmelt experiment - NO_3^--N, Ca, Mg, K

Calcium, magnesium and potassium were measured using an atomic absorption spectrometer (SP9, Pye Unicam, Cambridge). Three recordings per sample were taken and a mean calculated. Potassium was analysed by flame emission and calcium and magnesium by atomic absorption. For calcium and magnesium the aspiration tube was split to allow 0.1% lanthanum solution to be aspirated along with the sample. This acted as a releasing agent to minimise the effects of phosphate in samples. Calibration standards were made up from:

K - 0.1907 g dm^{-3} KCl (100 mg dm^{-3} (ppm))

Ca - 0.4995 g dm^{-3} Ca CO_3 (200 mg dm^{-3} (ppm))

Mg - 0.1000 g dm^{-3} Mg ribbon (oxide free) + 2 cm^3 conc. HCl made up to 1 dm^{-3}

A range of standards was made up and tried against some samples and an appropriate set of three standards, normally between 2-20 mg dm^{-3} used.

NO_3^--N, NH_4^+-N and PO_4^{3-}-P were all measured using a flow injection analyser (5020, Tecator, Sweden). In the analysis of NO_3^--N, the nitrate was first reduced to nitrite (originally assumed to be negligible) in a cadmium reductor. Acidic sulphanilamide was added to form a diazo compound which then reacted with N- (1-naphtyl) - ethylenediamine dihydrochloride to produce a purple azo dye. The intensity of this dye was proportional to the nitrate concentration, and was analysed at 540 nm.

PO_4^{3-}-P was determined by injecting the sample into a carrier stream (H_2O) and merging with a second carrier (5 ml conc. H_2SO_4 dm^{-3}) to avoid matrix effects. The combined stream was then mixed with an acidic ammonium molybdate solution to form a heteropoly acid which was reduced to molybdenum blue by adding acidic stannous chloride. The blue colour was measured at 690 nm.

NH_4^+-N was determined by mixing with sodium hydroxide. The joint stream passed along a PTFE membrane in a gas diffusion cell and the ammonia gas formed diffused through the membrane into an acid/base indicator stream. The indicator colour change was measured at 590 nm.

The volumes of carrier solutions and reagents were controlled by the diameter of the pump tubing used to supply them. Two injections per sample were analysed for all analyses on the "tecator" and the mean taken. Standards were made up from:

NO_3 - 0.6068 g dm^{-3} Na NO_3 (100 mg dm^{-3} (ppm))

PO_4 - 0.4394 g dm^{-3} K H_2 PO_4 (100 mg dm^{-3} (ppm))

NH_4 - 0.3820 g dm^{-3} NH_4 Cl (100 mg dm^{-3} (ppm)).

Again a range of standards was made up, tried against some samples and three appropriate standards used.

Recalibration of the "tecator" took place every 10 samples and of the spectrometer every nine samples.

4.2 Analysis of needles.

Needles were analysed for NH_4^+-N, PO_4^{3-}-P, Ca, Mg, K. Needles were dried in an oven at 75°C for four days and then ground in an ultracentrifugal mill (Retscht, Germany) to pass through a 0.5 mm screen. Approximately 0.1 g was accurately weighed out into hard glass test tubes and 2 cm^3 of concentrated H_2SO_4 and 1 cm^3 of hydrogen peroxide (100 volume) were added. The test tubes were then placed on a heating block at 340 °C for 6 h. The tubes were then allowed to cool and the solution was transferred to a 50 cm^3 volumetric flask and made up to the mark with distilled water. Samples were analysed against standard solutions made up as detailed for throughfall and cloudwater. 3.6 cm^3 conc. H_2SO_4 was added to standards in 100 cm^3 volumetric flasks (an estimated 10% of the 2 cm^3 in samples at 50 cm^3 was lost during digestion). The first carrier in the analysis of $PO_4$3--P was changed to 36 cm3 conc. H_2SO_4 dm^{-3} and the second to 4 N Na OH.

4.3 pH measurement.

About 1.5 cm^3 of samples collected by the vacuum-pipette system was transferred from the test tubes into small polystyrene thimbles. The pH was then measured with a combination microelectrode (Russell Electrodes, Auchtermuchty) and pH meter (EIL 7055, Electronic Instruments Ltd.,Chertsey, Surrey). The electrode was calibrated using 0.1 N (0.05 M) $H_2 SO_4$ (0.004904 g cm^{-3}) diluted to pH 4.0 and pH 3.0. The use of dilute acid rather than conventional buffers avoided a large residual liquid junction potential by keeping the standard and test solutions at similar ionic strength. Standards and test solutions were measured at the same temperature. Calibration took place each day samples were measured and at regular intervals throughout measurements. The response time of samples varied and enough time was given to allow readings to stabilize. Measurements were taken to within 0.01 pH units with an accuracy of ± 0.02 pH units.

Measurements of the pH of droplets on needles or artificial shoots were made with a micro-combination pH electrode (M1-410 Microelectrodes Inc., Londonderry, New Hampshire) with a tip diameter of 1.2 mm and pH meter (7045/46, Electronic Instruments Ltd., Chertsey, Surrey). Standards and operating procedure were as for the Russell Electrode. Calibration for field work and windtunnel work took place in the field or in the windtunnel respectively. Measurements were taken to 0.01 pH units with an accuracy of ± 0.02 pH units.

APPENDIX 5

Scoring record of SEM (Experiment two)

To assess the epicuticular wax structure of Scots pine needles, slides of the high magnification scanning electron micrographs of the needle stomata were projected onto a screen. A visual estimate of fusion and degredation of the wax tubules was made. An arbitrary scale of zero to five, representing pristine structure with tubules unfused to amorphous structure with no tubules distinguishable, was used. Observer 1 was myself and observer 2 was Dr A Crossley.

pH	Year (1 = Present) (2 = Previous)	Surface (+ = Abaxial) (- = Adaxial)	Score	
			Observer 1	Observer 2
5.6	1	+	3	3
			1	1
			3	4
			2	2
			2	2
	1	+	1	1
			0	0
			1	1
			0	0
			1	2
	1	-	0	0
			1	0
			1	1
			1	0
			1	1
	2	+	3	4
			3	3
			4	4
			3	4
			3	4
	2	+	3	3
			4	4
			3	4
			4	4
			4	4
	2	-	3	2
			3	3
			3	3
			3	3
			3	2

Appendix 5 (cont'd)

pH	Year (1 = Present) (2 = Previous)	Surface (+ = Abaxial) (- = Adaxial)	Score	
			Observer 1	Observer 2
3.0	1	+	1	0
			2	2
			2	2
			3	3
			2	1
3.0	1	+	2	1
			1	2
			2	2
			2	2
			2	2
	1	-	2	2
			1	1
			1	1
			0	0
			2	2
	2	+	2	2
			4	4
			3	3
			4	4
			4	4
	2	+	2	2
			2	2
			3	3
			2	2
	2	-	2	2
			3	2
			3	3
			3	2
			4	4
2.5(live)	1	+	2	2
			2	2
			1	1
			3	3
			1	2
	1	+	2	3
			1	2
			2	2
			3	3
			2	3

Appendix 5 (cont'd)

pH	Year (1 = Present) (2 = Previous)	Surface (+ = Abaxial) (- = Adaxial)	Score	
			Observer 1	Observer 2
	1	-	2	1
			1	2
			1	2
			2	1
			2	2
2.5 (live)	2	+	3	4
			4	4
			4	4
			3	3
			4	4
	2	+	4	4
			3	4
			4	4
			3	4
			3	3
2.5	2	-	3	3
			3	4
			2	2
			3	2
			2	2
2.5(Necrotic)	1	+	2	2
			3	3
			3	4
			1	1
	1	+	2	2
			1	1
			2	3
			1	2
			2	2
	1	-	0	1
			0	0
			1	1
			1	2
			0	0

pendix 6 pH values of samples from experiment 4.

SITKA SPRUCE

ime nutes	pH 5.6 Pre-flushing Date			pH 5.6 Post-flushing Date										pH 3.0 Pre-flushing Date			pH 3.0 Post-flushing Date									
	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/6	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/
0		6.55	5.62						6.55				5.75					2.97	2.94							2.
1-5										5.41					2.90											
6-10					7.25	6.35									2.81											2.
1-15		6.31											5.15									2.93				
6-20																										
1-25				6.79											2.87								2.96			
6-30				4.46																2.94						
1-35													5.64			2.75										
6-40			6.45																							2.
1-45					6.51																2.76	2.92				
6-50																2.70										
1-60											4.97													2.89		
1-70											4.92							2.90								
1-80																										
1-90							5.99																		2.74	
1-100																										
1-110																	2.53									
1-120												6.53					2.63									

endix 6 pH values of samples from experiment 4.
nt'd)

	SITKA SPRUCE													SCOTS PINE												
	pH 2.5													pH 5.6												
	Pre-flushing			Post-flushing										Pre-flushing			Post-flushing									
me	Date			Date										Date			Date									
utes	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/6	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/
0		2.15					2.37	2.35					2.48							5.98	6.42		5.18			4.
-5									2.41									6.13								
-10																										
-15																5.58						5.86				
-20													2.45		6.54											
-25								2.36		2.38												6.12				
-30																										
-35																					6.12					
-40			2.06									2.38		6.53												
-45			2.11																							
-50												2.21									6.34					
1-60							2.34													5.85						
1-70											2.38													5.79		
1-80			2.02																							
1-90														4.39											5.90	
1-100																										
1-110							2.28					2.28														
1-120																	4.59									

endix 6 pH values of samples from experiment 4.
nt'd)

SCOTS PINE

	pH 3.0													pH 2.5												
	Pre-flushing			Post-flushing										Pre-flushing			Post-flushing									
	Date			Date										Date			Date									
me utes	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/6	25/3	27/3	30/3	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/
0									2.91				2.98							2.42	2.33					
-5			2.73			2.88									2.31				2.32				2.38			
-10																			2.26							
-15															2.19											
-20				2.70																						
-25			2.65	2.66	2.95																					
-30					2.93										2.17	2.16					2.29					
-35	2.74									2.89																
-40																							2.38			
-45		2.72																				2.35				
-50	2.67				2.93																					
1-60														2.32									2.38			
1-70					2.87																			2.31		
1-80														2.18						2.28						
1-90												2.64														
1-100							2.79																			
1-110																				2.13				2.22		
1-120												2.42														

endix 6 pH values of samples from experiment 4.
nt'd)

PARAFILM

	pH 5.6													pH 3.0												
	Pre-flushing			Post-flushing										Pre-flushing			Post-flushing									
	Date			Date										Date			Date									
me utes	25/3	27/3	1/4	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/6	25/3	27/3	1/4	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/
0			6.36	6.71				6.34									2.89									
1-5				4.39																						
6-10																										
1-15																								2.96		
6-20																										
1-25								6.54																		
6-30																										
1-35																										
6-40										6.60																
1-45																		2.95								
6-50																										
1-60					6.35																2.76					
1-70			6.61		7.58																					
1-80																									2.82	
1-90																										
1-100											6.44													2.86		
1-110																										
1-120												6.58														

Appendix 6
(Cont'd)

PARAFILM

pH 2.5

Time Minutes	Pre-flushing Date			Post-flushing Date									
	25/3	27/3	1/4	8/5	11/5	15/5	18/5	20/5	22/5	27/5	29/5	1/6	3/6
0			2.34						2.47				
1-5													
6-10													
11-15			2.28						2.46				
16-20													
21-25													
26-30													
31-35							2.31						
36-40									2.43				
41-45													
46-50										2.38			
51-60									2.41				
61-70													
71-80													
81-90			2.00										
91-100											2.37		
101-110													
111-120													