

Effects of Hydrogen Peroxide in Mist on Vegetation.

by

Ceris Ann Jones

This thesis is submitted for the degree of

Doctor of Philosophy

and for the

Diploma of Imperial College.

Imperial College of Science, Technology and Medicine

University of London

September, 1991

Abstract.

Hydrogen peroxide (H_2O_2) is a photochemical oxidant which shows a strong seasonal pattern, with the highest concentrations in the summer and lowest in the winter. H_2O_2 is very soluble and so rapidly enters into solution in cloud and rainwater and aqueous phase concentrations up to 250uM have been recorded at high elevation sites.

The aims of this project were to obtain information on concentrations of H_2O_2 which affect plant growth and physiology and to increase understanding of the mechanisms involved. Two herbaceous species, pea (*Pisum sativum*) and broad bean (*Vicia faba*) and seedlings of two tree species, oak (*Quercus petraea*) and Sitka spruce (*Picea sitchensis*) were investigated, using a range of H_2O_2 concentrations, 0 - 100uM, in a pH4 mist.

Experiments with herbaceous species (6h mist exposure per week for 4 weeks) provided strong evidence of reductions in growth rate in both species at 50uM. Leaf, stem and total plant dry weight were significantly decreased ($P < 0.05$) at this H_2O_2 concentration compared with the control. There was some evidence of reduced growth at 20uM H_2O_2 , but this was not statistically significant. In contrast, experiments with the two tree species conducted outdoors for 5 months (6h exposure overnight, twice every fortnight), provided no clear evidence of adverse effects on growth at 20uM or 50uM H_2O_2 . There appeared to be a stimulation of stem dry weight in Sitka spruce at 50uM H_2O_2 (compared with the control), but at 100uM H_2O_2 , there was a significant reduction in total needle dry weight and total above ground dry weight.

Gas exchange measurements on oak leaves consistently showed higher values of leaf conductance in the 100uM treatment than in control seedlings, but there were no significant effects on net photosynthesis. Surface wax content of Sitka spruce needles, expressed on dry weight basis, was substantially reduced at 100uM, although the effect was not statistically significant.

Analysis of droplets containing H_2O_2 placed on the surface of broad bean leaves showed an increase in droplet conductivity as droplet H_2O_2 content decreased. During the experiment with tree seedlings, surface droplets were collected from the seedlings at the end of the exposure. For a small number of individual events, the hydrogen ion (H^+) content of droplets collected from the 50 and 100uM treatments was significantly greater than the control for both species. For Sitka spruce, this increase in droplet H^+ was positively correlated with droplet sulphate (SO_4^{2-}) content.

H_2O_2 at concentrations representative of high elevation sites can affect plant physiology and may be a contributing factor in forest decline.

Acknowledgements.

First, I would like to thank my three supervisors Dr. Mike Ashmore, Prof. Nigel Bell and Dr. Geoff Dollard for all their help, advice and encouragement during this project.

Secondly, my thanks to the Air Pollution Group at Silwood Park for their friendship and being a great bunch of people to work with. Some deserve a special mention. Many thanks to Cathy Garretty for all her technical and tree advice, Nigel (The Oracle) Ainsworth for always having time to help me unscramble my brains and Mike Holland for handy hints on dealing with Sitka spruce. A million thanks to Paul Evans for buying the chocolate hob-nobs and generally keeping me sane.

I'd also like to thank Eastcote Hockey Club, especially the women's 1st XI and the Friday Night Club, for restoring my sanity at weekends, Carolyn for always having a bottle of Baileys in the cupboard and Spence for her statistical expertise. Special thanks to Desmond for listening to me rant and rave on numerous occasions and having endless patience.

Finally I'd like to thank my Mam and Dad for all the support that they've given me and eternal gratitude to those who came to the rescue in that last week before submission - Carolyn, Des, Paul, Richard and the person who invented the fax machine.

This project was carried out under the tenure of the Natural Environmental Research Council and was additionally funded by the Department of the Environment - from a C.A.S.E. award with A.E.A. Technology Harwell.

Ten Tall Oak Trees.

Ten tall oak trees
Standing in a line,
'Warships,' cried King Henry,
Then there were nine.

Nine tall oak trees
Growing straight and strong,
'Charcoal,' breathed the furnace,
Then there were eight.

Eight tall oak trees
Reaching towards the sky,
'Sizzle,' spoke the lightning,
Then there were seven.

Seven tall oak trees
Branches, leaves and sticks,
'Firewood,' smiled the merchant,
Then there were six.

Six tall oak trees
Glad to be alive,
'Barrels,' boomed the brewery,
Then there were five.

Five tall oak trees,
Suddenly a roar,
'Gangway', screamed the west wind,
Then there were four.

Four tall oak trees
Sighing like the sea,
'Floorboards,' beamed the builder,
Then there were three.

Three tall oak trees
Groaning as trees do,
'Unsafe,' claimed the council,
Then there were two.

Two tall oak trees
Spreading in the sun,
'Progress,' snarled the by-pass,
Then there was one.

One tall oak tree
Wishing it run,
'Nuisance,' grumped the farmer,
Then there were none.

No tall oak trees,
Search the fields in vain,
Only empty skylines
And the cold grey rain.

Richard Edwards

Table of Contents

	Page
Abstract	2
Acknowledgements	3
Table of Contents	4
List of Figures	10
List of Tables	13
List of Plates	17
Chapter 1 INTRODUCTION	18
1.1 A ROLE FOR HYDROGEN PEROXIDE IN FOREST DECLINE?	19
1.2 THE CHEMISTRY OF H₂O₂	20
1.2.1 The formation of H ₂ O ₂ in the atmosphere	20
1.2.2 The role of H ₂ O ₂ in acidification	24
1.2.3 Measurements of H ₂ O ₂	25
1.3 PLANT RESPONSE TO H₂O₂	27
1.3.1 The effects of wet deposited H ₂ O ₂ on tree seedlings	30
1.3.2 Reactions of H ₂ O ₂ at the leaf surface	32
1.4 AIMS OF THE PROJECT	33
Chapter 2 MEASUREMENT AND APPLICATION OF HYDROGEN PEROXIDE	35
2.1 MEASUREMENT TECHNIQUES	26
2.2 APPLICATION OF H₂O₂ FOR TWO MISTING SYSTEMS	38
2.2.1 Controlled environment cabinets	38
2.2.1.1 Chamber design	38

2.2.1.2 Mist application	41
2.2.1.3 Behaviour of H ₂ O ₂ within the misting system	42
2.2.2 Field misting system	42
2.2.2.1 Chamber design	42
2.2.2.2 Mist application	46
2.2.2.3 Behaviour of H ₂ O ₂ within the misting system	46
2.3 MEASUREMENT OF H ₂ O ₂ IN DROPLETS	48
 Chapter 3 EFFECTS OF HYDROGEN PEROXIDE ON HERBACEOUS SPECIES	50
3.1 INTRODUCTION	51
3.2 MATERIALS AND METHODS	54
3.2.1 Plant material	54
3.2.2 Mist chemistry	54
3.2.3 Exposure regimes	56
3.2.3.1 Experiment A	56
3.2.3.2 Experiment B	56
3.2.4 Measurements of growth	56
3.2.5 Examination of epicuticular wax struture	57
3.2.6 Cation leaching	57
3.2.6.1 Leaching of cations from leaves exposed to H ₂ O ₂ mist	58
3.2.6.2 Leaching from unexposed leaves	58
3.2.7 Effect of leaf surface on H ₂ O ₂ concentration	59
3.3 DATA ANALYSIS	60
3.3.1 Growth effects	60
3.3.2 Leaching effects	60

3.3.3 Effects of leaf surface on H ₂ O ₂ concentration	62
3.4 RESULTS	63
3.4.1 Effects of H ₂ O ₂ on growth	63
3.4.1.1 Experiment A	63
3.4.1.2 Experiment B	69
3.4.2 Cuticular effects	72
3.4.3 Leaching effects	72
3.4.3.1 Leaching from leaves exposed to H ₂ O ₂ mist	72
3.4.3.2. Leaching from leaves not exposed to H ₂ O ₂ mist	73
3.4.4 Changes in H ₂ O ₂ concentration on a leaf surface	77
3.4.4.1 Leaf surface v inert surface	77
3.4.4.2 Distilled water v H ₂ O ₂ droplets on a leaf surface	78
3.5 DISCUSSION	81
3.5.1 The effects of H ₂ O ₂ on growth	81
3.5.2 Interactions of H ₂ O ₂ with the leaf surface	86
 Chapter 4 EFFECT OF HYDROGEN PEROXIDE ON TREE SEEDLINGS	 92
4.1 INTRODUCTION	93
4.2 MATERIALS AND METHODS	95
4.2.1 Experimental design	95
4.2.2 Plant material	95
4.2.2.1 Initial size classing	95
4.2.2.2 Soil mixture	96
4.2.2.3 Pest and disease control	97
4.2.3 Environmental conditions	97
4.2.4 Mist chemistry	99

4.2.5 Exposure regime	99
4.2.6 Growth measurements	99
4.2.6.1 Non-destructive growth measurements and visible injury assessment	99
4.2.6.2 Destructive growth harvest	100
4.2.7 Effects of photosynthesis and gas exchange	101
4.2.8 Measurement of surface droplet chemistry	102
4.2.8.1 Surface droplet collection	102
4.2.8.2 Ion analysis of surface droplets	102
4.2.8.2.1 Divalent cations	102
4.2.8.2.2 Monovalent cations and anions	103
4.2.9 Nutrient analysis of EOS and final harvest trees	103
4.2.10 Wax content of Sitka spruce (using EOS trees)	103
4.2.11 Water loss in Sitka spruce (using EOS trees)	104
4.2.12 Timetable of measurement made during the experiment	104
4.3 DATA ANALYSIS	107
4.3.1 Non-destructive growth measurements	107
4.3.1.1 Growth in the 1989 season	107
4.3.1.2. Tree health in 1989	108
4.3.1.3 Leaf loss in oak	108
4.3.1.4 Spring buburst in 1990	108
4.3.2 Destructive harvests	108
4.3.3 Effects on photosynthesis and leaf conductance in oak	110
4.3.4 Effects on surface droplet chemistry	110
4.3.5 Nutrient analysis of EOS and final harvest trees	111
4.3.6 Wax content of Sitka spruce needles	111
4.3.7 Water loss in Sitka spruce	111

4.4 RESULTS	112
4.4.1 Mist application	112
4.4.2 Environmental conditions	112
4.4.2.1 Air pollution data for the 1989 season	112
4.4.2.2 Climatic data	112
4.4.3 Non-destructive and destructive growth measurements in oak	115
4.4.3.1 Non-destructive growth measurements	115
4.4.3.1.1 1989 measurements	115
4.4.3.1.2 EOS and final harvest measurements	115
4.4.3.1.3 Tree health in 1989	120
4.4.3.1.4 Autumn 1989 leaf loss and spring 1990 budburst	120
4.4.3.2 Destructive growth measurements	120
4.4.3.2.1 1989 EOS harvest	120
4.4.3.2.2 1990 final harvest	126
4.4.4 Non-destructive and destructive growth measurements in Sitka spruce	126
4.4.4.1 Non-destructive growth measurements	126
4.4.4.1.1 1989 measurements	126
4.4.4.1.2 EOS and final harvest measurements	131
4.4.4.1.3 Tree health in 1989 and 1990 spring budburst	131
4.4.4.2 Destructive growth measurements	131
4.4.4.2.1 1989 EOS harvest	131
4.4.4.2.2 1990 final harvest	131
4.4.5 Effects on gas exchange in oak	141
4.4.6 Surface droplet chemistry	146
4.4.7 Nutrient analysis of 1989 and 1990 harvest leaves and needles	159

4.4.7.1 1989 EOS harvest oak leaves	159
4.4.7.2 EOS and final harvest Sitka spruce	159
4.4.8 Wax content of Sitka spruce needles at the 1989 EOS harvest	163
4.4.9 Water loss in Sitka spruce at the 1989 EOS harvest	164
4.5 DISCUSSION	167
4.5.1 Effects on growth in oak	167
4.5.2 Effects on growth in Sitka spruce	170
4.5.3 Gas exchange in oak	173
4.5.4 Surface droplet chemistry	175
4.5.5 Seedling nutrient status	182
4.5.6 Wax content of Sitka spruce	185
4.5.7 Water loss in Sitka spruce	187
 Chapter 5 GENERAL DISCUSSION	 188
Appendix 1	197
Appendix 2	198
REFERENCES	199

List of Figures.

	Page
Fig.1.01. Measurements of H ₂ O ₂ and SO ₂ at Great Dun Fell in Cumbria.	26
Fig.2.01. Schematic diagram of the H ₂ O ₂ analyser.	37
Fig.2.02. Typical calibration curve for the H ₂ O ₂ analyser.	39
Fig.2.03. Diagram of the misting system within a controlled environment cabinet.	40
Fig.2.04. Comparison of the changes in concentration of sprayed and un-sprayed H ₂ O ₂ solutions over a three hour misting period in controlled environment cabinets.	43
Fig.2.05. Percentage loss in concentration for a range of H ₂ O ₂ solutions in 2 misting systems.	44
Fig.2.06. Comparison of the changes in concentration of sprayed and un-sprayed 100uM solutions over an 8 hour duration in the field misting system.	47
Fig.2.07. Calibration curve obtained for the H ₂ O ₂ analyser using the small volume adaptation.	49
Fig.3.01. Concurrent measurements of H ₂ O ₂ and hydrogen ion (H ⁺) concentration.	52
Fig.3.02. Regression analysis of the relationship between total plant dry weight and (leaf number x height), in <i>Pisum sativum</i> and <i>Vicia faba</i> .	61
Fig.3.03. Change in height and leaf number for <i>Pisum sativum</i> and <i>Vicia faba</i> from Expt.A during a four week exposure to H ₂ O ₂ .	65
Fig.3.04. Effects of H ₂ O ₂ on <i>Vicia faba</i> leaf growth.	67
Fig.3.05. Effects of exposure to H ₂ O ₂ on the growth of <i>Pisum sativum</i> and <i>Vicia faba</i> from Expt.A.	68
Fig.3.06. Effects of exposure to H ₂ O ₂ on the growth of <i>Pisum sativum</i> from Expt.B.	71
Fig.3.07. Age and treatment differences in cation leakage from pea leaves in 100uM H ₂ O ₂ solution.	75

Fig.3.08. Age and treatment differences in cation leakage from pea leaves in 100uM H ₂ O ₂ -pH4 solution.	76
Fig.3.09. The effect of leaf surface on H ₂ O ₂ and hydrogen ion concentration and conductivity of droplets.	80
Fig.4.01. Mean pollutant concentrations from May to September 1989.	113
Fig.4.02. Changes in extension growth in oak during the 1989 treatment season.	118
Fig.4.03. Non-destructive growth measurements of extension growth in oak at the 1990 final harvest.	122
Fig.4.04. Effect of H ₂ O ₂ on needle dry weight in Sitka spruce at the 1989 EOS harvest.	137
Fig.4.05. Effect of H ₂ O ₂ on needle and stem dry weight in Sitka spruce at the 1990 final harvest.	140
Fig.4.06. Effect of H ₂ O ₂ on leaf conductance in oak throughout the 1989 season.	144
Fig.4.07. Ion content of droplets collected from oak and Sitka spruce after misting.	147
Fig.4.08. Changes in H ⁺ droplet content with H ₂ O ₂ concentration on three occasions for oak and Sitka spruce.	152
Fig.4.09. Regression analysis of H ⁺ and SO ₄ ²⁻ droplet content for Sitka spruce.	154
Fig.4.10. H ⁺ droplet content for combined oak and Sitka spruce data.	155
Fig.4.11. Regression analysis of mean H ⁺ and SO ₄ ²⁻ droplet concentration for Sitka spruce collected throughout the season.	157
Fig.4.12. Change in weight over time of excised Sitka spruce shoots after exposure to H ₂ O ₂ .	164
Fig.4.13. Effect of year class on the mean rate of water loss from excised Sitka spruce shoots.	166
Fig.4.14. Extension growth in oak at the end of the 1989 season.	169
Fig.4.15. H ⁺ concentration in droplets collected from oak and Sitka spruce exposed to 0 and 100uM H ₂ O ₂ .	177

Fig.5.01. Effect of H_2O_2 on anti-oxidant levels in 1989 Sitka spruce needles at the 1990 final harvest.

195

List of Tables.

	Page
Table 1.01. Gas and aqueous phase measurements of H_2O_2 .	28
Table 1.02. The effects of wet deposited H_2O_2 on tree seedlings.	31
Table 2.01. Reagents used in the H_2O_2 analyser.	37
Table 2.02. Measurement of H_2O_2 in small volumes - voltage output achieved for a 100uM H_2O_2 solution using four different reagent:sample ratios.	48
Table 3.01. Comparison of greenhouse and external ambient temperatures.	55
Table 3.02. Ionic concentrations for a pH4 misting solution.	55
Table 3.03. t test values for the effects of H_2O_2 on height and leaf number in <i>Pisum sativum</i> and <i>Vicia faba</i> .	64
Table 3.04. F ratios for final harvest data from Expt.A for both <i>Pisum sativum</i> and <i>Vicia faba</i> .	66
Table 3.05. Mean relative growth rates for <i>Pisum sativum</i> and <i>Vicia faba</i> in Expt.A.	69
Table 3.06. F ratios for final harvest data from Expt.B. for <i>Pisum sativum</i> .	70
Table 3.07. Mean relative growth rate for <i>Pisum sativum</i> from Expt.B.	72
Table 3.08. K leakage from leaves of pea plants in Expt.B.	73
Table 3.09. Age and H_2O_2 treatment differences in cation leakage from pea leaves incubated in a H_2O_2 solution.	74
Table 3.10. Age and H_2O_2 treatment differences in cation leakage from pea leaves incubated in a H_2O_2 -pH4 solution.	74
Table 3.11. Comparison of cation leaching from leaves of all ages incubated in solutions with or without H_2O_2 .	77
Table 3.12. Changes in H_2O_2 and H^+ concentration and conductivity of droplets on a teflon-coated surface and the surface of a broad bean leaf.	79
Table 3.13. Changes in H_2O_2 and hydrogen ion concentration and conductivity of H_2O droplets and H_2O_2 - H_2O droplets on the surface of a broad bean leaf.	79

Table 3.14. Changes in leaf number and leaf area between initial and final harvests of pea plants from Expts.A and B.	82
Table 3.15. Comparison of the effects of H ₂ O ₂ treatment on plant growth.	85
Table 4.01. Timetable of all measurements performed during the experiment.	96
Table 4.02. A summary of insecticide and fungicide application throughout the 1989 season.	98
Table 4.03. Detailed sequence of measurements made during the experiment.	105
Table 4.04. Statistical analysis of pre-treatment extension growth in oak.	109
Table 4.05. A summary of mist application for both species.	112
Table 4.06. Daily mean, minimum and maximum temperatures and rainfall in the 1989 season.	114
Table 4.07. F ratios for pre-treatment non-destructive growth measurements in oak.	116
Table 4.08. Growth measurement dates.	116
Table 4.09. 1989 extension growth in oak.	117
Table 4.10. Non-destructive growth measurements for EOS harvest oak.	119
Table 4.11. Non-destructive growth measurements for final harvest oak.	121
Table 4.12. Mean scores for the occurrence of mildew on oak leaves in 1989.	123
Table 4.13. F ratios for leaf loss in oak in autumn 1989.	123
Table 4.14. Destructive growth parameters for oak at the 1989 EOS harvest.	124
Table 4.15. Destructive growth parameters for oak at the 1990 final harvest.	127
Table 4.16. Oak soil pH at all harvests.	126
Table 4.17. F ratios for pre-treatment non-destructive growth measurements of Sitka spruce.	129
Table 4.18. Sitka spruce extension growth during the 1989 season.	130
Table 4.19. Non-destructive growth measurements for 1989 EOS harvest Sitka spruce.	132
Table 4.20. Non-destructive growth measurements of 1990 final harvest Sitka spruce.	133

Table 4.21. Destructive growth parameters for Sitka spruce at the 1989 EOS harvest.	135
Table 4.22. Destructive growth parameters for Sitka spruce at the 1990 final harvest.	138
Table 4.23. Sitka spruce soil pH at all harvests.	141
Table 4.24. Net photosynthesis and leaf conductance (gl) in oak in 1989.	142
Table 4.25. Light and air temperature measurements for the gas exchange measurement days.	145
Table 4.26. Leaf conductance in oak on the evening of 15/9/89.	146
Table 4.27. F ratios for the effect of H ₂ O ₂ concentration on H ⁺ droplet content for oak and Sitka spruce.	151
Table 4.28. Effect of H ₂ O ₂ on combined oak and Sitka spruce H ⁺ droplet content.	151
Table 4.29. F ratios for the effect of H ₂ O ₂ on mean ion droplet content in oak and Sitks spruce.	156
Table 4.30. Effects of H ₂ O ₂ treatment on mean droplet concentrations of Zn ²⁺ in oak and H ⁺ in Sitka spruce.	156
Table 4.31. Cation:anion ratios in surface droplets collected from oak and Sitka spruce.	158
Table 4.32. Enrichment factors for ions collected from oak and Sitka spruce in 1989.	158
Table 4.33. Nutrient content of 1F and 2F oak leaves at the 1989 EOS harvest.	160
Table 4.34. Nutrient content of 1988 and 1989 Sitka spruce needles at the 1989 EOS harvest.	161
Table 4.35. Nutrient content of 1988, 1989 and 1990 Sitka spruce needles at the 1990 final harvest.	162
Table 4.36. Effect of H ₂ O ₂ concentration on wax content of Sitka spruce needles.	163
Table 4.37. F ratios for mean rate of water loss per day from excised Sitka spruce shoots.	165

Table 4.38. Deficiency values for foliar cation content in trees.	183
Table 4.39. Estimates of the amount of wax on a needle area basis for Sitka spruce.	186
Table 5.01. Categories of factors influencing declines of forest trees (after McLaughlin,1985).	190

List of Plates

	Page
Plate 1 Temporary misting chambers for the exposure of tree seedlings	45

Chapter 1

INTRODUCTION

1 INTRODUCTION.

1.1 A ROLE FOR HYDROGEN PEROXIDE IN FOREST DECLINE?.

Concern over the health of European forests was renewed in the early 1970s when a "new type of forest decline" (Neuartige Waldschaden) was observed in West Germany (Ashmore *et al*,1985; Blank,1985; McLaughlin,1985). This new damage had different symptoms from those traditionally associated with damage from sulphur dioxide (SO₂) and particulates, and it occurred in many rural areas where the concentrations of these pollutants were low (Ling and Ashmore,1987).

Symptoms of decline were first seen in silver fir (*Abies alba*) as premature defoliation and tree death. Since the late 1970s, similar symptoms have been seen on Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), beech (*Fagus sylvatica*) and oak (*Quercus* spp). The damage was initially restricted to high altitudes and older trees, but the symptoms have spread to lower altitudes and younger trees (Ashmore *et al*,1985; Blank,1985).

In the U.S.A., high elevation red spruce (*Picea rubens*) forests have shown symptoms of die-back for the past 15-20 years. The decline characteristics are those of a stress related disease - a lack of an obvious cause, loss of foliage and subsequent invasion of secondary organisms (Johnson and Siccama,1983).

There are some European - U.S. parallels in that conifer mortality is found over a wide geographical area and on a variety of soils. Drought and/or warm dry summers are associated with a loss of tree vigour and the degree of damage increases with increasing elevation (Johnson and Siccama,1983).

High elevation sites are subject to high levels of wet acidic deposition as rain and occult deposition. The latter includes water deposited through the interception of fog, mist and cloud and element concentrations are often greater than in rain (Mrose,1966; Dollard *et al*,1983; RGAR,1987; Fowler *et al*,1988; Lindberg *et al*,1988; Vong *et al*,1991a). In a review of cloudwater deposition to Appalachian forests in the U.S.A., Vong *et al* (1991a) cited evidence that cloudwater contributed approximately 25 - 50% of total (rain + cloud + dry) sulphate, nitrogen and hydrogen ion deposition at 1483m (a.s.l). There have been reports of regions at greater than 800m (a.s.l) being exposed to mist/cloudwater for parts of 250 days per year (Dollard *et al*,1983). The mean number of cloud/fog days per year for high altitude U.K. sites has been observed to increase with elevation (Gallagher *et al*,1990). At 556m (a.s.l) there were 50 cloud/fog days per year (for the period 1972 - 1980), while at 847m

(a.s.l), the number rose to 239 per year. All such observations gave rise to the hypothesis that wet deposited acidity (as sulphuric and nitric acids) was a contributing factor in forest decline.

At these high altitudes, it has also been observed that ozone (O₃) concentrations do not show the diurnal pattern seen at lower elevations, rather, high concentrations persist for a number of days (Ashmore *et al*,1985; Lindberg *et al*,1988). Visible O₃ damage to Californian forests in the U.S.A. has been observed (McLaughlin,1985). Combined exposure to O₃ and acid mist was shown experimentally to increase ionic leaching from tree seedlings (Krause *et al*,1986), but it was later suggested by Brown and Roberts (1988) that this effect was in part due to the production of N₂O₅ during O₃ formation.

However, "there is more pollution in the air than just sulphur dioxide (SO₂) and nitrogen oxides (NO_x), more in rain, fog and clouds than just hydrogen ions and photo-oxidants other than ozone" (Mallant *et al*,1986). Hydrogen peroxide (H₂O₂) is one such photochemical oxidant and high concentrations (up to 250uM) have been recorded at medium to high altitudes in mist and cloudwater (Olszyna *et al*,1988; Dollard *et al*,1988; Gunz and Hoffman,1990; Sakugawa *et al*,1990). Uncertainty surrounds its possible effects on plants as both detrimental and stimulatory effects have been found on coniferous and broadleaved species (Mallant *et al*,1986,1988; Masuch *et al*,1986,1989; Hanson and McLaughlin,1989).

1.2 THE CHEMISTRY OF HYDROGEN PEROXIDE.

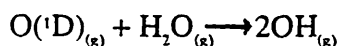
1.2.1 The formation of hydrogen peroxide in the atmosphere.

Hydrogen peroxide (H₂O₂), once described as being best known as an aid to pulchritude (Penkett,1986), is the product of atmospheric photochemistry in both clean and polluted air. There is the possibility that there may be a primary source of H₂O₂ e.g. in the incomplete combustion of natural gas (Gunz and Hoffman,1990), but the evidence is inconclusive. It is therefore generally assumed that H₂O₂ is formed by photochemical reactions in both gas and aqueous phases of the atmosphere.

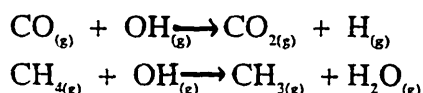
The production of H₂O₂ is a complex function of ambient concentrations of nitrogen oxides (NO_x), volatile organic compounds (VOC), ozone (O₃) and carbon monoxide (CO) and a number of meteorological factors, including solar radiation, temperature and water vapour content (Gunz and Hoffman,1990; Sakugawa *et al*,1990). Its formation is closely linked to the photolysis of O₃:



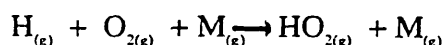
The reaction of the resultant O(¹D) atom with a water molecule, leads to the formation of the hydroxyl radical (OH):



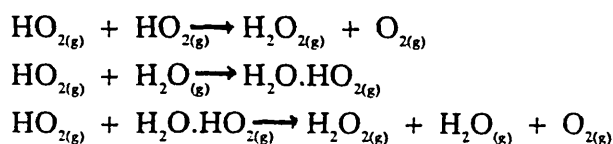
The major sources of the OH radical in daytime urban atmosphere are the photochemical dissociation of O₃ and the photochemical dissociation of aldehydes and ketones. The OH radical is central to a number of reactions in the atmosphere and is the major oxidant of carbon-containing molecules (Gunz and Hoffman, 1990) e.g. CO, methane (CH₄), other hydrocarbons, formaldehyde (HCHO). The major loss pathways for OH are:



H atoms in reaction with oxygen give rise to another free radical, the hydroperoxy radical (HO₂):



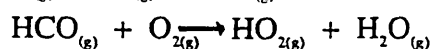
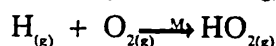
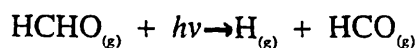
HO₂ radicals are also produced from reactions which follow the generation of CH₃. The major gaseous sources of H₂O₂ in the atmosphere involve HO₂ and hydrated HO₂ (H₂O.HO₂) radicals:



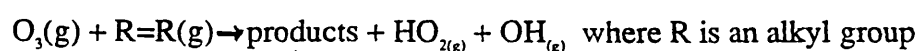
H₂O₂ production via the hydrated HO₂ radical proceeds at a faster rate than the self-reaction of HO₂. An enhancement by a factor of 2-3 in the second order rate constant has been found for relative humidity approaching 100%, compared with that at 50% relative humidity (Gunz and Hoffman, 1990; Sakugawa *et al*, 1990).

High CO concentrations favour H₂O₂ formation, as CO accelerates HO₂ formation by reacting with OH. It has been calculated that if CO concentrations were to increase twofold, the concentration of H₂O₂ would increase by 30% in unpolluted continental mid-latitude atmosphere (Thompson *et al*, 1989). In the same model, a doubling of CH₄ concentrations led to a 15% increase in H₂O₂ levels.

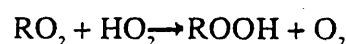
During daylight, a major source of the HO₂ radical is the photolysis of formaldehyde (Bufalini *et al*, 1972), although any source of H atoms can contribute to H₂O₂ formation (Kok *et al*, 1980):



The reaction of O_3 with alkenes, both natural and anthropogenic, can also lead to the formation of H_2O_2 and organic hydroperoxides, ROOH , where R is an alkyl group (Gab *et al*, 1985; Moller, 1988, 1989; Becker *et al*, 1990; Gunz and Hoffman, 1990; Hewitt *et al*, 1990a,b; Sakugawa *et al*, 1990), e.g.

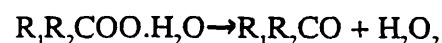
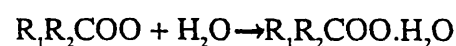


The addition of O_3 to the unsaturated $\text{C}=\text{C}$ bond leads to the formation of an initially energy rich Criegee biradical ($\text{R}_1\text{R}_2\text{COO}$), which on decomposition yields stable products and free radicals (OH , RO , HO_2 , RO_2 , etc). The subsequent cross or self-reactions of the peroxy radicals resulting from the decomposition of the Criegee intermediate can lead to the formation of hydroperoxides:



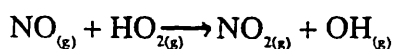
where R is an alkyl group, RO_2 is an organic hydroperoxy radical and ROOH the organic hydroperoxide

In the self-reaction of the HO_2 radical discussed earlier, the production of H_2O_2 was enhanced (twofold) in the presence of water vapour, through the formation of a $\text{H}_2\text{O} \cdot \text{HO}_2$ complex. Becker *et al* (1990) reported a ten fold increase in H_2O_2 yield from the ozonolysis of alkenes in the presence of water vapour. It was suggested that this could be due to a direct reaction between water and the Criegee biradical, the intermediate complex then decomposing to give H_2O_2 :

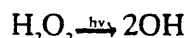
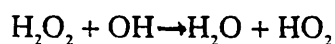


The reaction of O_3 with biogenic alkenes e.g. isoprene and terpenes emitted by a number of tree species, could be especially important in the forest atmosphere. There are reports of increased levels of H_2O_2 measured inside forests compared with outside (Jacob *et al*, 1987). It has been suggested that H_2O_2 and organic hydroperoxides produced in this way may contribute to forest decline (Moller, 1988, 1989; Becker *et al*, 1990; Hewitt *et al*, 1990a,b). High concentrations of volatile organic compounds therefore favour the production of H_2O_2 , as a result of increased generation of free radicals e.g. OH , RO , HO_2 , RO_2 (organic hydroperoxy radical).

The concentration of H_2O_2 in the atmosphere is sensitive to the levels of other pollutants. High NO_x concentrations tend to inhibit H_2O_2 formation, by scavenging free radicals e.g. the reaction of nitrogen oxide (NO) with HO_2 (Calvert *et al*, 1985):



Gas phase sinks for H_2O_2 include photolysis, reactions with the OH radical, S(IV) (as SO_2) oxidation, washout into aqueous phase, and deposition and decomposition (Kok, 1978a; Zika *et al*, 1982; Das *et al*, 1983; Radojevic, 1983; Gunz and Hoffman, 1990; Sakugawa *et al*, 1990). The gas phase reaction of H_2O_2 with OH radical and the photolysis of H_2O_2 are thought to be the major destruction pathways for H_2O_2 in air:



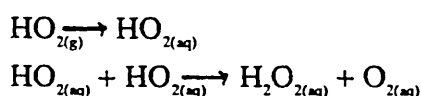
An estimate of the mean deposition velocity of gaseous H_2O_2 to vegetation (short grass) of $0.28 \pm 0.11 \text{ cm s}^{-1}$ has been calculated by Dollard *et al* (1989a). In the same study the data for O_3 yielded an average of $0.3 \pm 0.13 \text{ cm s}^{-1}$. Measurements of day and night-time fluxes of gaseous H_2O_2 to red spruce (*Picea rubens*) by Ennis *et al* (1990) were found to be almost identical, suggesting that stomatal uptake of H_2O_2 was negligible. It was concluded that the high reactivity and solubility of H_2O_2 could result in near total deposition to external plant surfaces.

The relatively high concentrations of NO_x , hydrocarbons and aldehydes in urban industrial areas tend to favour O_3 production, with the lower NO_x levels in rural areas and the remote troposphere favouring H_2O_2 formation (Kleinman, 1984; McElroy, 1986). Therefore H_2O_2 concentration will be influenced not only by the absolute concentrations of NO_x and volatile organic compounds (VOC) but also by the concentration ratio VOC: NO_x (Sakugawa *et al*, 1990). A high VOC: NO_x ratio will favour H_2O_2 production, whereas a low ratio will favour O_3 production.

Significant temporal correlations between H_2O_2 and O_3 concentrations in ambient air have been reported (Olszyna *et al*, 1988; Dollard *et al*, 1989a; Gunz and Hoffman, 1990). This may be due to their closely related origins in photochemical processes. Regression analysis of H_2O_2 and O_3 concentrations by Davies and Dollard (1989), Dollard *et al* (1989a) and Dollard *et al* (1991) gave H_2O_2 : O_3 ratios of 1:30ppb, 1:26ppb and 1:21ppb respectively. However, Kok *et al* (1978a) observed no fixed ratio of H_2O_2 to O_3 . In a number of cases, H_2O_2 concentrations were seen to decline more slowly than those of O_3 following the O_3 maximum.

H₂O₂ has been positively correlated with ambient temperature and dewpoint (Olszyna *et al*,1988) and with traffic density, except when winds were too high (Das *et al*,1983), but showed only weak correlation with relative humidity (Olszyna *et al*,1988).

The source of H₂O₂ in the aqueous phase is generally assumed to be the result of the scavenging of HO₂ from the gas phase. It is highly soluble in water, the Henry's Law constant (K_H) is in the range 1.4-1.7x10³ M atm⁻¹ (Martin and Damschen,1981; Yoshizumi *et al*,1984). However, there is evidence that H₂O₂ can be produced in situ in rain and cloudwater droplets (Chameides and Davis,1982; Zika *et al*,1982; Radojevic,1983; Gunz and Hoffman,1990). HO₂ radicals scavenged from the gas phase can generate H₂O₂:

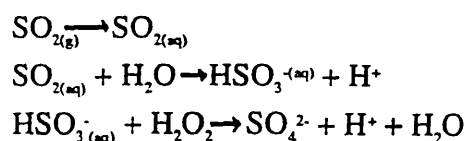


Chameides and Davis (1982) calculated that one H₂O_{2(aq)} molecule was produced for every 2.2 HO_{2(g)} radicals scavenged. McElroy (1986) considered this to be an overestimate and suggested that the production of one H₂O_{2(aq)} molecule for every four HO_{2(g)} scavenged was more reasonable. The rate determining step in the aqueous phase formation of H₂O₂ is the gas phase formation of HO₂ and not the self-reaction of aqueous HO₂ (McElroy,1986).

The sinks for aqueous phase H₂O₂ are the same as those for gas phase H₂O₂. The role of the former in the oxidation of S(IV) has received much attention and is discussed in the following section.

1.2.2 The role of hydrogen peroxide in acidification.

A major sink for aqueous phase H₂O₂ is in the oxidation of dissolved sulphur dioxide (SO₂). This reaction and the role of other oxidants such as O₂ and O₃, has been the subject of numerous studies, both in the laboratory and in the field. It is generally concluded that at pH<5, H₂O₂ is the principal oxidant of SO₂ (Penkett *et al*,1979; Daum *et al*,1983; Calvert *et al*,1985; Kelly *et al*,1985; Penkett,1986; Gunz and Hoffman,1990; Sakugawa,1990). This reaction is subject to acid catalysis, which offsets the decrease in SO₂ solubility with decreasing pH (Penkett *et al*,1979; Radojevic,1983; Calvert *et al*,1985; Daum *et al*,1988).It is limited by SO₂ and especially H₂O₂ concentration.



Altitude profiles have shown a maximum gas phase H_2O_2 concentration just above cloud tops (Heikes *et al*,1987). Vertical mixing of H_2O_2 from above with SO_2 from below, together with long range transport of H_2O_2 , can lead to in-cloud sulphate (SO_4) production (Heikes *et al*,1985,1987; Sakugawa *et al*,1990). The rate of the reaction may be controlled by this entrainment of upper level air (McElroy,1986; Chandler *et al*,1988a).

The rate of the oxidation reaction is very fast and accounts for the mutually exclusive nature of H_2O_2 and SO_2 (Daum *et al*,1983; Kelly *et al*,1985; Penkett,1986; Heikes *et al*,1987; Jacob *et al*,1987; Chandler *et al*,1988b; Daum,1988; Gervat *et al*,1988a,b; Clark *et al*,1990; Gallagher *et al*,1991). Measurements of H_2O_2 and SO_2 made at Great Dun Fell in Cumbria illustrate this phenomenon (Fig.1.01). Increases in SO_2 concentration are coincident with decreases in H_2O_2 concentration.

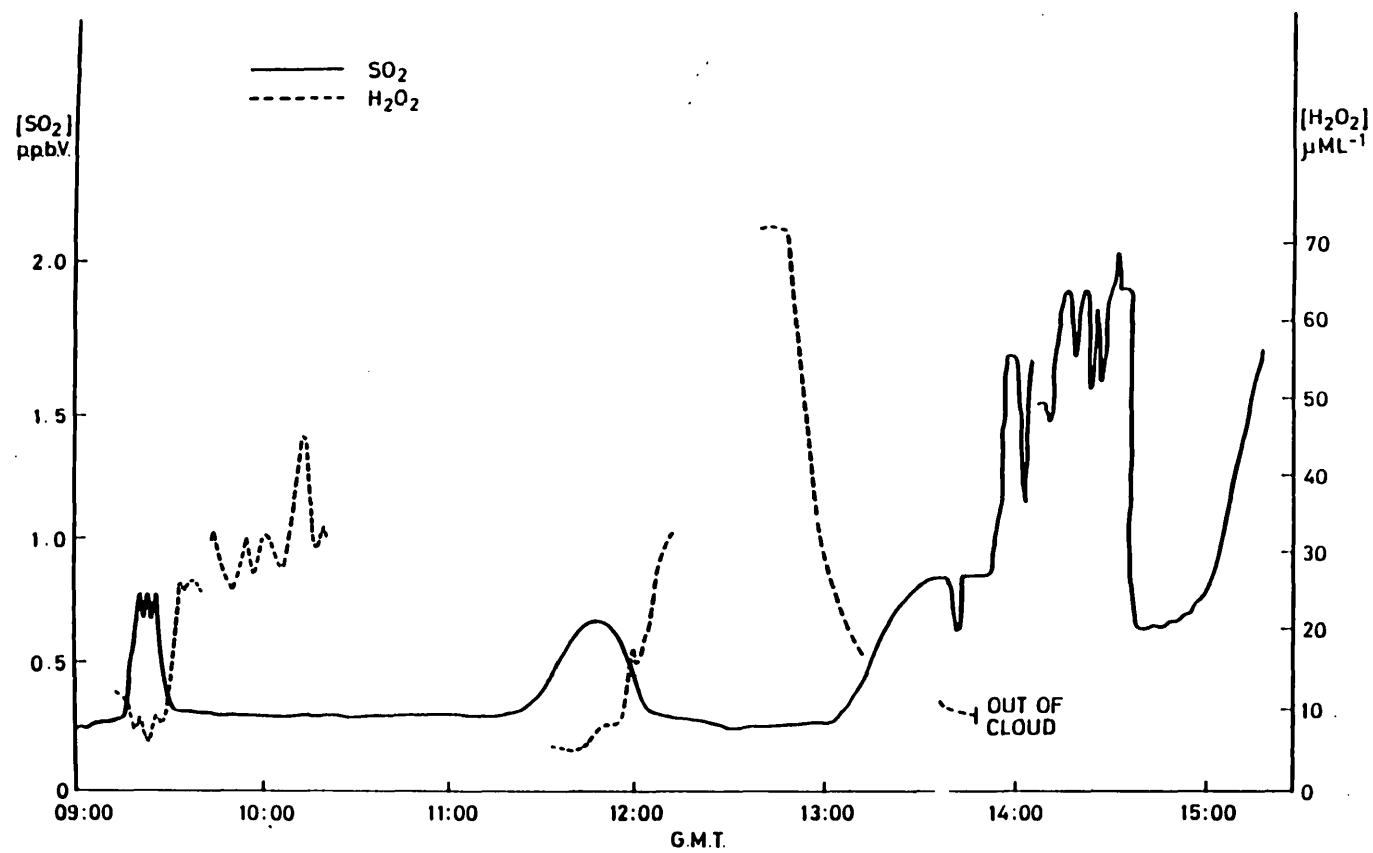
At $\text{pH} > 5$, ozone becomes the major oxidising agent, and the rate of this reaction increases with increasing pH (Penkett *et al*,1979). Therefore at $\text{pH} < 5$, values which are commonly found in fog and cloudwater, H_2O_2 has an important role in acidification, if concentrations are not limiting.

1.2.3 Measurements of hydrogen peroxide.

H_2O_2 levels in the atmosphere (like O_3) commonly show both diurnal and seasonal trends, with highest levels in the afternoon and in the summer respectively (Radojevic,1983; Das *et al*,1983; Yoshizumi *et al*,1984; Kelly *et al*,1985; Jacob *et al*,1987; Olszyna *et al*,1988; Davies and Dollard,1989; Dollard *et al*,1988,1989b,1991; Gunz and Hoffman,1990, Sakugawa *et al*,1990). Peaks of H_2O_2 concentration are often seen 1-2h later than those of O_3 and PAN (peroxyacetyl nitrate). This is expected as competition for HO_2 radicals by NO_x would inhibit H_2O_2 production until the NO_x concentration decreased to a level sufficiently low to allow the self reaction of HO_2 to generate H_2O_2 (Dollard *et al*,1989b).

The diurnal and seasonal trends reflect the dependence of H_2O_2 formation on light intensity and on its precursors. In the U.K. periods of enhanced photochemistry or photochemical episodes occur during summertime anticyclonic weather, when light south-easterly winds bring polluted air masses from continental Europe (Dollard *et al*,1989a). The enhancement of H_2O_2 formation by solar radiation (especially uv radiation) is of special interest in terms of global climate change, as stratospheric O_3 depletion could lead to an increase in H_2O_2 formation. Thompson *et al* (1989) calculated that a 10% decrease in stratospheric O_3 would increase H_2O_2 concentrations by 100% or more.

Fig.1.01. Measurements of H_2O_2 and SO_2 at Great Dun Fell in Cumbria on 19/5/86 (from Dollard *et al*, 1988).



There is a paucity of continuous, long term measurements of atmospheric H_2O_2 . Reports of H_2O_2 levels are usually the result of short-term investigations (Table 1.01). Maximum reported gas phase concentrations (summer) are in the range 3-5ppb (reviewed in Gunz and Hoffman, 1990; Sakugawa *et al*, 1990). Bufalini *et al* (1972) recorded 180ppb H_2O_2 under severe smog conditions, but this high concentration is now considered to be the result of the production of artifact H_2O_2 during analysis (Dollard *et al*, 1989a). In the UK, a maximum concentration of approximately 2ppb was reported during a photochemical episode in southern England (Dollard *et al*, 1988).

In general, aqueous phase concentrations of H_2O_2 are greater in mist and cloudwater than in rainwater (Table 1.01). However, Dollard *et al* (1989c) reported a concentration of 300uM for 2 mins during a thunderstorm of 45 mins duration.

High concentrations recorded in mist and cloudwater have usually been made at high elevation sites (Table 1.01). The maximum reported concentration is 247uM at Whiteface Mountain (1689m) in the U.S.A. (Olszyna *et al*, 1988). In the U.K., cloudwater concentrations in the region of 100uM, have been recorded at Great Dun Fell (847m) in Cumbria (Dollard *et al*, 1988).

Analysis of the H_2O_2 content of Greenland ice cores has shown that between 1970 and 1985, atmospheric H_2O_2 concentration has increased to approximately 50% above pre-industrial values (Sigg and Neftel, 1991) and model calculations have predicted a 20 -40% increase in H_2O_2 levels in remote regions between 1985 and 2035 (Thompson *et al*, 1989).

1.3 PLANT RESPONSE TO HYDROGEN PEROXIDE.

Interest in the effects of H_2O_2 on plants and especially tree species arose from observations of its chemical and spatial properties, including its production in photochemical air masses and its high solubility in water. High aqueous phase concentrations have been recorded at medium to high elevation sites. With the symptoms of forest dieback being greatest at such sites, it was possible that H_2O_2 could be a contributing factor in the decline.

H_2O_2 is also produced within plant cells in the detoxification of oxygen radicals and is itself toxic (Larson, 1988). There are a number of anti-oxidant enzymes, part of a plant's defence system, that are responsible for its destruction e.g. catalase, ascorbate peroxidase. It is not known if plants respond to exposure to externally applied H_2O_2 in the same manner.

Table 1.01. Gas and aqueous phase measurements of H₂O₂.

H ₂ O ₂ concentration	Comments	Reference
Gas phase (ppb)		
180	Maximum concentration in smog conditions in August.	Bufalini <i>et al</i> (1972)
10 - 30	Smog, July and August.	Kok <i>et al</i> (1978a)
0 - 1.3	Peak at 0930h coincident with traffic rush-hour.	Das <i>et al</i> (1983)
33ppt 700ppt	Winter maximum Summer maximum	Jacob <i>et al</i> (1987)
0.02 - 2.6 mean 0.57	At high elevation, 1689m.	Olszyna <i>et al</i> (1989)
<0.1 - 1.8	Photochemical episode, April/May.	Dollard <i>et al</i> (1989b)
<0.05 - 0.7	Yearly (1987) range for southern England	Davies and Dollard (1989)
Aqueous phase (uM)		
47	Maximum value during a summer shower.	Kok (1980)
9 - 75	Rain, February to July.	Zika <i>et al</i> (1982)
22 - 71	Cloudwater, September.	Daum <i>et al</i> (1983)
0.15 - 31	Rain.	Yoshizumi <i>et al</i> (1984)
0.1 - 63 0.1 - 110	Rain Cloudwater	Kelly <i>et al</i> (1985)
0.02 - 120 2.7 10.2	Rain Winter mean. Summer mean.	Lee <i>et al</i> (1986)

<0.04 - 40 <0.04 - 247	Rain Cloudwater At high elevation, 1689m.	Olzsyna <i>et al</i> (1989)
0.1	Cloudwater, at Great Dun Fell (847m), November.	Chandler <i>et al</i> (1988b)
0 - 8 <1 - 100	Autumn Spring Cloudwater at Great Dun Fell.	Dollard <i>et al</i> (1988)
13.7 300	Summer mean. Maximum concentration for 2 min during a 4.5 min thunderstorm.	Dollard <i>et al</i> (1989c)

1.3.1 The effects of wet deposited H₂O₂ on tree seedlings.

Investigation of plant response to wet deposited H₂O₂ has been restricted to the exposure of tree seedlings to a range of H₂O₂ concentrations in mist and the evidence is contradictory. The experimental results are summarised in Table 1.02.

The symptoms seen in Norway spruce (*P.abies*) and beech (*F.sylvatica*) seedlings - increased stomatal density, changes in the xylem and phloem fractions of the vascular bundle - were suggested to be indicative of water stress (Slanina *et al*,1985; Mallant *et al*,1986,1988; Masuch *et al*,1986). The fused wax rods and cracks in stomatal plugs of Norway spruce were suggested as possible causes for uncontrolled diffusion of water vapour (Masuch *et al*,1989). The production of phenols decrease the rate of transpiration and Norway spruce seedlings treated with H₂O₂ showed increased phenol production and changes in the ratio of different compounds forming the tannin (Slanina *et al*,1985; Mallant *et al*,1986,1988; Masuch *et al*,1986,1989). Similarly, beech seedlings exposed to H₂O₂ had high concentrations of oil drops in cells and this was considered by Masuch *et al* (1986) to be a xeromorphic adaptation.

For both beech and Norway spruce, the effect of H₂O₂ was dependent on age (Slanina *et al*,1985; Mallant *et al*,1986; Masuch *et al*,1986). Developing tissue was more susceptible than mature tissue, as significant effects of H₂O₂ treatment were seen on current year needles and young beech leaves. The response of mature tissue to H₂O₂ often showed the same trend, but was rarely significant.

Masuch *et al* (1989) saw significant effects of H₂O₂ treatment on Norway spruce at 25uM H₂O₂. Mallant *et al* (1988) reported significant effects on beech at 21uM H₂O₂, but concluded that although this concentration was not excessively high, the integrated exposure time (126h over 6 weeks) was probably greater than ambient. In this same experiment, an increase in tannin concentration in Norway spruce needles was seen at 6uM H₂O₂.

However, exposure of red spruce (*P.rubens*) to H₂O₂ concentrations up to 235uM showed no adverse effects of H₂O₂ treatment (Hanson and McLaughlin,1989). Stimulation of growth was seen at 59uM and 235uM H₂O₂ (Table 1.02) and it was concluded that wet deposited H₂O₂ was unlikely to contribute directly to red spruce decline. The authors did however, stress the danger in extrapolating from the seedling stage to natural forest stands.

The H₂O₂ concentrations given in Table 1.02 were the levels in mist at the start of the experiments, but an important consideration when working with H₂O₂ is its reactivity and potential for decay upon standing. In the work reported by Slanina *et al* (1985), Mallant *et al* (1986) and Masuch *et al* (1986) the intended H₂O₂ concentration was 118uM. However,

Table 1.02. The effects of wet deposited H₂O₂ on tree seedlings.

H ₂ O ₂ concentration (uM)	Species	Summary of H ₂ O ₂ effects
0, 118. ¹ (3h daily for 6 wks).	<i>Picea abies</i> and <i>Fagus sylvatica</i>	Current year needles and growing <i>P.abies</i> and <i>F.sylvatica</i> leaves more affected than mature tissue. Indications for both species of decreased capacity for transport of water and assimilation products due to vascular bundle changes.
0, 6, 21, 59, 147. ² (3h daily for 6 wks).	<i>Picea abies</i> and <i>Fagus sylvatica</i>	<i>F.sylvatica</i> leaves showed symptoms of water stress with an increase in stomatal number and xylem content of vascular bundle. Effects significant at 21uM. Needles of <i>P.abies</i> showed continuous increase in tannin content as function of H ₂ O ₂ concentration.
0, 25. ³ (3h, 4 days/wk for 8 wks).	<i>Picea abies</i>	Decrease in needle and tissue volume and increased stomatal density. Cracks in wax plugs of stomata. Change in the ratio of phenolic compounds. Decrease in the rate of water loss from needles.
0, 15, 59, 235. ⁴ (9h, 3 days/wk for 16 weeks).	<i>Picea rubens</i>	16% more root and current year shoot dry weight produced at 235uM H ₂ O ₂ than at 0 and 15uM. Mean relative growth rate at 59 and 235uM also significantly greater than at 0 and 15uM. No effect on photosynthetic rate, dark respiration or leaf conductance.

¹ Slanina *et al* (1985), Mallant *et al* (1986), Masuch *et al* (1986). ² Mallant *et al* (1988). ³ Masuch *et al* (1989). ⁴ Hanson and McLaughlin (1989).

during the first three weeks of their experiment, using the same mist stock solution, there was a decrease in H_2O_2 concentration due to decay. In the second half of their experiment, the mist solution was frequently renewed. This meant that the mean H_2O_2 starting concentration applied to the tree seedlings was approximately 80uM. Hanson and McLaughlin (1989) overcame this problem by preparing the misting solution in the afternoon prior to each early morning exposure.

This potential for decay upon standing applies not only to the starting concentration, but also to the change in concentration during the misting episode. Mallant *et al* (1986) and Masuch *et al* (1986) made no measurements of H_2O_2 during the misting episode, but Masuch *et al* (1989) measured the H_2O_2 concentration after each event. The levels of H_2O_2 were found to be $25\text{uM} \pm 7\text{uM}$ compared to the intended value of 29uM. Initial test runs by Hanson and McLaughlin (1989) showed that no appreciable degradation took place in H_2O_2 solutions over a 24h period.

1.3.2 Reactions of H_2O_2 at the leaf surface.

In the application of wet deposited H_2O_2 , the foliage would provide the first point of contact. There is only one report of H_2O_2 effects on epicuticular wax structure. Masuch *et al* (1989) observed fusion of wax rods and cracks in stomatal wax plugs of Norway spruce needles exposed to 25uM H_2O_2 mist. Changes in wax structure have been reported in a number of plant species in response to other pollutants (Riding and Percy, 1985; Barnes *et al*, 1988; Cape, 1988; Percy and Baker, 1990; Turunen and Huttenen, 1990).

There is the potential for further reactions at the leaf surface. These may include the reaction of H_2O_2 with atmospheric deposits on the foliage or catalytic decomposition of H_2O_2 by the leaf surface. Both processes would lead to H_2O_2 degradation. There is evidence that up to 90% of the H_2O_2 measured in cloudwater is removed by contact with red spruce (*Picea rubens*) foliage (Wolfe *et al*, 1987). Actual values for H_2O_2 concentration in the mist were not provided.

It is also known that H_2O_2 can be produced in the leaf and may play an important role in causing oxidant damage. There are a number of reports of increasing levels of foliar antioxidants in response to air pollution and other environmental stresses (Tanaka *et al*, 1985; Hausladen *et al*, 1990). It has been suggested that one such antioxidant, ascorbate peroxidase, may protect plant cells against the toxic build-up of H_2O_2 when exposed to oxidative stress (Tanaka *et al*, 1985; Mehlhorn, 1990). Tanaka *et al* (1985) exposed spinach (*Spinacea*

oleracea) to 100ppb O_3 and saw increases in the activity of ascorbate peroxidase. It was suggested that H_2O_2 which accumulates in O_3 fumigated leaves could induce enzyme synthesis.

In another study, mung beans (*Vigna radiata*) and peas (*Pisum sativum*) were exposed to ethylene before exposure to approximately 0.7M H_2O_2 and these plants produced significantly less visible injury after H_2O_2 treatment than those plants not receiving ethylene pre-treatment (Mehlhorn, 1990). The results suggested that ethylene production enhanced ascorbate peroxidase activity led to less injury.

1.4 THE AIMS OF THIS PROJECT.

As the preceding review illustrates, knowledge of the effects of H_2O_2 on plants is minimal and the evidence is contradictory. It is apparent however, that medium to high elevation sites in Europe and the U.S.A., where the symptoms of forest decline are greatest, may be subject to large inputs of wet-deposited H_2O_2 .

In the light of these observations this project aimed to investigate the effects of H_2O_2 in mist on herbaceous and tree species. The specific aims were:

1. To initially examine the potential effects of H_2O_2 on plants, by exposing herbaceous species, *Pisum sativum* and *Vicia faba*, to H_2O_2 mist.
2. To investigate the H_2O_2 sensitivity of coniferous and broadleaved U.K. tree species, which could under natural conditions be exposed to H_2O_2 .
3. To determine plant response to H_2O_2 by investigating growth and physiology and effects on plant nutrient status.

The first part of the project involved the exposure of herbaceous species to H_2O_2 under controlled environment conditions. These experiments were of four weeks duration and were aimed at elucidating any possible effects of H_2O_2 on the growth of these species. With the leaf surface being the first point of contact of wet deposited H_2O_2 , the susceptibility of plants to leaching was also investigated.

The information gained from this work was used to develop a programme for the long-term exposure (five months) of tree seedlings to a range of H_2O_2 concentrations. Dose-response data were obtained for growth, gas exchange and leaf surface chemistry. Investigations of effects on the epicuticular wax and plant nutrient status were also undertaken.

Details of the design and testing of the mist exposure systems are given in chapter 2. Chapters 3 and 4 are concerned with the experiments involving the herbaceous and tree seedlings respectively and in chapter 5, the results of all the experimental work are brought together and discussed with reference to the published literature.

Chapter 2

MEASUREMENT AND APPLICATION OF HYDROGEN PEROXIDE

2 MEASUREMENT AND APPLICATION OF HYDROGEN PEROXIDE.

2.1 MEASUREMENT TECHNIQUES.

Early analytical methods for aqueous phase H_2O_2 were wet chemical methods involving the formation of coloured titanium (IV) complexes (Bufalini *et al*, 1972). Various fluorescence reactions have also been utilised. Zika *et al* (1982) made use of the oxidation of scopoletin by H_2O_2 with horseradish peroxidase as catalyst. Horseradish peroxidase also catalysed the reaction of H_2O_2 and p-hydroxy phenyl acetic acid (POPHA) in the method of Lazrus *et al* (1985). This responds to both H_2O_2 and organic hydroperoxides, but is less sensitive to the latter, because of their lower solubility. The detection limit was recorded as 1.2nM H_2O_2 .

Chemiluminescent reactions have played a major role in the detection of H_2O_2 in both gaseous and aqueous phases. The method first described by Kok *et al* (1978b) depends on the chemiluminescence produced on oxidation of compounds with the acyl hydrazide group (specifically cyclic diacyl hydrazides) like luminol, 5-amino-2,3-dihydrophthalazine-1,4-dione. The mechanism of the reaction is uncertain, but the light emitting species is thought to be an excited state of the aminophthalate ion. The reaction needs an alkaline solution, a powerful oxidising agent and for an efficient reaction, a catalyst.

Kok *et al* (1978b) used a copper (Cu II) catalyst, but transition metal ions are known to interfere with H_2O_2 detection. Ibusuki (1983) showed that iron (Fe III) and manganese (Mn II) at typical concentrations in rainwater can cause significant negative interference to the H_2O_2 signal.

Methods using enzymes as catalysts have received some attention, particularly those involving haem derivatives like peroxidase. Yoshizumi *et al* (1984) used haemin as a catalyst for the luminol/ H_2O_2 oxidation, which gave a detection limit below $0.1 \mu\text{g l}^{-1}$. Microperoxidase (consisting of a haem ring attached to a polypeptide chain, 11 amino acids long) was the catalyst of choice of Ames (1983), as this showed stability when compared to other peroxidases and was not particularly sensitive to pH. Another useful chemiluminescent reaction is the oxidation of oxalic acid esters by H_2O_2 (Klockow and Jacob, 1986).

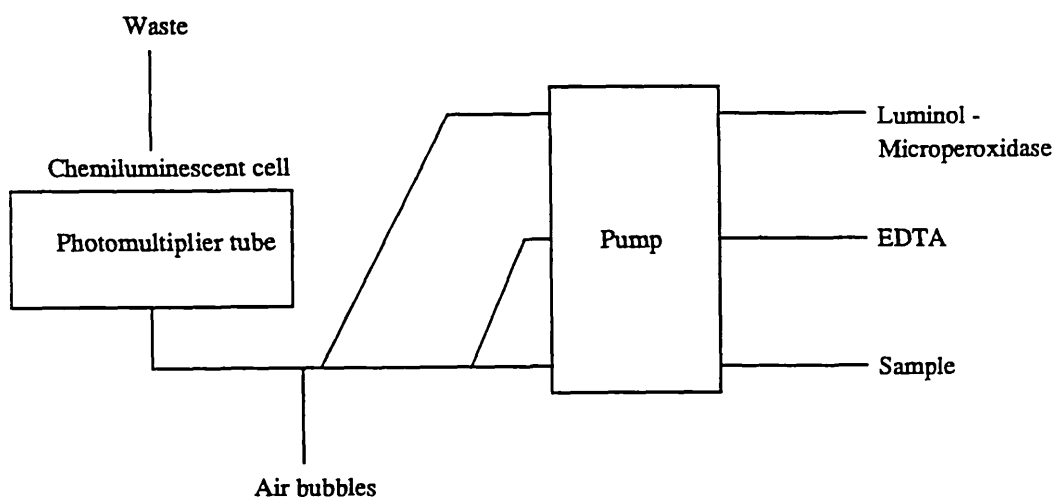
The aqueous phase analyser used in this study was assembled by A.E.A Technology Harwell, after the method described by Ames (1983). The method of Ames (1983) has been compared with the fluorescence technique of Lazrus *et al* (1985) with both giving very similar results (Chandler *et al*, 1988a; Dollard *et al*, 1988).

The reagents used in the analyser in its adapted form consist of a microperoxidase catalyst, $\text{NaHCO}_3/\text{NaOH}$ buffer and EDTA addition. The preparations are given in Table 2.01.

Table 2.01. Reagents used in the H₂O₂ analyser.

Reagents	Concentration	Flow rate ml/min	Comments
Luminol	200uM	0.42	
Microperoxidase	2uM	0.42	Type MP-11, Sigma Chemical Company.
NaHCO ₃ /NaOH buffer	0.05M/0.1M	0.42	pH optimum 10.5
EDTA	400uM	0.10	Removes interference from transition metal ions.

Fig.2.01. Schematic diagram of the H₂O₂ analyser.



The analyser is shown schematically in Fig.2.01. Solutions are fed in by a peristaltic pump. The sample and EDTA are mixed first and then meet the luminol and catalyst prior to entry to the cell. The chemiluminescence reaction reaches a peak after 10s, coincident with entry into the reaction cell. Any bubbles originating in the solution lines prior to entering the chemiluminescence cell are recirculated through the pump and expelled as waste. The detection limit is 50nM.

The analyser was routinely calibrated prior to each misting event. A standard calibration curve was prepared from a series of dilutions of 30% (100 volumes) H_2O_2 . All dilutions were performed using deionised water. Concentrations ranged from 0.1 to 100uM. The analyser was connected to a Rikadenki (R-50 series) chart recorder. The voltage output to the chart recorder was cross-checked periodically with a variable voltmeter. Fig.2.02 shows one such calibration curve, in which both H_2O_2 concentration and voltage output are expressed on a log scale.

2.2 APPLICATION OF H_2O_2 FOR TWO MISTING SYSTEMS.

An important consideration when working with H_2O_2 is its reactivity and potential for decay upon standing. Additionally, the reaction between ozone and water is a source of artifact H_2O_2 production and can be promoted at glass surfaces (Heikes,1984). The presence of impurities and reactive surfaces in the misting system would give rise to uncertainty with regards to the actual concentration of H_2O_2 reaching the plants.

It was therefore important to determine whether any changes in H_2O_2 concentration occurred during the misting period.

2.2.1 Controlled Environment cabinets.

2.2.1.1 Chamber design.

Two controlled environment cabinets, Fisons model 250PG (Fisons Scientific Apparatus, Loughborough), were adapted to allow misting over a short time scale, Fig.2.03. Within each cabinet, a Perspex chamber (0.6 x 0.6 x 0.7m) was constructed. The top panel (roof) of this Perspex chamber was built with a hole in its centre so as to allow the misting system to be inserted into the chamber. The front panel was similarly equipped with two 'port-holes' and could also be detached totally if needed. This enabled easy manipulation of misting system and plants alike.

The nature of the cabinets enabled control of temperature and photoperiod, these conditions being kept equivalent to the minimum conditions in the greenhouse i.e. 16h photoperiod, 17°C day temperature, 10°C night temperature. Lighting was provided by two banks of

Fig.2.02. Typical calibration curve for the H₂O₂ analyser.

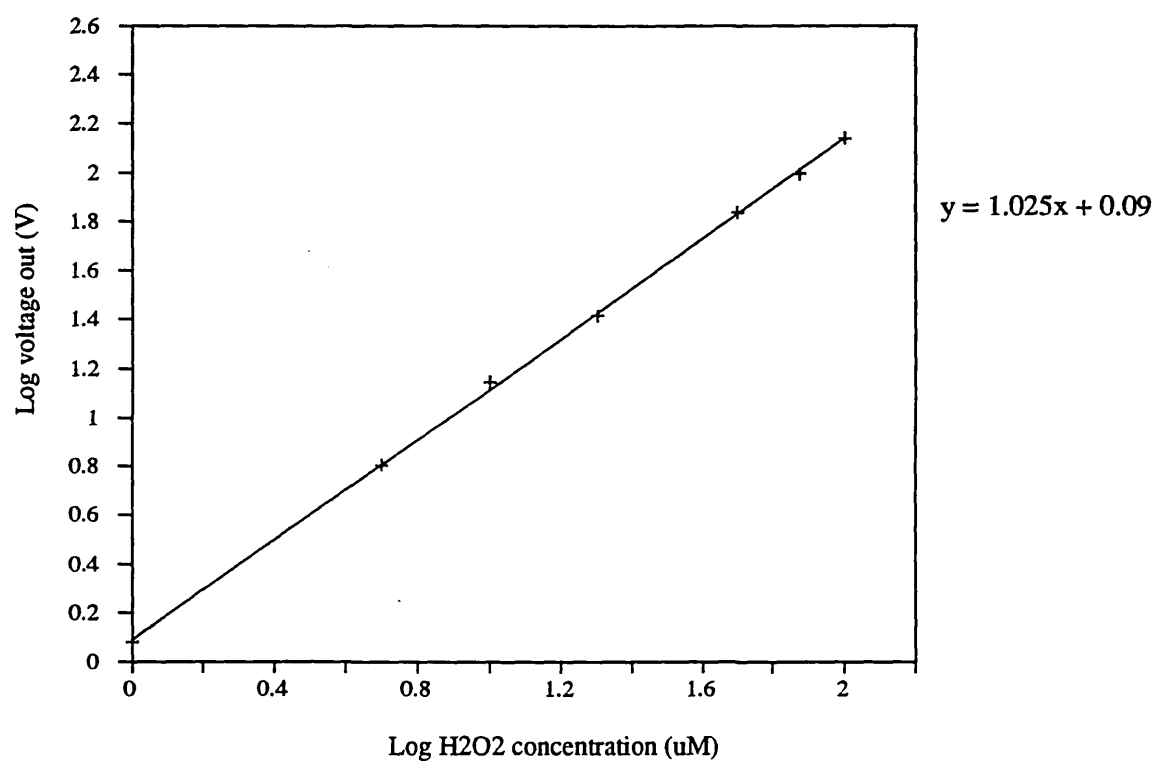
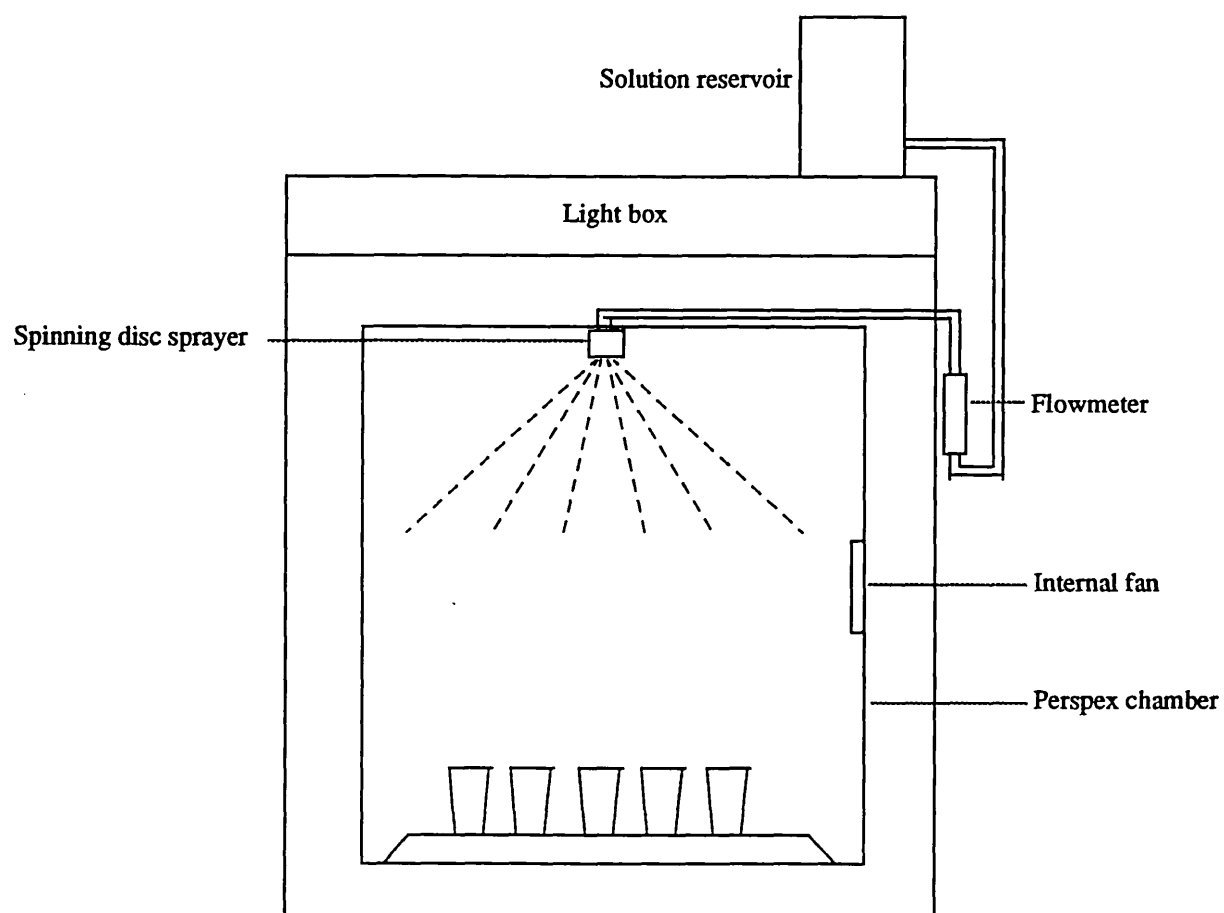


Fig.2.03. The misting system within a controlled environment cabinet.



fluorescent tubes suspended above the chambers. Photon flux density at plant height (30cm) was approximately $600 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$. Reflective foil placed on the inner walls of the cabinets ensured a more even light distribution. Relative humidity reached 100% in the chamber within 5-10 min of the first application of mist and was maintained over the misting period (three hours). A small fan attached to one wall of the perspex chamber circulated internal air to improve distribution of mist.

2.2.1.2 Mist application.

The mist solution was stored in external 10l reservoirs situated above the cabinet. A flowmeter ($0\text{-}100 \text{ ml min}^{-1}$; Platon flowbits, Basingstoke) was used to set the rate of flow at 30 ml min^{-1} from this reservoir. The flowmeter connected internally via 8mm diameter PVC tubing to a spinning disc delivery system (Micron micro-ulva, Controlled Droplet Application Ltd., Wantage) suspended from the roof of the perspex chamber. The voltage delivered to this spray head by a d.c.power supply was set at 12V.

Droplet size may be characterised by the volume median diameter (vmd) and the number median diameter (nmd). Vmd is the diameter which divides a spray into two equal parts by volume. Nmd is the median diameter of droplets by number, without any reference to the volume of the droplets. Considerable emphasis is given to small droplets in calculating the nmd, whereas a few large droplets, which can account for a large proportion of the total volume of the spray, may influence the value of the vmd.

The droplet size distribution was determined by a laser method (Arnold, 1983). With the 12V supply giving a disc spin speed of $11,000 \text{ rev min}^{-1}$ and the flow rate set at 30 ml min^{-1} , droplet diameter ranged from $20 - 133 \mu\text{m}$, with vmd $48 \mu\text{m}$ and nmd $35 \mu\text{m}$. However, these measurements were made outside the misting chambers, due to the nature of the laser technique. Therefore, temperature and humidity differences between the chambers and those employed during the laser technique could affect the size distribution of droplets expelled from the spinning disc under normal operating conditions. Fog and cloud droplets are typically less than $60 \mu\text{m}$ in diameter (Jacobson, 1984).

This system delivered approximately 2000 ml h^{-1} to the perspex chamber. However, mist losses occurred as a result of droplet impaction against the sides and roof of the chamber. The amount of mist impacting directly onto vegetation within the chamber would vary with plant size and density of the foliage. An attempt was made to estimate the amount of spray impacting directly onto the chamber walls. This was largely accounted for by impaction on the upper 20cm of the chamber, as the swathe width of the sprayer was greater than 40cm. A series of collectors was placed at two heights (35 and 55cm) on separate occasions within the chamber and mist was collected over a 15 minute misting period. The mean volume of liquid

collected in the vessels was 8 and 9ml at 35 and 55cm respectively, compared with 12.5ml if no loss occurred. The estimated mean (at both heights) loss of volume was approximately 30%. Although this figure would not necessarily be accurate when plants were in the chamber, it did provide an indication of the magnitude of the loss. The determination of the mist characteristics and calculation of loss by impactation were undertaken by C.Garretty (pers.comm.).

It was also noted that deposition of mist was slightly higher to the area directly in front of the circulation fan and so this area was not used.

2.2.1.3 Behaviour of H_2O_2 within the misting system.

The change in concentration with time for the misting system within the controlled environment cabinets was monitored for a 3h duration (1300-1600) over five episodes. A comparison was made between sprayed and unsprayed solutions. The sprayed solution was collected inside the misting chamber using a conical flask and funnel. The unsprayed solution was sampled from the solution container and therefore did not pass through the misting system. The loss in concentration of 20uM and 50uM H_2O_2 (both in a pH4 solution) with time is shown in Fig.2.04.

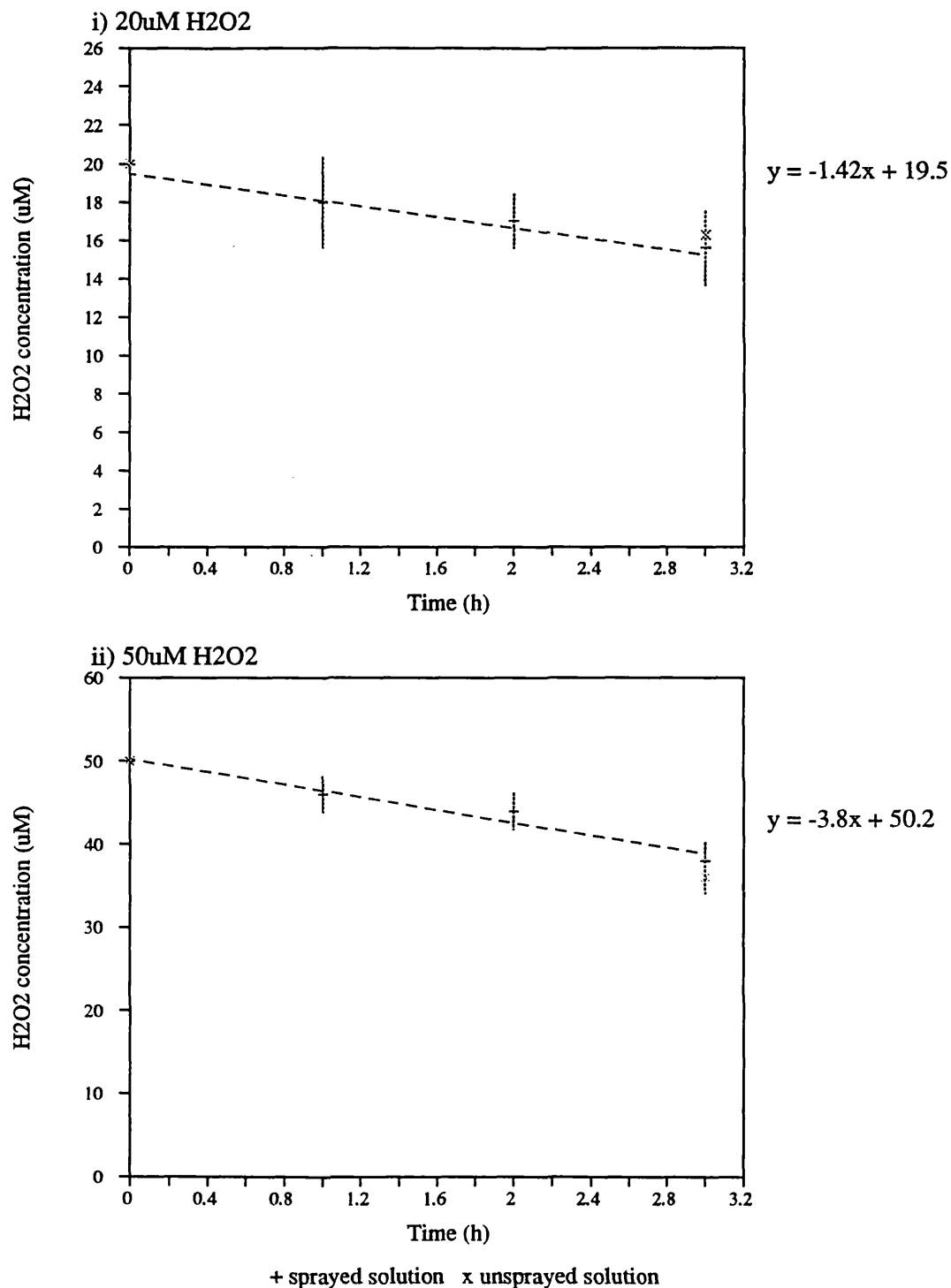
The values represent the mean concentration over misting events undertaken before the experiment started together with the first two experimental events (after which time the size of the plants limited the space available in the chamber). The negligible and insignificant difference when comparing sprayed and unsprayed solutions at both concentrations, shown in Fig.2.04, demonstrated no loss through the system. The percentage loss in concentration for both 20 and 50uM H_2O_2 mists is shown in Fig.2.05. The 22-24% loss in concentration by the end of the 3h misting period, was independent of the initial concentration, and was attributed to natural decay, although there may have been some reaction in the storage vessel.

2.2.2 Field Misting System.

2.2.2.1 Chamber design.

Stainless steel struts provided a frame on which temporary polythene sheeting could be attached for the duration of the exposure. These struts were secured at the base to concrete blocks. The dimensions of each chamber, 8 in total, were 2m x 1.2m x 2.4m (length x width x height), with the plants raised on a platform 40cm off the ground (Plate 1). These chambers are as those used by Ashmore *et al* (1988,1989).

Fig.2.04. Comparison of the changes in concentration of sprayed and unsprayed H₂O₂ solutions over a three hour misting period in a controlled environment cabinet.



Standard errors are shown (of 5 replicate runs).

Fig.2.05. Percentage loss in concentration for a range of H₂O₂ solutions in two misting systems.

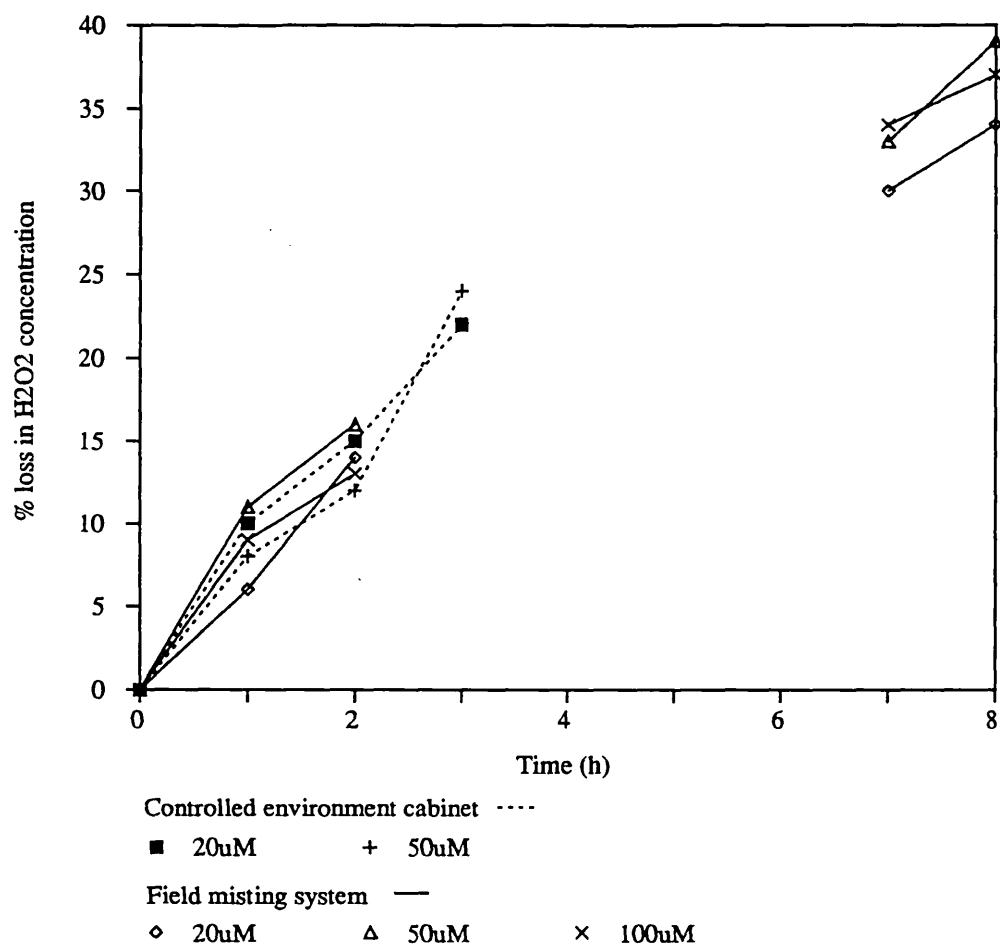


Plate 1. Temporary misting chambers for the exposure of tree seedlings.



2.2.2.2 Mist application.

The mist solution was stored in a reservoir suspended from one horizontal strut (Plate 1). This was connected by PVC tubing to a Defensor spinning disc humidifier situated in the centre of the platform. Any metal surfaces in these humidifiers were covered with PTFE tape to reduce the possibility of metal-catalysed H_2O_2 decomposition.

Droplet diameter ranged from 5-30 μm and the median droplet diameter was 11 μm (Ashmore *et al*, 1988). Relative humidity was measured within the chambers and reached 100%, although it is not known whether this was maintained over the duration of each misting event. A timer switch ensured that mist was automatically delivered at the set time.

These humidifiers delivered mist at a rate of approximately 420 ml h^{-1} . Over the planned 6h misting period about 2500ml would be deposited to surfaces within the chambers. This low volume meant that determination of loss through impaction on non-plant surfaces would be difficult to estimate. It was noted however, that after each misting event, the foliage of all seedlings in the chambers was thoroughly wetted, with little or no 'drip' off the plants.

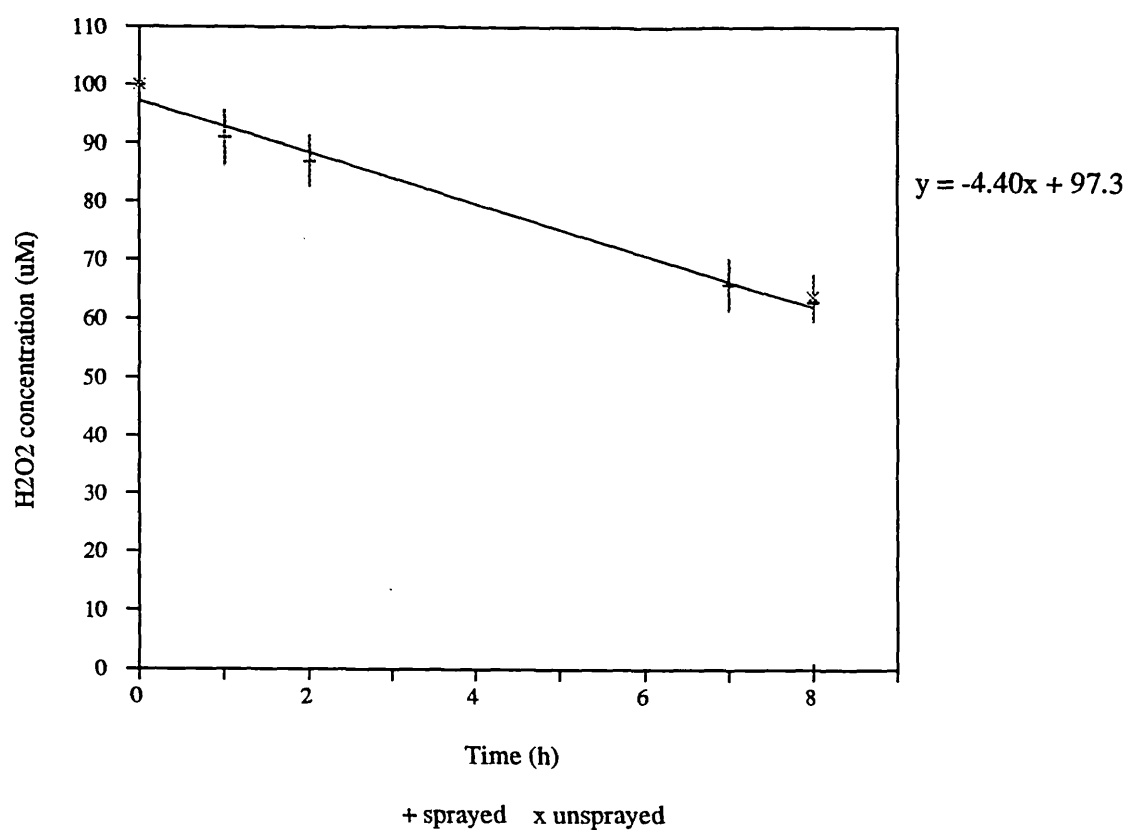
2.2.2.3 Behaviour of H_2O_2 within the misting system.

The changes in concentration with time for the field misting system were monitored over a minimum 8h duration on five occasions before the experiment began. The concentrations under consideration were 20, 50 and 100 μM H_2O_2 . As for the controlled environment system, sprayed and unsprayed solutions were compared. The decay in concentration after 8h for all treatments ranged from 34-39% and was independent of the starting concentration over the episodes monitored (Fig.2.05). A visual comparison of the data from both misting systems (Fig.2.05) suggested a similar rate of H_2O_2 loss in the controlled environment cabinets and in the field misting system.

In the field misting system there was negligible and insignificant difference in H_2O_2 concentration between sprayed and unsprayed solutions, indicating that there was no loss through the system. Fig.2.06 shows the mean loss in concentration of 100 μM H_2O_2 (in a pH4 solution).

In the field, there was concern over the concentrations of other atmospheric pollutants e.g. SO_2 , which in reacting with H_2O_2 , could result in a loss of H_2O_2 during the treatments. The field site at Silwood Park is in a rural area and previous summers have recorded low SO_2 concentrations. The mean SO_2 concentration in 1988 was 0.3ppb, with the range of daily means from 0 - 2.6ppb, but the SO_2 monitor was not operational in the year of treatment (1989). It was therefore decided to try to restrict the misting events to days when the winds came from a westerly direction, as SO_2 concentrations are then normally much lower. This approach was generally successful.

Fig.2.06. Comparison of the changes in concentration of sprayed and unsprayed 100uM H₂O₂ solutions over an 8 hour misting period in the field misting system.



Standard errors are shown (for 5 replicate runs).

2.3 MEASUREMENT OF H₂O₂ IN DROPLETS.

Use of the H₂O₂ analyser in its continuous flow mode was suitable for measuring changes in concentration for large solution volumes, as described in the section above. However, when considering the changes which could take place within a mist droplet on a leaf surface, this mode was impractical. Structural modifications to the analyser were performed at Harwell by Dr.G.J.Dollard which would enable the determination of H₂O₂ in small volumes.

Solutions were no longer pumped into the reaction cell. Adaptations made to the photo-multiplier housing enabled a narrow-bore capillary tube (7.5cm long, 7mm external diameter, 4mm internal diameter, approximate volume 0.35ml) to be inserted into the reaction cell, through a metal tube. The reagents (microperoxidase/luminol and EDTA) and sample were pre-mixed in the capillary tube, using a syringe fitted with a length of PTFE tubing (so that the total volume in the tube could be withdrawn into the syringe and thoroughly mixed) before insertion into the reaction cell. This method of pre-mixing proved to be more efficient than an earlier method of simply inverting the tubes. This gave more reproducible results within the available 10s time-interval (the interval to maximum reaction).

Initial investigations involved changing the ratio of the reagent and sample solutions in order to maximise the signal for any one H₂O₂ concentration. Table 2.02 shows the voltage output achieved for a 100uM solution (in water) using four different reagent:sample ratios. The ratio finally decided upon and thus used in all relevant experiments was:

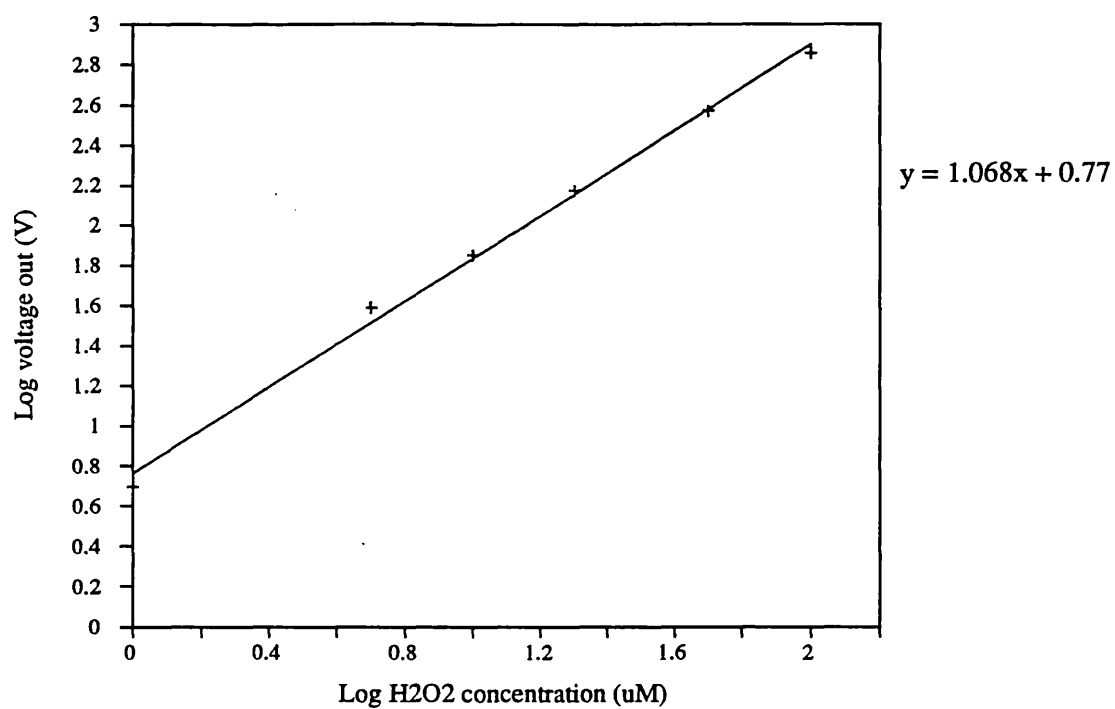
0.125ml sample, 0.125ml microperoxidase/luminol, 0.05ml EDTA

A calibration curve obtained using the small volume adaptation is shown in Fig.2.07.

Table 2.02. Measurement of H₂O₂ in small volumes; voltage output achieved for a 100uM H₂O₂ solution using four different reagent:sample ratios.

Sample volume ml	Microperoxidase volume ml	EDTA volume ml	Voltage output V
0.1	0.1	0.1	2.2
0.125	0.125	0.1	1.375
0.125	0.125	0.05	2.775
0.15	0.15	0.05	2.025

Fig.2.07. Calibration curve obtained for the H₂O₂ analyser using the small volume adaptation.



Chapter 3

EFFECTS OF HYDROGEN PEROXIDE ON HERBACEOUS SPECIES

3 EFFECTS OF H₂O₂ ON HERBACEOUS SPECIES.

3.1 INTRODUCTION.

Little is known of the effects of H₂O₂ on any type of vegetation and so, with minimal background knowledge on which to base the work, the initial aims were simple: first, to establish an experimental system which could be adapted to accommodate a range of species and provide reproducible conditions and secondly, to establish whether any effects, adverse or otherwise, were produced. It was for these reasons that the effects of short-term episodic exposure to H₂O₂ on two herbaceous species, pea, *Pisum sativum* and broad bean, *Vicia faba*, were investigated.

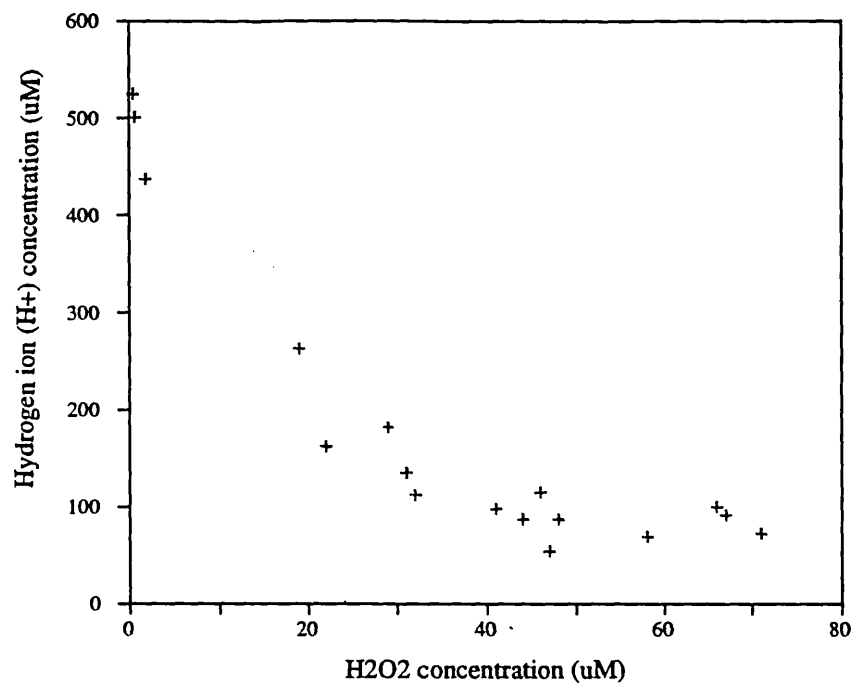
These species provided easily grown, reasonably uniform experimental material, thereby lending themselves to short term misting experiments. They were also chosen because of the difference in their leaf surface properties. *Pisum sativum* has non-wettable leaves due to crystalline epicuticular wax, while *V. faba* has wettable leaves resulting from amorphous epicuticular wax (Percy and Baker, 1987). With H₂O₂ being applied as a mist, the cuticle would be the first point of contact. It was therefore possible that plants with different cuticular properties would show varying susceptibility.

The choice of an experimental H₂O₂ concentration was made difficult by the lack of long term measurements of aqueous phase H₂O₂ levels in the field. Concentrations up to 247 μM have been recorded in mist and cloudwater at high elevation sites (reviewed in section 1.2.3). In the U.K., however, peak concentrations at Great Dun Fell in Cumbria were in the region of 100 μM (Dollard *et al*, 1988). Therefore, rather than apply concentrations which might occur only infrequently, it was decided to employ treatments with 20 μM and 50 μM H₂O₂.

The pH chosen to be used in conjunction with these two concentrations and with zero H₂O₂ (control), was pH4. This value (hydrogen ion, H⁺, concentration = 100 μM) has commonly been recorded in mist and cloudwater (Wisniewski, 1982; Daum *et al*, 1983; Waldman *et al*, 1983).

Concurrent measurements of H⁺ and H₂O₂ concentrations in mist/ cloudwater reported in the literature are shown in Fig.3.01. The inverse relationship arises from the role of H₂O₂ as a principal oxidant of SO₂ (see section 1.2.2): the oxidation of SO₂ by H₂O₂ would lead to a loss of H₂O₂ and an increase in H⁺ concentration. The H₂O₂ - H⁺ combinations chosen for this experiment, 20 or 50 μM H₂O₂ with 100 μM H⁺, fall within reported levels (Fig.3.01).

Fig.3.01. Concurrent measurements of H₂O₂ and H⁺ concentration in mist and cloudwater.



From the results of Daum et al (1983) and Kelly et al (1985).

Using the experimental system described earlier (section 2.2.1) the effects of H_2O_2 on herbaceous species were to be investigated under controlled environment conditions. Throughout the duration of treatment, the plants would be assessed for any symptoms of visible damage. Effects on growth would be determined by dry weight analysis at a final harvest. The structure of the epicuticular wax was to be examined, as the leaf surface would be the first point of contact of wet deposited H_2O_2 . In addition, any changes in the permeability of the leaf surface could lead to leaching from the foliage, so that cation loss from leaves was also examined.

In Chapter 2, examination of the changes in H_2O_2 concentration through the misting system was undertaken, but this gave no indication as to what might happen upon impaction at the plant surface. Therefore, the final consideration was to determine any changes in H_2O_2 concentration occurring at the leaf surface.

3.2 MATERIALS AND METHODS.

3.2.1 Plant Material.

Pisum sativum (cv Douce Provence) seeds were germinated on damp filter paper in PVC containers and incubated in the greenhouse for 4-5 days before planting. The seeds of *V.faba* (cv Sutton) had a tendency to rot and so were surface-sterilised by immersing for 10 min in 10% sodium hypochlorite. They were then allowed to soak in distilled water for 24h before planting. The seeds of both species were planted in plastic pots of volume 0.75l, with 2 seeds per pot.

A compost of peat and sharp sand in a ratio of 3:1, fertilised with a standard mix (given in Appendix 1) was used in all short term experiments. This compost has been successful in growing a range of species at Silwood Park. Ca and Mg fertilisers added were of GPR (General Purpose Reagent) grade, because of variation in the composition of Magnesian limestone obtained from garden centres. The use of such standard chemicals provided reproducible Ca/Mg levels.

The plants were maintained in a greenhouse of 16h photoperiod, with day temperature set at 17°C, night temperature at 10°C. The photon flux density from metal halide lamps was 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. There were differences in growing conditions for the experiments under discussion. Experiment A was set up in February 1988, so that the actual temperature was close to the preset value. Experiment B began in July 1988 when the mean daily temperature was 16.7°C. There is a linear relationship between greenhouse temperature and external ambient temperature (V.C.Brown, pers.comm). Table 3.01 shows the mean greenhouse temperature for a range of ambient temperatures. The temperature difference increases with increasing ambient temperature. Therefore the mean daily temperature experienced by the plants in the greenhouse would have been approximately 23.5°C. Photon flux density would also have exceeded the minimum provided by the lamps for a part of the photoperiod.

3.2.2 Mist Chemistry.

The ions added to distilled water to prepare the misting solution are the major ions found in mist and cloudwater (Mrose, 1965; Gascoyne and Patrick, 1981; Waldman *et al*, 1983, 1985; Jacob *et al*, 1984). The ionic concentration of the final pH4 solution are shown in Table 3.02.

Solutions were not stored between events primarily due to the natural decay of H_2O_2 . For this reason, a fresh misting solution was made up before each exposure. The required H_2O_2

Table 3.01. Comparison of greenhouse and external ambient temperatures.

Ambient temperature °C	Mean greenhouse temperature °C	Temperature difference °C
17	23.5	6.5
18	24.7	6.7
20	27.2	7.2
22	29.7	7.7

Table 3.02. Ionic concentrations for a pH4 misting solution.

Ion	Concentration ueq l ⁻¹
H ⁺	100
K ⁺	10
Na ⁺	25
NH ₄ ⁺	60
Ca ²⁺	80
Mg ²⁺	25
Cl ⁻	85
NO ₃ ⁻	70
SO ₄ ²⁻	135

concentrations, 0uM, 20uM, 50uM, were made up in the ion stock solution. The initial H₂O₂ concentration in each treatment was measured before the start of each mist application using the H₂O₂ analyser (section 2.1).

3.2.3 Exposure Regimes.

Two short term exposure experiments were designed.

3.2.3.1 Experiment A.

25 *Pisum sativum* and *V.faba* plants per treatment were exposed to a control (pH4) or 50uM H₂O₂-pH4 mist for three hours (1300-1600) twice a week for a total of four weeks. Plants from one species were exposed to the treatments in two separate chambers simultaneously. This regime gave 8 three hour applications (24h in total). The misting system in the controlled environment cabinets delivered approximately 2000ml h⁻¹ (as discussed in section 2.2.1.2), so that the total volume of mist applied over the four week period was approximately 48l.

The plants were randomly placed in the chambers approximately 5h before misting occurred and remained there until the morning after misting, so as not to disturb the droplets remaining on the leaf surface. The plant pots were placed on a platform (2.5cm from the floor of the chamber) so that they would not have to stand in the solution collecting at the chamber base. Misting began when the plants were five weeks old and was completed at nine weeks.

3.2.3.2 Experiment B.

In this experiment, 40 three week old *P.sativum* plants per treatment were exposed to a control (pH4) mist or one of two H₂O₂ treatments, 20uM-pH4 or 50uM-pH4. The timing and frequency of application, along with the duration of exposure, were as for Expt.A. The limitation of having only two chambers meant that only two treatments were exposed to mist simultaneously. This regime gave 8 three hour applications and the total volume applied over the four week period was approximately 48l.

Misting commenced three weeks after planting and was completed with the plants seven weeks old. It was not possible to perform this experiment with *V.faba* because of problems with raising the equivalent number of plants.

3.2.4 Measurements of growth.

The plants were grouped into two size classes, based on height and leaf number, 3-4 days before the experiment commenced. The plants from each size class were then allocated at random to the treatment groups or to an initial harvest. This procedure was intended to ensure

uniformity of initial size distribution for all treatments. The initial harvest was performed on 10-15 plants per species at the time of the first misting episode. Height, leaf number, leaf area, as well as fresh and dry weight of leaves, stems, roots and any reproductive structures, were registered. All plant parts were dried at 80°C for three days. Leaf area was measured using a Digithurst Microeye image analysis system (Digithurst Ltd., Royston, Herts) driven by an IBM PC-AT computer. In Expt.B, only half the total number of plants were used for area determination.

For the duration of the experiment, weekly measurements were taken of height and leaf number, along with observations of any damage symptoms. The final harvest was undertaken one week after the last misting episode. The parameters recorded were the same as for the initial harvest.

3.2.5 Examination of epicuticular wax structure.

Leaf segments were taken from pea plants (5 per treatment) used in Experiment A for examination under a scanning electron microscope (SEM). One segment was taken from each of two leaves representing three age classes: the youngest, fully unfurled leaf, a middle-aged leaf (at a node at approximately half the height) and the oldest leaf showing no signs of senescence. All segments were taken from the same area of the leaf, at the time of the final harvest.

A 11mm cork borer was used to remove the segments and these were mounted on aluminium SEM stubs pre-coated with a thin layer of silicone sealant. The samples were pre-frozen by dipping the stub in liquid nitrogen for 30s and then dried in a freeze drier with a cooled stage (Edwards) for 24h, following the method of Watterson (1989). The probability of the material cracking during its preparation was reduced by this method.

After freeze-drying, all samples were coated with gold in a Polaron sputter coater for 9 min. Examination of the material was achieved using a Phillips SEM operated at 12keV, 30nm spot size and 0°C specimen tilt. This voltage was found to produce the highest resolution without damaging the surface of the sample.

The effects of H₂O₂ treatment on epicuticular wax structure were not investigated in Expt.B.

3.2.6 Cation leaching.

The effects of H₂O₂ on the leaching of potassium (K⁺), calcium (Ca²⁺) and magnesium (Mg²⁺) from pea leaves were investigated. The procedure involved removing a leaf from a plant and sealing the cut end with paraffin wax, before quickly immersing it leaf in distilled water to remove any loose surface contamination. The leaves were then placed in 5cm x 10.5cm

plastic bags, containing 30ml of the solution under consideration. Bags containing solution but no leaves served as controls. The open end of the bag was heat sealed. The leaves were incubated at 5°C for 24h under constant illumination in the controlled environment cabinets.

The leachate was analysed for K^+ , Ca^{2+} or Mg^{2+} as required using a Thermo-Jarrell Ash S-12 Atomic Absorption/Emission Spectrophotometer. The leaves were then oven-dried at 80°C for 24h and the ions leached expressed on a $mg\ g^{-1}$ leaf dry weight basis.

3.2.6.1 Leaching of cations from leaves exposed to H_2O_2 mist.

The effect of exposure to a H_2O_2 mist on leaching was investigated in Expt.B. One leaf was removed from each of 20 plants per treatment, at the end of the four week exposure period. The leaf age was standardised by taking one of those present at the fifth node from the base of the plant. The leaves were incubated in distilled water. The solution was analysed for K^+ content by flame emission.

3.2.6.2 Leaching from unexposed leaves.

The effect of H_2O_2 concentration on unexposed plants (not used in Expts.A or B) was also investigated. Three leaves of different ages were removed from each of 20 three week old *P.sativum* plants. The ages were classified as:

young (Y) - youngest fully unfurled leaf.

middle aged (M) - a leaf taken from a node halfway up the plant.

old (O) - oldest leaf showing no signs of senescence.

The leaves were incubated in one of four solution treatments:

1. distilled water
2. distilled water-100 μ M H_2O_2
3. pH4 - the artificial mist solution, as described in section 3.2.2.
4. pH4-100 μ M H_2O_2 .

Leaves incubated in the two distilled water treatments were from the same set of plants but a different set, grown two weeks later, was used for the two pH4 treatments. The leachate was analysed for K^+ , Ca^{2+} and Mg^{2+} content.

3.2.7 Effect of leaf surface on H_2O_2 concentration.

The reaction of H_2O_2 at the leaf surface was investigated by applying individual droplets (0.125ml volume) to the surface of a *V.faba* leaf, or to a control, teflon-covered glass surface. The experiments were undertaken in a CT room at 18°C and 70% relative humidity. At set time intervals, droplets were withdrawn from both surfaces using a Pasteur pipette. The H_2O_2

concentration was measured using the small volume adaptation to the H_2O_2 analyser (section 2.3). pH and conductivity of the droplets were measured using a Horiba Cardi-Compact pH meter and conductivity meter, respectively.

Initially, the behaviour of 100uM H_2O_2 (in distilled water) droplets on the leaf and teflon-covered surfaces was investigated. It was only possible to use three plants in this study, due to the number of measurements that had to be made in the available time interval. At hourly time intervals, one droplet was collected from each of the experimental plants and from the control surface. However, due to evaporation of the droplets with time, it was necessary to sample droplets from more than one leaf per plant as the experiment progressed.

This was followed by a comparison of the differences between H_2O and 100uM H_2O_2 droplets on the leaf surface only. A different set of five plants to the above, but of the same age, were used in this investigation. Each of these plants had both H_2O and H_2O_2 droplets on their leaves. The droplets were analysed for H_2O_2 concentration, pH and conductivity. Again, leaves of different ages were studied.

3.3 DATA ANALYSIS.

All data were analysed using the Genstat IV (Rothamsted Experimental Station) statistical package.

3.3.1 Growth effects.

The weekly measurements of height and leaf number of plants used in Expt.A. were analysed using a t test for differences between control and H_2O_2 treated plants. The data were log transformed to ensure that they were normally distributed.

A one-way analysis of variance was used to analyse the final harvest data from both Expts.A and B. The data were log transformed. In testing for potential covariates, the effect of initial leaf number on the final harvest data was found to be significant (at $P < 0.05$) in both experiments. Consequently, it was used as a covariate in the analysis of the harvest data and all values given in the results (section 3.4) are adjusted means. Specific leaf area ratio (SLAR) was calculated as leaf area/leaf dry weight, mean leaf area as leaf area/leaf number and mean leaf weight as leaf dry weight/leaf number. All were logged for analysis.

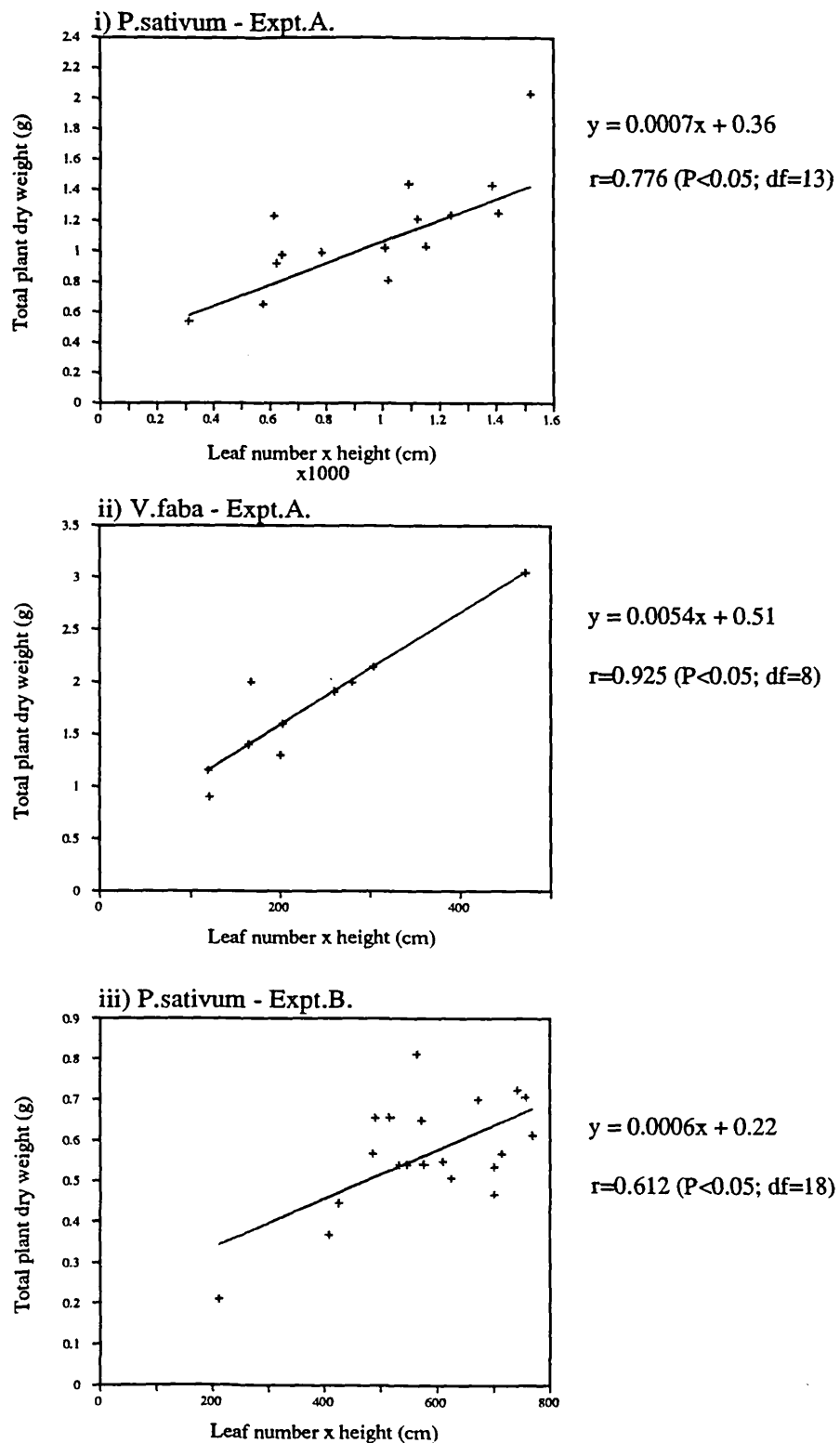
The results from Expt.A were analysed for differences between control and H_2O_2 treated plants. The incorporation of three treatments in Expt.B. meant that the treatment sum of squares was partitioned into a linear component, in order to test for a linear relationship between H_2O_2 and plant response. An example of the output from this analysis is given in Appendix 2. In cases where significant variance (F) ratios arose, determination of significant differences between the treatment means was achieved by calculation of the least significant difference, LSD (Sokal and Rohlf, 1981).

In both experiments, the mean relative growth rate (MRGR) was determined. From each initial harvest, the data for total plant dry weight were plotted against leaf number x height, and a regression line obtained (Fig.3.02). There was a significant positive correlation between the two growth parameters at all initial harvests. The regression lines were used to predict initial plant dry weight for the final harvest plants. MRGR was calculated as (final dry weight - initial dry weight)/duration of the experiment in days and logged for analysis.

3.3.2 Leaching effects.

The results of leaching from leaves exposed to H_2O_2 mist (in Expt.B) were log transformed and then subjected to a one-way ANOVA to test for differences between the treatments and for a linear relationship in the data.

Fig.3.02. Regression analysis of the relationship between total plant dry weight and (leaf number x height) in Pisum sativum and Vicia faba.



The data from leaves not exposed to a H_2O_2 mist were also log transformed. The two-way ANOVA used in this analysis tested the differences between the H_2O_2 treatments and between leaf age, as well as indicating any treatment-age interaction. No covariates were used in either analyses.

3.3.3 Effects of leaf surface on H_2O_2 concentration.

A one-way ANOVA was used to test the difference between the teflon-coated surface and the leaf surface and also the difference between H_2O and H_2O_2 droplets on a leaf surface. Initial measurements of pH were used as covariates for the pH data.

3.4 RESULTS.

3.4.1 Effects of H₂O₂ on growth.

3.4.1.1 Experiment A.

The results of this experiment were complicated by the age of the plants, with the older leaves showing signs of senescence at an early stage in the investigation. With the delay incurred by the breakdown of the controlled environment cabinets, the plants were already five weeks old when misting began, with some 25% of the pea plants already flowering.

Two weeks into the experiment, 90% of the pea plants in both treatments had produced pods, at an average of three per plant and 85 - 90% of the beans were in flower. In both species, older leaves were showing signs of senescence and turning yellow, but there was no difference between the treatments.

There was no significant effect of H₂O₂ treatment on height or leaf number of either species at any time during the experiment or at the final harvest (Table 3.03 and Fig.3.03).

At the final harvest leaf area measurements indicated a significant H₂O₂ effect in broad bean with H₂O₂ treated plants having significantly less leaf area (30%) than the controls, but specific leaf area ratio (SLAR) was unaffected (Table 3.04 and Fig.3.04). Mean leaf area (MLA) and mean leaf weight (MLW) of bean were also significantly affected by H₂O₂ (Table 3.04). Exposure to H₂O₂ significantly decreased the leaf area per leaf (MLA) by 29% and the dry weight weight per leaf (MLW) by 24% when compared to the control (Fig.3.04). In pea, these parameters were not significantly affected by H₂O₂ (Table 3.04).

There was no significant difference in flower and pod dry weight, between control and treated plants for both species (Table 3.04 and Fig.3.05). For pea, only root dry weight was significantly affected by exposure to H₂O₂ (Table 3.04 and Fig.3.05i), with H₂O₂ treated plants having 21% less root dry weight than the controls. There was some evidence that all other dry weight parameters in pea also decreased as a result of H₂O₂ exposure, but these were non-significant. In broad bean, leaf, stem root and total plant dry weight were significantly decreased by 27%, 24%, 31% and 27%, respectively, as a result of H₂O₂ treatment (Table 3.04 and Fig.3.05ii).

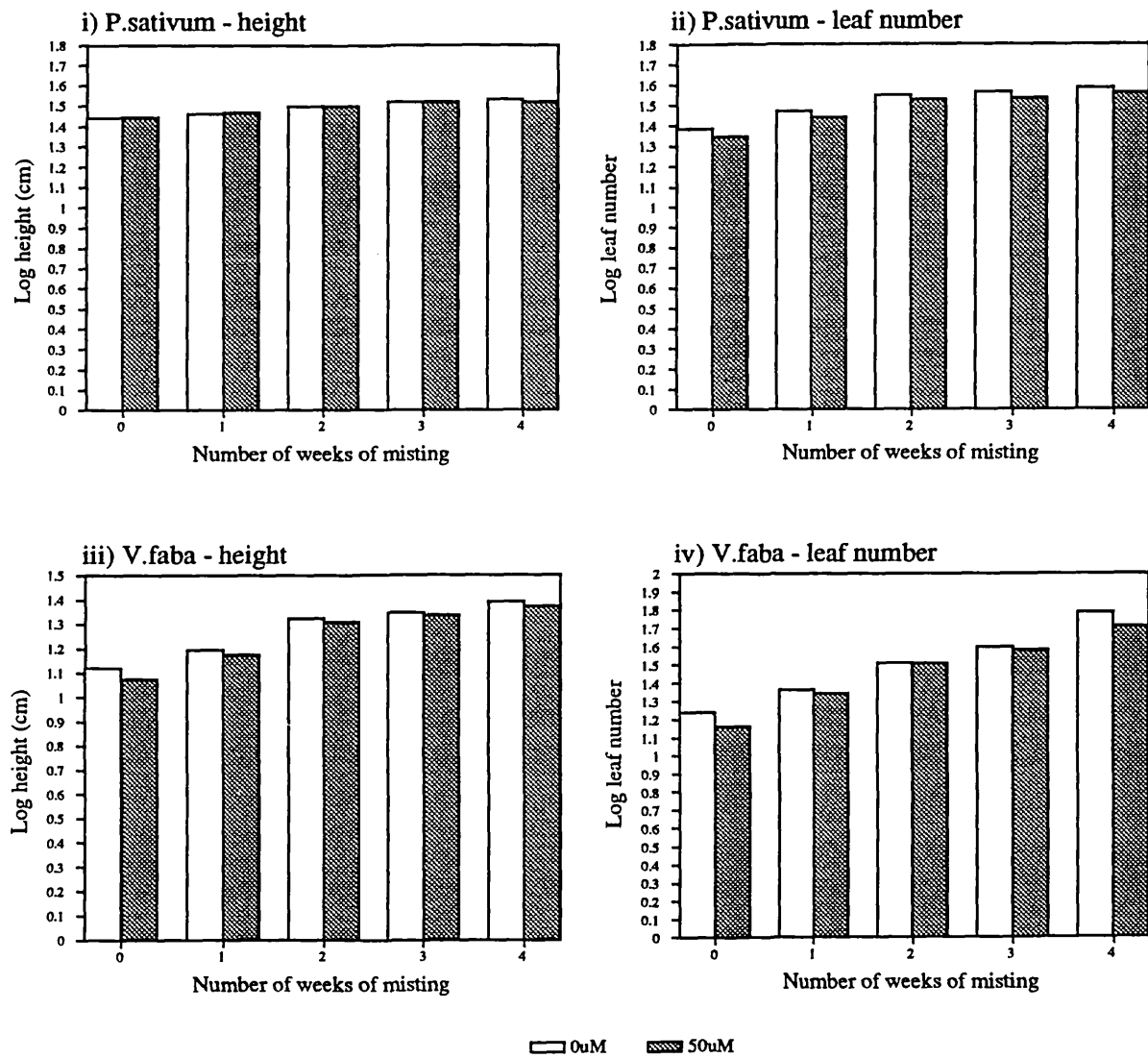
Mean relative growth rate (MRGR) in pea was not significantly affected by exposure to H₂O₂ (Table 3.05). In broad bean, MRGR for the 50uM treated plants was significantly decreased by 48% compared to the controls (Table 3.05).

Table 3.03. t test values for the effects of H₂O₂ on height and leaf number in *Pisum sativum* and *Vicia faba*.

Number of weeks of misting	Value of t (df=48)			
	<i>Pisum sativum</i>		<i>Vicia faba</i>	
	Height	Leaf number	Height	Leaf number
0	0.20	1.37	1.19	1.32
1	0.06	0.51	0.57	0.74
2	0.03	0.28	0.44	0.06
3	0.40	1.22	0.25	0.39
4 (final harvest)	0.01	1.05	0.70	1.38

All values of t shown in the table are non-significant.

Fig.3.03. Change in height and leaf number in Pisum sativum and Vicia faba from Expt.A during a four week exposure to H₂O₂ mist.



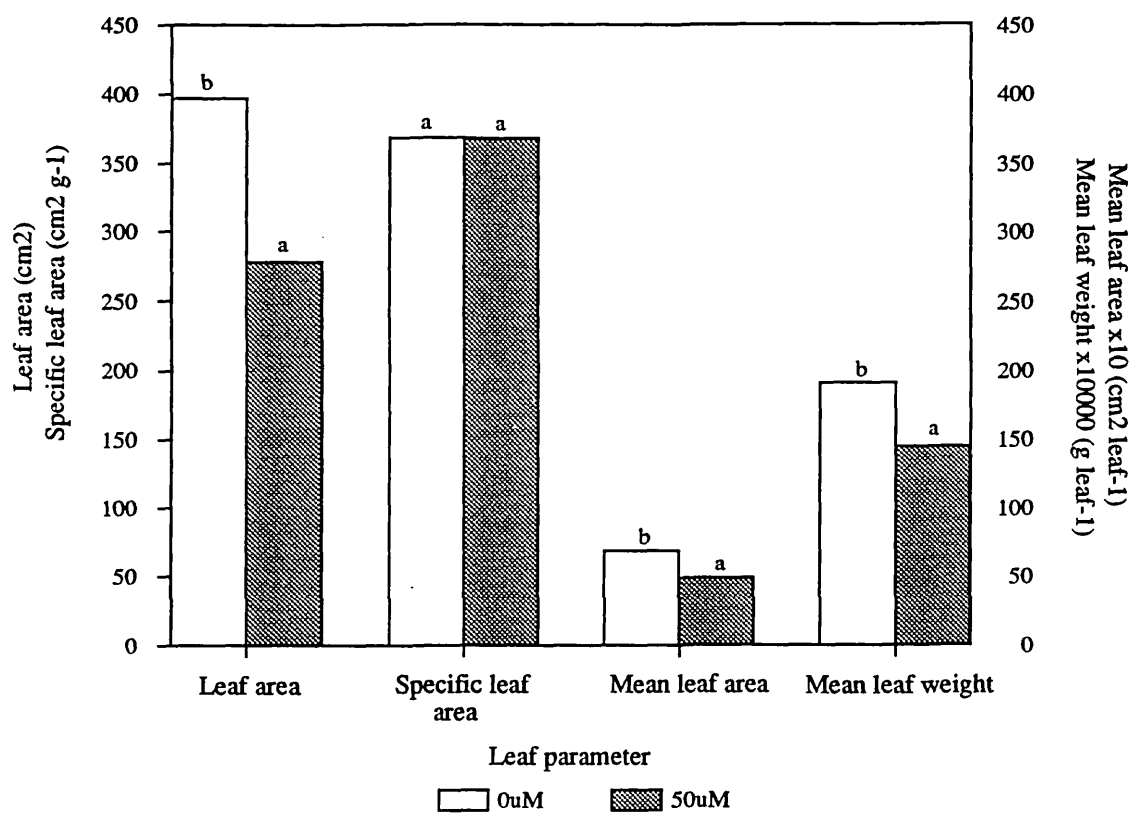
There were no significant differences between the means at any time during the experiment.

Table 3.04. F ratios for final harvest results from Expt.A for both *Pisum sativum* and *Vicia faba*.

Parameter	<i>P.sativum</i> (df=1,47)	<i>V.faba</i> (df=1,47)
Leaf area	2.38 ns	7.27 **
SLAR	2.28 ns	0.76 ns
MLA	1.08 ns	4.58 *
MLW	1.17 ns	11.95 **
Flower and pod dry weight	0.59 ns	0.02 ns
Leaf dry weight	1.26 ns	10.64 **
Stem dry weight	0.01 ns	7.91 **
Root dry weight	4.03 *	7.23 **
Total plant dry weight	1.11 ns	9.22 **

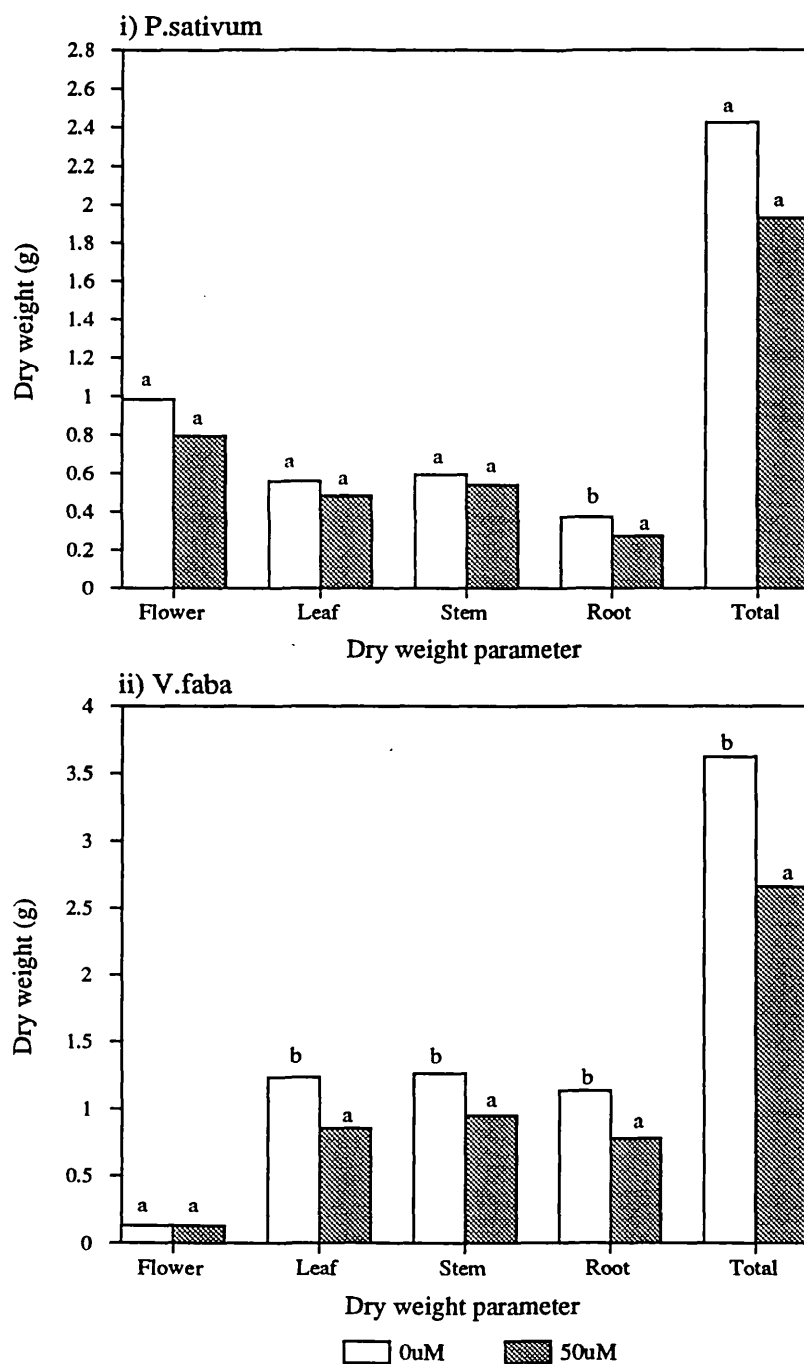
* and ** indicate significant F ratios at $P<0.05$ and $P<0.01$ respectively. ns indicates non-significant F ratios at $P<0.05$. The results were obtained from logged data with initial leaf number as a covariate.

Fig.3.04. The effects of H₂O₂ on *Vicia faba* leaf growth.



For any one parameter, means with different letters are significantly different at $P < 0.05$.

Fig.3.05. Effects of exposure to H₂O₂ on the growth of Pisum sativum and Vicia faba from Expt.A.



For any one parameter, means with different letters are significantly different at $P < 0.05$.

Table 3.05. Mean relative growth rate for *Pisum sativum* and *Vicia faba* in Expt.A.

Species	F ratio (df=1,48)	MRGR (g day ⁻¹)	
		0uM	50uM
<i>P.sativum</i>	3.71 ns	0.058	0.040
<i>V.faba</i>	13.66 **	0.071	0.037

ns and ** indicates non-significance and significance at $P < 0.05$ and $P < 0.01$ respectively.

Throughout the four week experiment the plants were assessed for any signs of visible damage, but none were seen.

3.4.1.2 Experiment B.

At the final harvest there was no effect of H_2O_2 on plant height, but there was a significant reduction in leaf number (Table 3.06). This reduction was not shown as a significant difference between treatments, but rather, by a significant linear trend within the data. A significant F ratio for the linear component does not necessarily indicate a significant difference between treatments. It does however, imply that the relationship between the treatment means and H_2O_2 concentration can be described by a linear equation. Leaf number was significantly affected by H_2O_2 (Table 3.06) and showed an inverse linear relationship with H_2O_2 concentration (Fig.3.06i). Calculation of the LSD indicated a significant difference (11%) between the means of control and 50uM treatments.

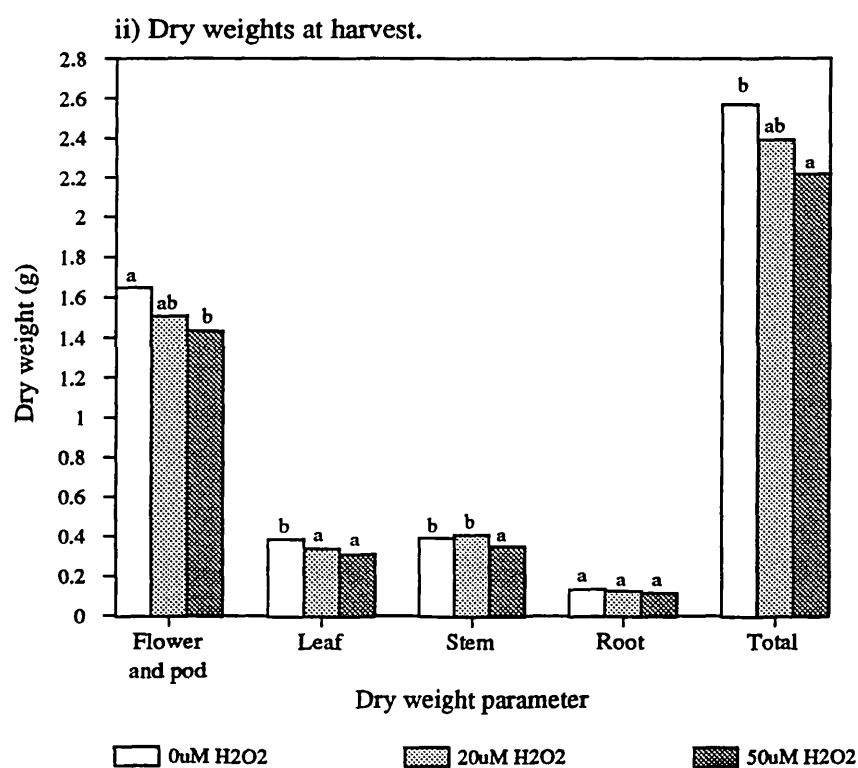
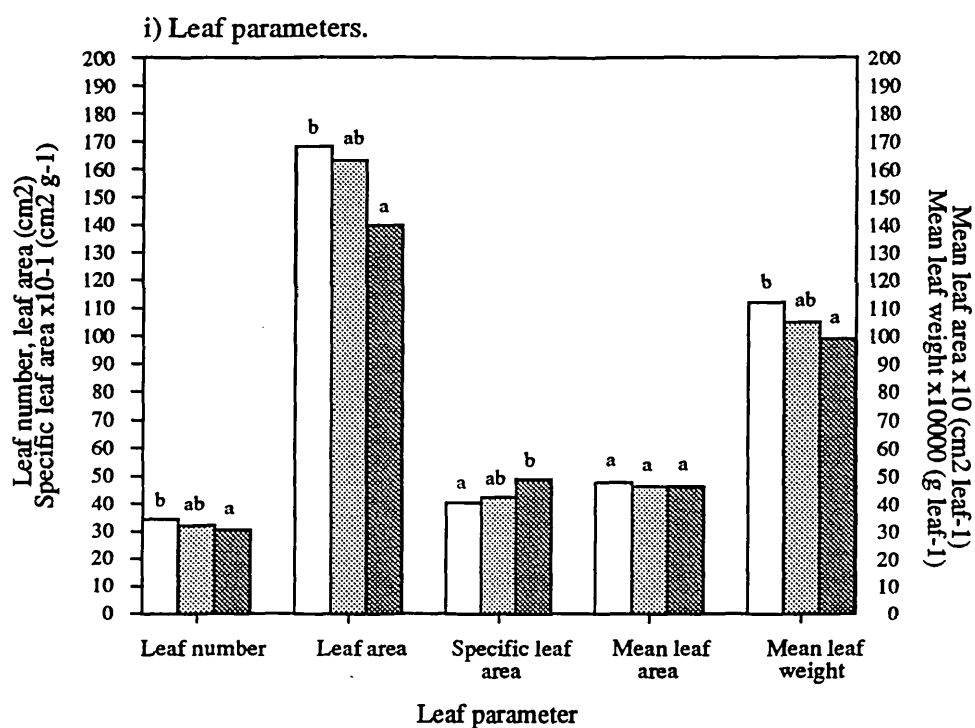
Exposure to H_2O_2 had a significant effect on leaf area and specific leaf area, SLAR (Table 3.06). For leaf area there was a significant negative linear relationship with H_2O_2 , but for SLAR the relationship was positive (Fig.3.06i). There were significant differences of 17% for leaf area and 21% for SLAR between control and 50uM treatments. Mean leaf area (MLA) was not significantly affected by H_2O_2 treatment, but mean leaf weight (MLW) showed a significant linear response to H_2O_2 exposure (Table 3.06). Leaf dry weight per unit leaf (MLW) was significantly decreased by 12% in the 50uM treatment compared to the control (Fig.3.06i)

Table 3.06. F ratios for final harvest data from Expt.B for *Pisum sativum*.

Parameter	Treatment (df=2,116*)	Linear (df=1,116*)
Height	0.11 ns	0.08 ns
Leaf number	3.17 *	6.30 *
Leaf area	2.72 ns	5.26 *
SLAR	3.16 ns	5.58 *
MLA	0.25 ns	0.45 ns
MLW	2.41 ns	4.31 *
Flower and pod dry weight	2.61 ns	5.30 *
Leaf dry weight	4.23 *	9.04 **
Stem dry weight	3.51 *	5.34 *
Root dry weight	1.47 ns	2.80 ns
Total plant dry weight	3.28 *	6.97 **

* and ** indicate significant F ratios at $P<0.05$ and $P<0.01$ respectively. ns denotes non-significance. * the numbers in brackets at the head of the columns represent the degrees of freedom associated with the F ratios, except for those for leaf area, SLAR and MLA, where df=2,56 or 1,56. These results were obtained for logged data with initial leaf number as a covariate.

Fig.3.06. Effects of exposure to H₂O₂ on the growth of *Pisum sativum* from Expt.B



For any one parameter, means with different letters are significantly different at $P < 0.05$.

Leaf dry weight was significantly decreased in the 20uM and 50uM treatments by 12% and 19%, respectively, when compared to the control. The data for leaf dry weight gave significant F ratios for both treatment and linear components (Table 3.06 , Fig.3.06ii). Flower and pod dry weight was 13% less in the 50uM treatment than in the controls and this was described by a significant linear trend (Table 3.06, Fig.3.06ii).

Analysis of stem and total plant dry weight gave significant treatment and linear F ratios (Table 3.06, Fig.3.06ii). Stem dry weight in the 50uM treated plants was significantly decreased by 10% and 14% compared to the control and 20uM treatment respectively. Total plant dry weight showed a 15% reduction in the 50uM treatment compared to the control. Root dry weight was not significantly affected by exposure to H₂O₂ (Table 3.06, Fig.3.06ii).

MRGR was not affected by exposure to H₂O₂. Both treatment and linear component F ratios were non-significant (Table 3.07).

Table 3.07. Mean relative growth rate for *Pisum sativum* in Expt.B.

F ratio		MRGR treatment means (g day ⁻¹)		
Treatment (df=2,117)	Linear (df=1,117)	0uM	20uM	50uM
2.02 ns	3.67 ns	0.070	0.062	0.059

ns indicates non-significance at P<0.05.

No visible damage symptoms were observed on the pea plants after four weeks misting. This result was consistent with that seen in Expt.A.

3.4.2 Cuticular effects.

No differences in leaf epicuticular wax structure or stomatal wax organisation were seen between control and H₂O₂ treated pea plants.

3.4.3 Leaching effects.

3.4.3.1 Leaching from leaves exposed to H₂O₂ mist.

There was a suggestion that previous exposure to H₂O₂ in Expt.B, increased K⁺ leakage from leaves incubated in distilled water (Table 3.08). However, the F ratios for both treatment and linear components were non-significant.

Table 3.08. K⁺ leakage from leaves of pea plants from Expt.B, incubated in distilled water.

F ratio		K ⁺ leakage (mg g ⁻¹) for 24h		
Treatment (df=2,57)	Linear (df=1,57)	0uM	20uM	50uM
1.07 ns	2.15 ns	1.308	1.559	1.684

ns denotes non-significance.

3.4.3.2 Leaching from leaves not exposed to H₂O₂ mist.

Of the three cations analysed (K⁺, Ca²⁺ and Mg²⁺) only K⁺ leakage showed a significant H₂O₂ treatment effect (Table 3.09, Fig.3.07i). Pea leaves (all ages) leached 57% more K⁺ on incubation in 100uM H₂O₂ solution, compared with leaves in H₂O only. Mg²⁺ leakage was also greater into the H₂O₂ solution, but this was non-significant.

Leaf age (both treatments) had no significant effect on K⁺ leakage, but there were differences in the leaching of Ca²⁺ and Mg²⁺ with age (Table 3.09, Fig.3.07ii). The loss of Ca²⁺ from old leaves was 100% and 185% greater than from young and middle-aged leaves respectively. For Mg²⁺, leakage was 51% and 46% greater from young and old leaves, respectively, compared to middle-aged leaves. There was no H₂O₂ - age interaction for any of the cations.

The response of leaves incubated in 100uM-pH4 solution differed from the above. Only Ca²⁺ leakage showed a significant effect of H₂O₂ treatment (Table 3.10, Fig.3.08i). Leaves incubated in 100uM H₂O₂-pH4 leached significantly more Ca²⁺ (112%) than leaves in pH4 solution only. The leaching of all three cations showed significant differences with leaf age (Table 3.10, Fig.3.08ii). Young leaves leached 72% and 190% more K⁺ than middle-aged and old leaves, respectively. These young leaves also lost 61% more Ca²⁺ compared to middle-aged leaves and 200% more Mg²⁺ than both middle-aged and old leaves. There were no significant treatment - age interactions.

When all results from leaves (of all ages) not exposed to H₂O₂ mist were averaged across the H₂O and pH4 treatments, it was seen that cation leaching, especially K⁺ and Ca²⁺, was substantially greater from leaves treated with H₂O₂ (Table 3.11). No statistical comparison of all four treatments was undertaken as the distilled water treatments and the pH4 treatments involved two different sets of plants.

Table 3.09. Age and H₂O₂ treatment differences in cation leakage from pea leaves incubated in H₂O₂ solution.

Cation	F ratio		
	Treatment (df=1,79)	Age (df=2,79)	Treatment-age (df=2,79)
K ⁺	16.05 **	0.43 ns	0.34 ns
Ca ²⁺	0.24 ns	12.53 **	0.12 ns
Mg ²⁺	3.16 ns	5.86 **	0.42 ns

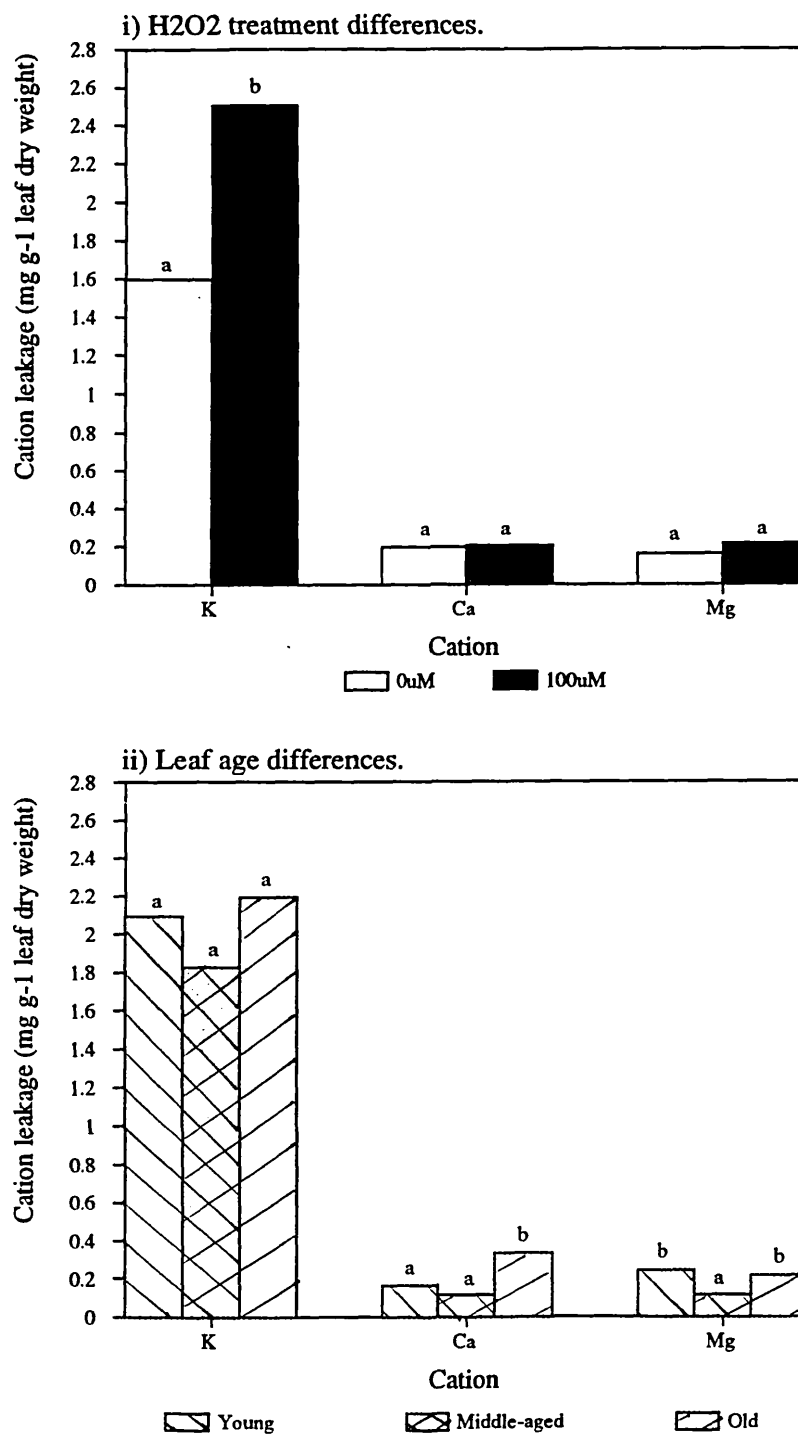
ns and ** indicate non-significance at P<0.05 and a significant F ratio at P<0.01 respectively. The numbers in brackets represent the degrees of freedom associated with each F ratio.

Table 3.10. Age and treatment differences in cation leakage from leaves incubated in H₂O₂-pH4 solution.

Cation	F ratio		
	Treatment (df=1,108)	Age (df=2,108)	Treatment-age (df=2,108)
K ⁺	0.10 ns	17.54 **	0.12 ns
Ca ²⁺	15.32 **	3.74 *	1.33 ns
Mg ²⁺	0.05 ns	23.13 **	0.23 ns

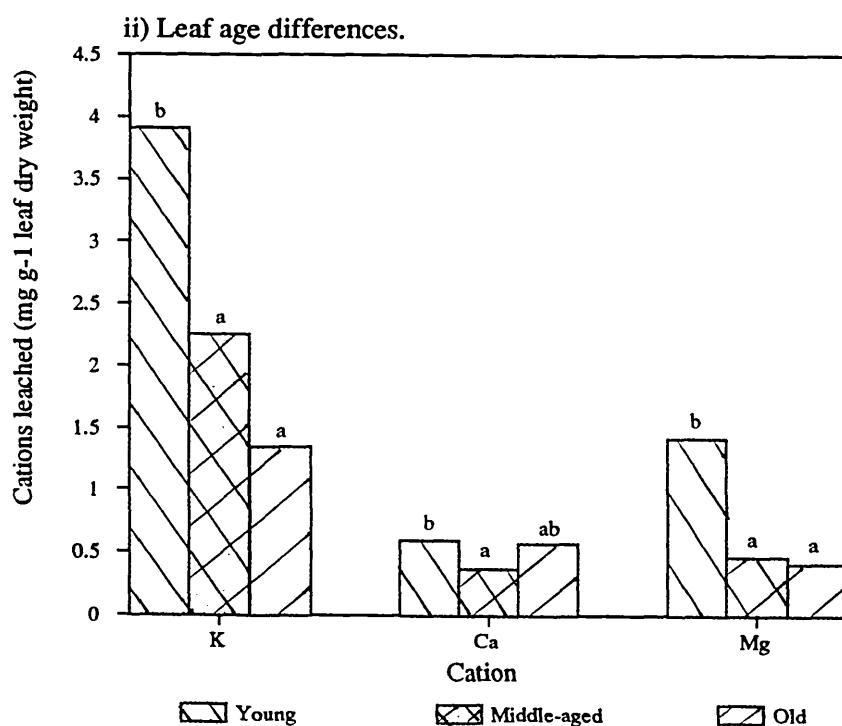
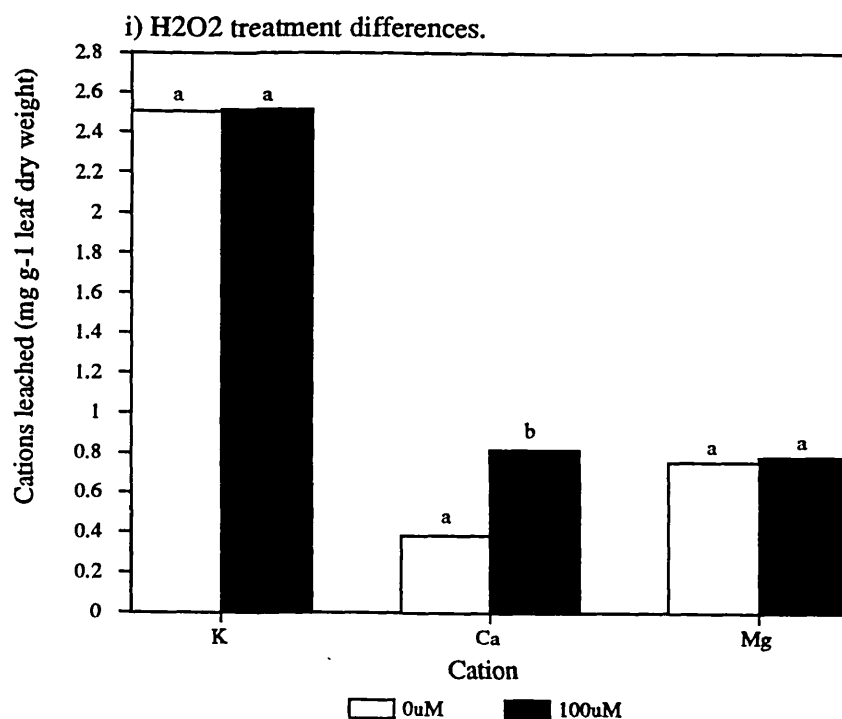
* and ** indicate significant F ratios at P<0.05 and P<0.01 respectively, ns denotes non-significance. The numbers in brackets represent the degrees of freedom associated with each F ratio.

Fig.3.07. Age and treatment differences in cation leakage from leaves of Pisum sativum in 100uM H₂O₂ solution.



Within any one category, means with different letters are significantly different at $P < 0.05$

Fig.3.08. Age and treatment differences in cation leakage from leaves of Pisum sativum in 100uM H₂O₂-pH4 solution.



Within any one category, means with different letters are significantly different at $P < 0.05$.

Table 3.11. Comparison of cation leaching from pea leaves of all ages incubated in solutions with or without H₂O₂.

Treatment	Ion leakage (mg g ⁻¹ leaf dry weight)		
	K ⁺	Ca ²⁺	Mg ²⁺
- H ₂ O ₂	3.174	1.007	0.783
+ H ₂ O ₂	5.027	1.502	0.851
% difference	58	49	8

3.4.4 Changes in H₂O₂ concentration on a leaf surface.

3.4.4.1 Leaf surface v inert surface.

Droplets evaporated from both leaf and teflon surfaces within three to four hours. Fig.3.09i shows mean H₂O₂ and H⁺ concentration and conductivity measurements made over the 4h investigation. On the teflon-coated surface, there was a loss in H₂O₂ concentration up to 60 min, followed by an apparent increase. On the surface of the bean leaf however, H₂O₂ concentration decreased over the time scale of the experiment. There was no increase in concentration as seen for the inert surface. The difference in H₂O₂ concentration between the two surfaces becomes significant at 120 min and remains significant at 180 and 240 min (Table 3.12).

H⁺ concentration and conductivity of droplets on the teflon surface slowly increased with time (Fig.3.09i). On the leaf surface, H⁺ droplet concentration increased at 60 min and then decreased, with conductivity gradually increasing with time. The difference in H⁺ droplet concentration between the two surfaces was significant at 60, 180 and 240 mins and the difference in conductivity was significant at all time intervals (Table 3.12).

3.4.4.2 Distilled water v H₂O₂ droplets on a leaf surface.

There was no production of H₂O₂ in the distilled water droplets sitting on the leaf surface. In the 100uM H₂O₂ droplets, there was a decrease in concentration with time (Fig.3.09ii). The H⁺ concentration of the distilled water droplets fluctuated, while the conductivity gradually increased with time (Fig.3.09ii). For the 100uM H₂O₂ droplets, H⁺ concentration increased at 60 min, but then decreased. The conductivity of these droplets increased with time and to a greater extent than the distilled water only droplets (Fig.3.09ii).

The difference in droplet H_2O_2 concentration was significant at 60 and 120 min but not at 180 min (Table 3.13). There were no differences in droplet H^+ concentration or conductivity at any time (Table 3.13).

Table 3.12. Changes in droplet H_2O_2 and H^+ concentration and conductivity on a teflon-coated surface and on the surface of a bean leaf.

Time (min)	F ratio		
	H_2O_2 (df=1,4)	H^+ (df=1,3)	Conductivity (df=1,4)
60	0.72 ns	18.72 *	16.00 *
120	39.57 *	5.78 ns	100.00 **
180	266.53 **	15.57 *	30.25 **
240	715.99 **	83.24 *	48.00 *

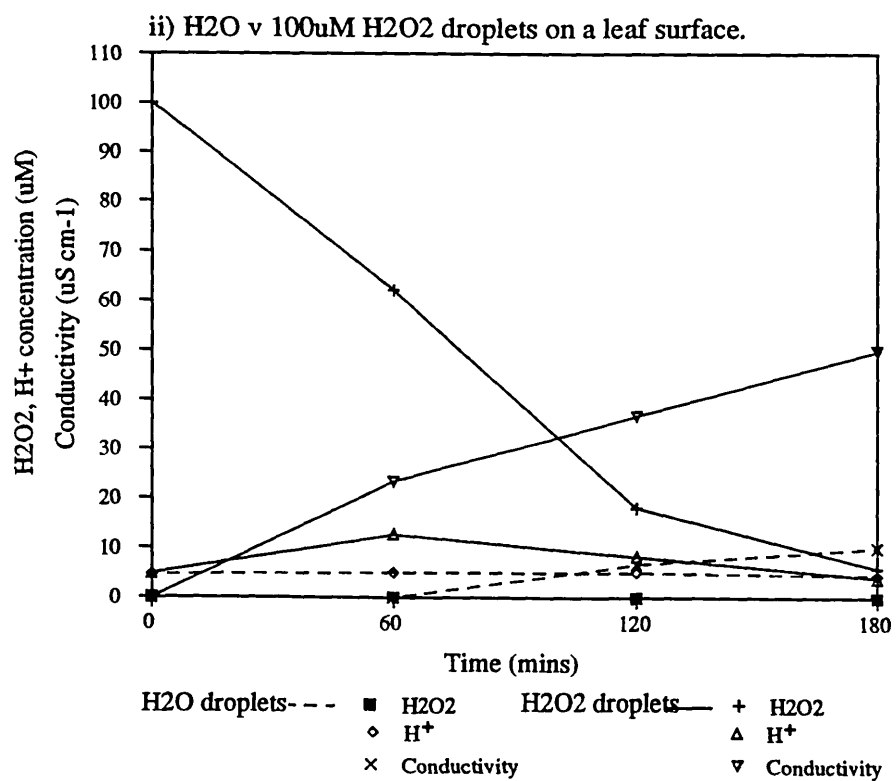
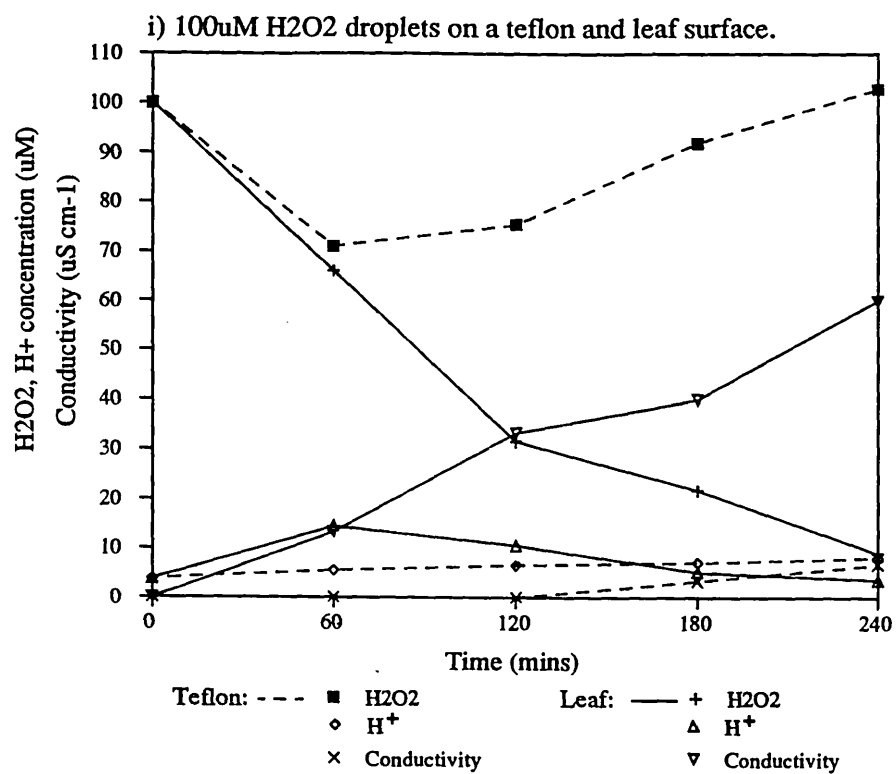
* and ** indicate significant F ratios at $P < 0.05$ and $P < 0.01$. ns denotes non-significance at $P < 0.05$. The results for H^+ droplet concentration were obtained using initial H^+ concentration as a covariate.

Table 3.13. Changes in H_2O_2 and H^+ concentration and conductivity of distilled water and H_2O_2 droplets on a leaf surface.

Time (min)	F ratio		
	H_2O_2 (df=1,4)	H^+ (df=1,3)	Conductivity (df=1,4)
60	283.91 **	6.90 ns	7.00 ns
120	31.36 **	1.81 ns	5.79 ns
180	5.14 ns	0.54 ns	6.00 ns

** indicates significant F ratio at $P < 0.01$. ns denotes non-significance at $P < 0.05$. The results for H^+ droplet concentration were obtained using initial H^+ concentration as a covariate.

Fig.3.09. Effect of leaf surface on H₂O₂ and H⁺ concentration and conductivity of droplets.



3.5 DISCUSSION.

3.5.1 The effects of H_2O_2 on growth.

The final harvest results from both Expts.A and B indicated a general detrimental effect of H_2O_2 on plant growth, for both pea and broad bean. Despite the concern over the validity of comparing Expts.A (exposure of 5 week old peas and broad beans) and B (exposure of 3 week old peas) due to the difference in the age of the plants, the results were not dissimilar.

The adverse effect of treatment in Expt.B increased as the H_2O_2 concentration increased, thereby giving rise to the large number of linear relationships seen in the results. On all occasions when significant linear trends were found in the data, there was only a significant difference between control and 50uM treated plants, except for stem dry weight, when there was also a significant difference between 20uM and 50uM treatments.

In bean (from Expt.A) the significant decrease in leaf area in the 50uM H_2O_2 treatment was not the result of a decrease in leaf number, but rather, due to a significant reduction in the area per unit leaf (MLA). The decrease in leaf dry weight resulted from reductions in the dry weight per unit leaf (MLW) and leaf area. SLAR was unchanged as leaf area and leaf dry weight were affected equally by H_2O_2 treatment.

This was in contrast to the results for pea from Expt.B. Here, the significant decrease in leaf area was due to a reduction in the number of leaves and not the result of a decrease in the MLA. The significant reduction in leaf dry weight seen at both 20uM and 50uM H_2O_2 was the result of a decrease in MLW and leaf number. The decrease in leaf dry weight was slightly greater than the reduction in leaf area, so that SLAR was significantly increased after exposure to H_2O_2 .

The age of the plants could have been important in determining the differences seen for pea in Expts.A and B. Pea plants in Expt.A were at an advanced stage of development at the start of the experiment and the leaf growth was unaffected by H_2O_2 treatment. For the pea plants from Expt.B, which were at an earlier stage of development at the start of the experiment, most leaf parameters (except for MLA) were significantly affected by exposure to H_2O_2 . Table 3.14 shows the changes in leaf number and leaf area between initial and final harvests in both experiments.

The difference in leaf number and area between initial and final harvests was greater in pea plants from Expt.B than in Expt.A (Table 3.14). The large increase in leaf area seen in Expt.B was not only due to an increase in leaf number, but also to an increase in the area per unit leaf (MLA). The values in Table 3.14 were averaged across all treatments and in Expt.A

Table 3.14. Changes in leaf number and leaf area between initial and final harvests for pea plants in Expts.A and B.

Leaf parameter	Harvest [#]		% change
	Initial	Final	
Leaf number: Expt.A	29	36	24
Leaf number: Expt.B	23	32	39
Leaf area (cm ²): Expt.A	135	199	48
Leaf area (cm ²): Expt.B	96	157	63
MLA (cm ² leaf ⁻¹): Expt.A	4.8	5.2	9
MLA (cm ² leaf ⁻¹): Expt.B	3.9	4.9	21

[#] These values represent the means across all treatments.

these parameters were not significantly affected by H_2O_2 . However, in Expt.B leaf number and leaf area were significantly reduced by exposure to 50uM H_2O_2 and the values in Table 3.14 include this effect. If the changes in Expt.B were expressed only for control pea plants then the increases in leaf number and leaf area between initial and final harvests would be 75% and 50%, respectively. Values of mean relative growth rate (MRGR) for Expts.A and B also reflect the greater growth achieved by the latter during the experimental period. MRGR (averaged across all treatments) for pea plants from Expt.B was 23% greater than that attained in Expt.A.

These results suggest that plants in a more active phase of growth may be more susceptible to exposure to H_2O_2 . There are reports of developing tissue being more sensitive to the effects of H_2O_2 than mature tissue. Young beech (*Fagus sylvatica*) leaves, still growing during exposure to H_2O_2 concentrations up to 147uM (in a solution of pH4) for six weeks, showed a number of adverse effects of treatment including decreases in leaf area and leaf dry weight (Masuch *et al*,1986; Mallant *et al*,1988). In these experiments, leaves already fully grown, showed lesser, non-significant changes. Current year needles of Norway spruce (*Picea abies*) exposed to 118uM H_2O_2 for six weeks were shown by Mallant *et al* (1986) and Masuch *et al* (1986) to be more susceptible to H_2O_2 treatment than first year needles. It is difficult to compare directly previous work on the effects of H_2O_2 on trees with those on herbaceous species obtained here. However, such comparisons can provide indications of general plant response to H_2O_2 .

Temperature may also have been a factor in bringing about the differences seen between pea plants in Expts.A and B. Expt.A was undertaken in February 1988 and mean greenhouse temperatures were 1°C. Expt.B began in July 1988 when mean greenhouse temperatures were approximately 23°C. The temperature at which plants develop has been shown to influence their response to simulated acid rain. Caporn and Hutchinson (1987) observed that exposure to simulated acid rain of pH3 led to a 29% reduction in total plant dry weight of cabbage (*Brassica oleracea*) grown at 20°C compared with that at pH5.6. However, for plants grown at 10°C, treatment with pH3 rain only reduced total dry weight by 8.5%. It was therefore possible in this study, that the higher temperatures during Expt.B could have contributed to the greater response to H_2O_2 displayed by these pea plants.

Adverse effects of H_2O_2 treatment on flower production were only significant in Expt.B, with a 13% reduction in flower and pod dry weight in the 50uM treated plants compared with the controls. Stem dry weight and total plant dry weight in broad bean from Expt.A and pea from Expt.B were significantly reduced at 50uM H_2O_2 . Decreases in root dry weight were only

seen in Expt.A, but in both species. These results are summarised in Table 3.15. All plants treated with H_2O_2 showed a reduction in mean relative growth rate (MRGR), but the effect was only significant in broad bean.

These results are in contrast to those of Hanson and McLaughlin (1989), where treatment of red spruce seedlings (*Picea rubens*) with a range of H_2O_2 concentrations 0 - 235uM (in a solution of pH4), for 9h, three times a week for 16 weeks, led to a significant increase in root and current year shoot growth of those trees in the highest H_2O_2 treatment. MRGR at 59uM and 235uM H_2O_2 was also significantly greater than at the lower H_2O_2 concentrations (0uM and 15uM). Again, it is difficult to compare directly the growth responses of tree seedlings and herbaceous species. The differences between these two groups of plants may be sufficient to account for the contradiction in the results. However, the frequency and duration of exposure to mist, chemical composition of mist and growing conditions may also have contributed to these differences.

The response to H_2O_2 by both bean (from Expt.A) and pea (from Expt.B) were generally consistent (Table 3.15). There were some differences e.g. exposure to 50uM H_2O_2 significantly decreased leaf number and flower and pod dry weight in pea but not in bean. Age and temperature differences (as discussed earlier) between the two experiments may have contributed to the variation in response.

However, there are a number of reports of herbaceous species differences in response to acidity (Lee *et al*,1981; Evans *et al*,1983; Irving,1983; Musselman and Sterrett,1988). In a review of the effects of acid precipitation on the yield of 34 crop varieties (28 species), Irving (1983) noted that six species (including radish, *Raphanus sativus* and beet, *Beta vulgaris*) exhibited a negative response to acidity, eight species (including tomato, *Lycopersicon esculentum* and timothy, *Phleum pratense*) showed a positive response and 17 showed no effect (including bush bean, *Phaseolus vulgaris* and pea, *Pisum sativum*). It was therefore possible that in this study, species differences between pea and broad bean gave rise to the variation observed in the results.

No visible damage symptoms after treatment with H_2O_2 were seen in either experiment. This is consistent with the results of Slanina *et al* (1985), Mallant *et al* (1986,1988), Masuch *et al* (1986,1989) and Hanson and McLaughlin (1989), although these groups were working with tree species.

Table 3.15. Comparison of the effects of H₂O₂ treatment on plant growth.

Parameter	Expt.A [#]	Expt.B
Leaf number	0	-
Leaf area	-	-
Specific leaf area	0	+
Mean leaf area	-	0
Mean leaf weight	-	-
Flower dry weight	0	-
Leaf dry weight	-	-
Stem dry weight	-	-
Root dry weight	-	0
Total plant dry weight	-	-

+ and - represent significant effects of H₂O₂ treatment (at P<0.05 at least), where the effects are positive or negative compared to the control respectively. 0 represents no significant effect of H₂O₂ treatment. [#] the results from Expt.A represent those obtained with broad bean, with only root dry weight being significantly reduced in pea.

3.5.2 Interactions of H₂O₂ with the leaf surface.

There were no effects on epicuticular wax structure of pea and broad bean leaves used in Expt.A. Some damage to epicuticular wax structure has been reported after H₂O₂ treatment in other experiments. Masuch *et al* (1989) reported cracks in the wax plugs of stomata of current-year Norway spruce needles after exposure to 25uM H₂O₂-pH4 for three hours, four days a week for 8 weeks. Wax structure is known to be disrupted by the application of simulated rain without H₂O₂ or acid (Baker and Hunt,1986), with 14 and 45% of the total deposit of pea leaves being detached by 1 and 9mm of rain, respectively.

In this study with peas and broad beans, it is unlikely that any damage to the leaf surface structure could have been repaired in the time interval between misting events. In a review by Juniper (1960), very few species had been observed to recover from surface damage (mechanical) and in the majority of waxy species e.g. legumes, recovery did not occur. Percy (1987) removed the wax deposits from rape (*Brassica napus*) leaves at a stage with approximately 20% of full leaf expansion and examined the degree of wax regeneration. It was concluded that regeneration was not a viable recovery mechanism, for not only was less wax produced but the chemical composition of the wax had changed. Therefore, it seems likely that wax regeneration in the broad beans and especially, pea plants used in Expt.A did not occur. Although no effect of H₂O₂ was seen on wax structure, there may have been changes in the wax content of leaves, but this was not investigated.

Detached leaves of pea plants from Expt.B exposed to H₂O₂ mist leached more K⁺ into a distilled water solution than control leaves, but this was not significant. These leaves were washed with distilled water prior to immersion, so that contamination from surface deposited materials was less likely. In addition, this pre-wash may also have removed some of the readily exchangeable cations (which include K⁺) from the leaf surface.

It would have been difficult to assess the impacts of increased leaching on these experimental plants as the ionic content of the leaves was not investigated. Although the results were not significant, there was a suggestion that exposure to H₂O₂ mist may have increased plant susceptibility to K⁺ loss.

K⁺ is known to be readily leached from plants (Tukey,1970) and most tissue K⁺ is symplastic in origin, but there are no reports in the literature of the effects of H₂O₂ on the leaching of cations. Evans *et al* (1981) investigating the response of *Phaseolus vulgaris* leaves to simulated acid rain suggested that the leaching of cations from leaves was the result of either exchange mechanisms in the course of mineral absorption or altered membrane permeability.

The slight increase seen in K⁺ leakage from pea leaves in this study after exposure to H₂O₂ mist, may have been the result of a change in the rate of cation exchange or of increased membrane permeability.

In contrast to the results from Expt.B, pea leaves (all ages), not exposed to H₂O₂ mist, but incubated in a 100uM H₂O₂ solution, leached significantly greater amounts of K⁺ than control leaves (in H₂O only). There was no effect of H₂O₂ incubation on the leaching of Ca²⁺ and Mg²⁺. Incubation of pea leaves (not exposed to H₂O₂ mist) in a 100uM H₂O₂-pH4 solution increased the leaching of Ca²⁺, but not K⁺ and Mg²⁺, when compared to the controls (in pH4 solution only).

The results from the incubation studies were obtained at a higher H₂O₂ concentration of 100uM and past misting history did not have to be taken into consideration. The quantity of Ca²⁺ and Mg²⁺ leached in pH4 solutions was greater than in distilled water, with little effect on K⁺ leakage. The incubation time of 24h was relatively long, but if substantial cell breakdown was occurring, especially at pH4, greater K⁺ leakage would have been expected as K⁺ is mainly symplastic in origin.

However, it was evident that the chemical composition of the H₂O₂ incubating solution influenced the leaching of a particular cation. In a 100uM H₂O₂ solution, the leaching of K⁺ was significantly increased, but in a 100uM H₂O₂-pH4 solution, Ca²⁺ leakage increased significantly. These leaves were again washed before incubation so that surface deposits and some readily exchangeable cations would have been removed.

There are reports of changes in K⁺ and Ca²⁺ leaching with changes in pH. Fairfax and Lepp (1975) saw a decrease in K⁺ leakage from *Nicotiana tabacum* leaves when the pH of simulated rain decreased, but the leaching of Ca was greater at pH3 than at pH6.7. They suggested that as Mecklenberg *et al* (1966) had shown Ca²⁺ loss from leaves to occur by a passive process of ion exchange, the leaching of Ca²⁺ was solely dependent on H⁺ concentrations in rainfall. The decrease in K⁺ loss with pH was less readily explained, but it was possible that Ca²⁺ was more readily leached than K⁺ or alternatively, Ca²⁺ depletion by leaching and replacement by H⁺ could lead to a reduction in K⁺ uptake and a decrease in the K⁺ available for leaching.

The increase in Ca²⁺ loss but lower rate of K⁺ loss at lower pH was also seen by Evans *et al* (1981), who exposed *Phaseolus vulgaris* leaves to simulated acid rain and by Leonardi and Fluckiger (1989) for beech (*Fagus sylvatica*) seedlings. Leonardi and Fluckiger (1989) used the work of Dittrich *et al* (1979) to explain the minor leaching of K⁺ at low pH: an

electroneutral H^+/Cl^- uptake by the cells due to an increased H^+ concentration in the apoplast would be followed by a re-export of H^+ , thereby generating the electrochemical gradient for K^+ uptake by the symplast. As a consequence less K^+ would be leached from the apoplast.

In another study involving the application of pH3 droplets to *Artemisia tilesii* leaves, both Mg^{2+} and Ca^{2+} were much more readily leached than K^+ (Adams and Hutchinson, 1984). The preferential leaching of any one ion has also been shown to be dependent on the species under consideration. Ca^{2+} was the prevalent ion entering pH3 raindrops on cabbage and sugar beet leaves, whereas K^+ was the predominant cation in raindrops on radish and sunflower (Hutchinson *et al*, 1986).

In this study with pea leaves, a decrease in pH led to increased loss of Ca^{2+} and Mg^{2+} , but little change in K^+ . This is in agreement with the results from other studies discussed above. However, the leaching of Ca^{2+} at pH4 was significantly increased by the presence of H_2O_2 . If the mechanism of Ca^{2+} loss from leaves is one of passive ion exchange for H^+ , then the results suggest that H_2O_2 may have increased the rate of Ca^{2+} exchange by increasing the concentration of H^+ in the external solution. The production of H^+ could have been achieved by the reaction of H_2O_2 with contaminants remaining on the leaf surface after the pre-wash.

For the pH4 treatments (with and without H_2O_2) the leaching of ions was generally greater from young leaves than from older leaves. This result was surprising as it has been suggested that older leaves are more susceptible to leaching than younger leaves (Tukey, 1970). In leaves of *Phaseolus vulgaris*, the entire exchangeable Ca pool is the primary source of leached Ca (Mecklenberg *et al*, 1966), so that young tissues, accumulating Ca within cells and cell walls were not so readily leached, as the amount of exchangeable Ca was reduced. In mature tissues there is a reduction in this rate of accumulation, leaving a larger amount available for leaching.

The results of Mallant *et al* (1986) and Masuch *et al* (1986) with beech and Norway spruce seedlings suggested that young developing tissue was more susceptible to the effects of H_2O_2 . However, the leaching results in this study gave no indication of a H_2O_2 treatment - age interaction.

Wolfe *et al* (1987) suggested that H_2O_2 is destroyed as soon as it comes into contact with a leaf surface. The results here, for the surface of a bean leaf, indicated a more gradual decline in concentration: the initial loss in concentration over the first 60 min was probably partly due to natural decay with time and was also seen on the teflon-coated surface. No such

continuous decrease in concentration was seen on the teflon-coated surface. The apparent increase in H_2O_2 observed on this surface after 60 min was possibly due to the effects of evaporation, leading to a concentration of H_2O_2 in the droplet.

The gradual destruction of H_2O_2 on the leaf surface could have been due to reaction with surface contaminants. The plants were grown in a greenhouse in unfiltered air and the leaves were not washed before the application of the H_2O_2 droplets. It would therefore seem likely that a proportion of the H_2O_2 concentration lost in the droplets would be due to reaction with surface compounds. Alternatively, the absorption of gases from the atmosphere e.g. SO_2 , by the droplets and consequent oxidation by H_2O_2 , would also lead to a reduction in H_2O_2 concentration. These experiments were conducted in CT (controlled temperature) rooms where the quantity of such gases was unknown. It would be expected, however, that such a reaction would occur irrespective of the surface retaining the droplet. No decrease was seen in the H_2O_2 concentration of droplets on the teflon-coated surface, so that the probability of such an oxidation reaction taking place is remote.

It may also be possible that the external application of H_2O_2 stimulated the production of anti-oxidant enzymes within the leaf, which were ultimately responsible for its breakdown. H_2O_2 produced during normal cell metabolism is toxic to cells and there are a number of protective mechanisms for its removal (Larson, 1988). These involve the action of enzymes like catalase and ascorbate peroxidase. An increase in ascorbate peroxidase activity has been shown in spinach plants (*Spinacia oleracea*) fumigated with ozone (Tanaka *et al*, 1985). It was suggested that the H_2O_2 which accumulated in O_3 -fumigated leaves might induce the synthesis of ascorbate peroxidase and therefore the plants would protect themselves through the induction of specific H_2O_2 scavenging enzymes.

There are no known investigations of the response of plant anti-oxidant status to externally applied H_2O_2 . However, among the functions attributed to plant peroxidases are the assembly of cell wall constituents and this implied the transport of peroxidase molecules out of the cell and control of their activities within the wall space (Heath and Castillo, 1988). Extracellular peroxidase activity in *Sedum album* leaves has been shown to increase nearly three-fold over that of control plants after exposure to 400ppb O_3 for 2h (Castillo *et al*, 1984). It was possible that the application of wet deposited H_2O_2 to bean leaves in this study may have stimulated a similar response.

The increase in conductivity with time of H_2O_2 droplets on a leaf surface may have been the result of a combination of factors. An increase in evaporation with time would have led to a concentration of chemical species in the droplets. Reaction with, or dissolution of surface

contaminants and leaching from the leaf itself would also have increased droplet conductivity. The slight increase in conductivity of droplets on the teflon-coated surface with time, suggests that evaporation plays a part in the results seen at the leaf surface. No differences were observed in the rate of droplet evaporation from the teflon and leaf surfaces. The larger conductivity value for the H_2O_2 droplets, compared to the distilled water only droplets on a leaf surface, suggested the reaction of H_2O_2 and surface compounds or increased leaching in response to H_2O_2 application.

There were changes in the H^+ concentration of H_2O_2 droplets on a leaf surface with time. The increase in H^+ over the first 60 min coincided with a decrease in H_2O_2 concentration and only after this time did H^+ droplet content decrease as the H_2O_2 continued to disappear. On the control teflon-coated surface there was a gradual increase in H^+ , which was attributed to evaporation leading to a concentration of H^+ in the droplets. A similar reaction of increased H^+ was seen for droplets on a parafilm control surface by Adams and Hutchinson (1984).

It appeared that in the first 60 min, the reaction of H_2O_2 at the leaf surface, produced greater H^+ than that expected from the effects of evaporation alone, as there was little change in the H^+ content of droplets on the teflon-coated surface in this time. The reaction of H_2O_2 with dry deposited material at the leaf surface may have produced acids, thereby increasing H^+ concentration. This reaction was not simply a case of solubilisation of surface deposits as there was little change in the H^+ content of water-only droplets on the leaf surface in this time. Only after 60 min was the H^+ neutralised by the leaf. The neutralisation achieved not only offset the H^+ produced by H_2O_2 , but also the increase in concentration of H^+ in droplets by evaporation as seen for the teflon-coated surface.

The results from the leaching experiment discussed earlier, suggested that in 100uM H_2O_2 solution, K^+ leaching was significantly increased. In this case, where 100uM H_2O_2 droplets were placed on the leaf surface, it was possible that the leaching of K^+ was partly responsible for the neutralisation of H^+ produced and the increase in conductivity observed.

There have been a number of experiments investigating the neutralisation of acidic (but not H_2O_2) droplets by leaf surfaces. The degree of neutralisation has been related to plant species. The rate of increase of droplet pH from a value of 4 was greatest for red kidney bean (*P. vulgaris*) and wheat (*Triticum aestivum*) giving the greatest pH change, but corn (*Zea mays*) and timothy (*Phleum pratense*) had little effect over four hours (Craker and Bernstein, 1984). In another study, *Artemisia tilesii* had the greatest neutralising ability at pH3, the pH rising to 3.9 after 75 mins contact, while *Spinacea oleracea* leaves were

acidified to pH2.8 before drying (Adams and Hutchinson,1984). Larsen (1986) reported that a number of species were capable of buffering rain of pH3.5, with mustard (*Sinapsis alba*) having the greatest ability and rape (*Brassica napus*) the least.

Leaf age has also been shown to have an effect on hydrogen ion neutralisation (Adams and Hutchinson,1984), with older leaves of spinach (*S.oleracea*) having considerably higher droplet pH than recently expanded and younger leaves. However, the same workers have also reported (Adams and Hutchinson,1987) no significant effect of leaf age for sunflower (*Helianthus annuus*), radish (*Raphanus sativus*) or beet (*Beta vulgaris*). In measuring the response of broad bean leaves to H₂O₂ droplets in this study, no distinction was made between the ages of the leaves. It may be that leaves of different ages would have given a smaller or greater response than that seen.

Neutralisation ability is also dependent on the wettability of the leaves which determines how much liquid is retained and the degree of interaction between retained droplets and the leaf surface (Hutchinson *et al*,1986; Adams and Hutchinson,1987). The two species used in this study, pea and broad bean, have different leaf surface characteristics. Pea leaves are smooth and waxy and wetted with difficulty, but the leaves of species like broad bean are wetted easily (Tukey,1970). Hutchinson *et al* (1986) showed that cabbage and other brassicas, with a thick layer of epicuticular wax, had poor neutralising ability. Brassicas in general have a dense layer of epicuticular wax and so are very hydrophobic. The large contact angle (i.e. small contact area) limits the degree of neutralisation leading to the formation of lesions. The importance of leaf wettability was reinforced by Adams and Hutchinson (1987) who reported that cabbage and sugar beet were wetted and leached with difficulty and had poor neutralising ability. However, radish and sunflower produced much higher droplet pH.

In summary, H₂O₂ applied as a mist did have an effect on the growth of the two herbaceous species considered and this effect was not limited to any particular plant part. There were no visible damage symptoms, nor any disruption of epicuticular wax structure. Leaching of ions was increased during short-term incubation but was unaffected after a long-term misting exposure. Complex chemical reactions may have been taking place at the leaf surface, associated with the destruction of H₂O₂.

Chapter 4

EFFECTS OF HYDROGEN PEROXIDE ON TREE SEEDLINGS

4 EFFECT OF H₂O₂ ON TREE SEEDLINGS.

4.1 INTRODUCTION.

The experiments with herbaceous species (chapter 3) had indicated a general detrimental effect of short-term episodic exposure to H₂O₂ on plant growth and so, the aims for further work were to use species which could, under natural conditions, receive high levels of exposure to H₂O₂ in mist at high elevation sites.

There were two considerations when choosing the tree species for investigation:

- i) that the study should encompass one deciduous and one coniferous species in order to examine the effect of H₂O₂ on these two very different types of tree.
- ii) the species chosen should be important upland species in the U.K.

The species chosen for this experiment were Sitka spruce, *Picea sitchensis* and sessile oak, *Quercus petraea*; both are found at elevations up to 500m in the U.K. (Clapham *et al*, 1962).

This investigation again used concentrations commonly recorded at high elevation sites. The range of H₂O₂ concentrations chosen for application were, 0, 20, 50, 100uM. Exposure was to be episodic but for a long duration (five months). It was hoped that this would provide a regime more typical of the exposure trees would get in the field. pH4 (hydrogen ion, H⁺, concentration = 100uM) was chosen to be used in conjunction with these four concentrations.

The experiments with herbaceous species had indicated an effect on plant growth. Using the experimental system described earlier (section 2.2.2), the effects of H₂O₂ on tree seedlings were investigated. Previous experiments exposing tree seedlings to H₂O₂ had suggested both negative and positive effects of treatment (reviewed in section 1.3). Slanina *et al* (1985), Mallant *et al* (1986, 1988) and Masuch *et al* (1986, 1989), observed detrimental effects on Scots pine, *Pinus sylvestris* and beech, *Fagus sylvatica* at concentrations up to 118uM H₂O₂-100uM H⁺, whereas Hanson and McLaughlin (1989) reported stimulation of the growth of red spruce, *Picea rubens* at 235uM H₂O₂-100uM H⁺.

In this experiment the effects of H₂O₂ on the growth of Sitka spruce and oak seedlings throughout the season of treatment were investigated. It was intended that the seedlings be maintained overwinter until the first flush of the following season, as the adverse effects of environmental stresses have often been seen as poor growth in the subsequent season (Kozlowski *et al*, 1991). The physiological response of oak to H₂O₂ treatment was investigated by measuring gas exchange at different times during the season.

It was hoped that collection and analysis of droplets remaining on the leaf/needle surface after misting would give some indication of interactions between H_2O_2 and the surface of the foliage. Chemical analysis could show whether ions were being exchanged with the misting solution. Any changes in the chemistry of the droplets could also be due to reaction with surface contaminants deposited on the leaf/needle surface prior to misting.

The ions to be analysed were those considered to be the most likely to be found in the droplets as a result of the above processes. Potassium (K^+), calcium (Ca^{2+}), Magnesium (Mg^{2+}) and manganese (Mn^{2+}) are readily leached from plants (Tukey, 1970). Sodium (Na^+) is easily mobilised, but its major source in throughfall is believed to be from sea-aerosol deposition (Parker, 1983). Zinc (Zn^{2+}) has been shown both to be taken up and leached by tree species (Parker, 1983). Ammonium (NH_4^+) analysis was also undertaken because of its influence on cloudwater pH and on the rate of the oxidation of SO_2 (Gallagher *et al*, 1990).

The major anion in this investigation was considered to be sulphate (SO_4^{2-}). In view of the rapid reaction between H_2O_2 and SO_2 (reviewed in section 1.2.2) it was possible that any correlation between an increase in droplet SO_4^{2-} (or H^+) content and H_2O_2 concentration could be due to oxidation of SO_2 . Other anions analysed were chloride (Cl^-) and nitrate (NO_3^-).

Although the earlier work with herbaceous species had suggested no effect of H_2O_2 treatment on epicuticular wax structure, Masuch *et al* (1989) observed cracks in the wax plugs of stomata of *Picea abies* seedlings after exposure to H_2O_2 mist. It was therefore possible that the H_2O_2 applied in this experiment could also affect the structure and amount of wax, although only the latter was investigated.

4.2 MATERIALS AND METHODS.

4.2.1 Experimental design.

Twenty trees per species were assigned to each of the four treatments, 0, 20, 50 and 100uM. Each treatment was subsequently split into two replicate blocks, so that every block consisted of 10 seedlings per species. These blocks were then randomly distributed among the 8 experimental chambers.

The duration of treatment was from May to September 1989. Table 4.01 summarises all measurements made for both species during the experiment. Details of how these measurements were performed are given in subsequent sections.

4.2.2 Plant material.

4.2.2.1 Initial size classing.

The species chosen were Sitka spruce, *Picea sitchensis* (Bong.) Carr and sessile oak, *Quercus petraea* (Mattuschka). The Sitka spruce were 2 year old seedlings, falling within the height range 25-45cm and the oak, also 2 years old, of height 25-35cm.

Initial size classing of the seedlings took place in February 1989. For Sitka spruce, measurements of basal diameter and total shoot length (tsl) were used to group the seedlings. Similar trees were arranged into 10 groups (each group containing 9 trees). The seedlings in these groups were selected at random and assigned to each of the 9 experimental blocks. One block of 10 trees was taken for an initial harvest, while the remaining 8 blocks were assigned to each of the four treatments.

A different procedure was used for size classing the oak. The seedlings were first grouped according to height, basal diameter and tsl. The extremes for each parameter were rejected. This meant that the shortest and tallest seedlings and those with the smallest and greatest basal diameter and shoot length were discarded. For those that remained, measurements of basal diameter and tsl were used to group the seedlings. These were then assigned to the 9 experimental blocks as described for Sitka spruce.

4.2.2.2 Soil mixture.

Both species were potted up in November 1988 into 4l plastic pots. A compost of peat and sharp sand in a ratio of 3:1, neutralised with calcium carbonate and containing a slow release

Table 4.01. Timetable of all measurements performed during the experiment.

Date	Measurements
Feb 89	Initial size classing.
May 89	Pre-treatment non-destructive growth measurements. Initial harvest.
May - Sept 89	Non-destructive growth measurements, every 5 weeks. Surface droplet collection, on the second morning of each 2-day event. Measurements of gas exchange in oak.
Sept 89	End of season (EOS) harvest, with both non-destructive and destructive growth measurements.
Dec 89	Sitka spruce re-potted.
Apr - May 90	Final harvest.

NPK fertiliser (Osmocote) and a fertiliser mix of major and trace elements (based on UCD1 standard mix, Matkin and Chandler, 1957), was used for both species. The seedlings were maintained outside over the winter of 1988/1989.

This same soil mixture was used to repot the Sitka spruce into 10l pots in December 1989 to allow the roots to expand.

Initial soil pH was determined by mixing a ratio of 2:1 by volume, soil:distilled water. This mixture was allowed to stand for 1h and the pH then measured. One sample from each of the initial harvest trees was used for pH determination. The mean soil pH was 5.30 for Sitka spruce and 5.32 for oak.

4.2.2.3 Pest and disease control.

It had been hoped to avoid spraying the seedlings against any insect infestation or disease outbreak to prevent any possible interaction between fungicide/pesticide and mist treatment. However, an aphid outbreak in April, mainly on Sitka spruce (causative organism, *Elatobium abietinum*), but to a lesser extent on oak, meant that both species were treated with a systemic insecticide (Rapid, ICI) at the beginning of April and also three weeks later. Three types of aphid were identified on the oak (by Dr.S.McNeill at Silwood Park), *Tuberculoides annulatus*, *Thelaxes dryophila* and *Myzocallis costanica*. The oak were also susceptible to other forms of attack; weevil and leaf miner damage were noted, but by far the greatest problem was the recurrent outbreak of mildew (*Micosphaera alphitoides*) throughout the season. To combat this, the trees were sprayed four times from May to August in an attempt to contain the disease. The fungicide used was Benlate (50% Benomyl). A summary of insecticide/fungicide application throughout the 1989 season is given in Table 4.02.

Once misting had begun, care was taken over which days the insecticide/fungicide was applied. Application was performed on days (represented by # in Table 4.02) which were as far removed as possible from H₂O₂ exposure dates. All insecticides/fungicides were applied in the early to late evening to reduce the possibility of scorching the leaves or needles.

4.2.3 Environmental conditions.

Continuous air pollution monitoring at the Silwood Park field site was undertaken as part of a contract under the European Open Top Chamber Programme by Ms.D.Shah. This consisted of measurements of ambient O₃ using a Dasibi 1003-AH and NO_x by a Monitor Laboratories NO_x analyser, model 8840. These measurements were made throughout the 1989 season. Measurements of SO₂ were not possible because of problems with the monitor.

Table 4.02. A summary of insecticide and fungicide application throughout the 1989 season.

Date	Species	Pest or Disease	Treatment	Active agent	Concentration of spray
3/4/89	Sitka spruce	<i>Elatobium abietinum</i>	Rapid	Pirimicarb	50 g l ⁻¹
4/5/89	Sitka spruce	<i>E.abietinum</i>	Rapid	"	"
	Oak	Aphids	"	"	"
20/5/89	# Oak	Mildew	Benlate	Benomyl	1.13 g l ⁻¹
8/7/89	# Oak	Mildew	"	"	"
25/7/89	# Oak	Mildew	"	"	"
24/8/89	# Oak	Mildew	"	"	"

see text for explanation.

An automatic weather station provided a record of a full range of meteorological measurements.

4.2.4 Mist Chemistry.

The ions added to distilled water to prepare the pH4 misting solution were as described in section 3.2.2. As before, solutions were not stored between events, rather, a fresh solution was made up each day prior to every evening exposure. The required H₂O₂ concentrations, 0, 20, 50 and 100uM, were made up in the ion stock solution. The initial H₂O₂ concentration in each treatment was measured before the start of the experiment using the H₂O₂ analyser, as discussed in section 2.2.1.

4.2.5 Exposure regime.

Exposures were of 6h duration overnight (2200-0400) for 2 consecutive nights per fortnight for the period May to September, 1989. Over the 6h misting period, approximately 2500ml were deposited to surfaces within the chamber. Each treatment received 18 events, giving a total exposure time of 108 hours and a total volume deposited of approximately 48l.

The volume of mist applied was not sufficient to adequately wet the soil. In addition, rainfall in 1989 was low, so that the seedlings were watered (with tap water) as required.

Seedlings were arranged within the chamber at approximately equal distances from the humidifier situated in the centre of the chamber. No seedlings were placed within 20cm of the humidifier or positioned beneath the horizontal stainless steel struts (frame for the chamber roof).

To overcome any chamber differences, the seedlings within each block were moved within and between the chambers at regular intervals throughout the 1989 season.

4.2.6 Growth measurements.

4.2.6.1 Non-destructive growth measurements and visible injury assessment.

All seedlings were measured prior to treatment beginning and then at five week intervals throughout the growing season. Parameters recorded include height, basal diameter, total shoot length (noting year classes and 1989 flushes) and leaf number for oak. These measurements were also made at the 1989 end of season (EOS) and 1990 final harvests.

Autumn leaf fall in oak was monitored from 22/8/89 until it stopped in late December. Collection of the leaves was difficult as the trees were not in enclosed chambers and so leaf fall was assessed by counting, every seven days, the number of leaves that remained on the tree.

Spring budburst in 1990 was monitored on seven dates, beginning on the 24/4/90 through to completion on 11/5/90 for oak and 22/5/90 for Sitka spruce. The assessment was made on an arbitrary scale of 0-10 for both species, based on the number of buds burst and the degree of bud opening.

The occurrence of any damage symptoms resulting from treatment and frequency of insect or fungal infection were surveyed during the season, as an indication of tree health. The frequency of infections was recorded on an arbitrary scale e.g. for mildew outbreaks on oak, the severity of the disease was noted on a scale of 1 to 4, where

1 represented mildew occurring on 0 - 25% of leaves,

2 = mildew occurring on 26 - 50% of leaves,

3 = mildew occurring on 51 - 75% of leaves and

4 = mildew occurring on 76 - 100% of leaves.

4.2.6.2 Destructive growth measurements.

An initial harvest was performed in May 1989 on 10 seedlings per species prior to the first misting episode. Height, basal diameter, tsl, along with needle or leaf, stem and root dry weights, were recorded.

In September 1989, a sub-sample of trees from both species, 5 per treatment, were chosen at random and harvested (EOS harvest). The remainder were maintained over winter and harvested in May 1990. Measurements of leaf/needle area, leaf/needle, stem (separated into year classes and 1989 flushes) and root dry weight were made.

Leaf/needle areas were measured with a modified EEL photoelectric leaf area meter, based on a Digithurst Microeye image analysis system (Digithurst Ltd., Royston, Herts.), driven by an IBM PC-AT computer. For oak, all leaves were used for the area measurements. For Sitka spruce, a sub-sample of 50 needles per year class was used. Holland *et al* (1988) investigated the needle sample size required to give an acceptable estimate of the needle area of the whole tree. They took 5% as being an acceptable error and found that the number of needles needed were 45 for Sitka spruce.

For dry weight estimation, all plant parts were put in paper bags and oven-dried at 80°C for 3 days.

Measurements of soil pH at both harvests were made as discussed in section 4.2.2.2. One sample from each tree at the EOS harvest (five per treatment) and one sample from five randomly selected trees per treatment at the final harvest, were taken for analysis. All the soil from one pot was thoroughly mixed and a sample of approximately 10g was taken from this mixture.

4.2.7 Effects on photosynthesis and gas exchange.

All gas exchange measurements were made using an ADC LCA-2 analyser (ADC Ltd., Hoddeson, Herts.). This incorporates an infra-red gas analyser (IRGA), leaf cuvette, air supply unit with mass flowmeter and a data logger. The analyser in its entirety will be referred to as the LCA.

The LCA uses an open system where plant material is placed in a cuvette where there is a measured flow of air of known water vapour and CO₂ content. Gas exchange rates are determined from the flow rate and concentration differences between inlet and exhaust air. Cuvette air temperature, % relative humidity in cuvette, CO₂ concentration in air entering and leaving cuvette and photon flux density incident on cuvette are measured. Leaf conductance (mol m⁻² s⁻¹), net photosynthetic rate (umol m⁻² s⁻¹) and sub-stomatal cavity CO₂ concentration (ubar) are calculated by the data logger.

The leaf chamber available was designed for broad-leaved plants so that only oak could be measured. Readings were taken 35s after a leaf had been inserted into the chamber. Both pre- and post-misting measurements were planned, but this was not always possible. Measurements were taken four times during the season: 20/6/89 (post-misting), 31/7/89 (pre-misting), 3/8/89 (post-misting) and 6/9/89 (post-misting).

Measurements were made between 1130 and 1400 and where possible on days with continuous strong sunlight, in the hope that the effects of varying light intensity could be excluded and that the results would all be obtained at or near light saturation. Seedlings from each treatment and each block were measured alternately. The order in which measurements were made varied through the season.

The choice of leaves was restricted to those first flush (1F) leaves not shaded or near the apex, which had suffered no form of damage. It was then possible to use two leaves per tree from 10 trees per treatment. The production and condition of second flush (2F) leaves were highly variable and as such were excluded from the experiment.

A gas exchange measurement round was made on the night of 15/9/89, when minimal stomatal opening was expected. The seedlings which had been measured through the season from the 0 and 100uM treatment only were taken inside an unheated building from 1700. This was in order to prevent dew formation on the leaves. All windows were left open. The flow rate on the mass flow unit was reduced to 250 ml min⁻¹, to compensate for the reduction in CO₂ fluxes. The need for a light source for manipulation of the LCA was overcome by placing a green filter over a low intensity light. Light induction of stomatal opening has action maxima in the blue and red regions (Hart,1988) but extremely high quantum fluxes of green light are required (Farquhar and Sharkey,1982). Photon flux density under this regime was registered as 0. The measurements were made from 2200-2300.

4.2.8 Measurement of surface droplet chemistry.

4.2.8.1 Surface droplet collection.

Evaporation of the droplets remaining on the leaf/needle surface with increasing temperature, meant that droplets had to be collected as soon as possible after the end of the episode (0400). Droplet collection began at 0430 on the morning following the second misting episode and was completed by 0800. The droplets were gently shaken from the trees into pre-washed vials. The order of collection was varied each time, so that no one block was, for example, continually collected first.

Due to the low volume of liquid deposited on the seedlings, the droplets collected from five trees per species within a block were bulked to form one sample, so that for any treatment there were only four samples. A funnel and conical flask positioned away from the seedlings acted as a control collector.

After collection of all samples for each day, the pH of each was measured using an Alpha pH500 meter. pH determination of the mist deposited in control collectors was not possible due to the low volume collected. The samples were then filtered using Whatman 42 filters to remove particulate matter e.g. spruce needles and then stored at -4°C.

4.2.8.2 Ion analysis of surface droplets.

4.2.8.2.1 Divalent cations.

Analysis of Ca²⁺, Mg²⁺, Mn²⁺ and Zn²⁺ ions was undertaken using a Thermo-Jarrell Ash S-12 atomic absorption/ atomic emission spectrophotometer. The calibrations using a range of

standards were linear for all ions. Distilled water was used as a blank in all cases. 400ppm lanthanum chloride (LaCl_2) was added to the Ca^{2+} and Mg^{2+} assay to overcome phosphate interference (Allen, 1989).

4.2.8.2.2 Monovalent cations and anions.

Na^+ , K^+ , NH_4^+ , Cl^- , NO_3^{2-} and SO_4^{2-} content of droplets were determined using a Waters ion chromatograph (Waters Ltd, Hemel Hempstead, Herts.). This incorporates a HPLC pump (model 510), WISP (Waters Intelligent Sample Processor) - the automatic sample injection module, appropriate columns to separate the individual cations and anions (separate columns), conductivity detector (model 430) and a NEC APC-IV Powermate computer. The software (Baseline 810) enables chromatograms consisting of conductivity measurements over time, to be converted to ionic concentrations, by calibration against suitable standard solutions. Calibrations for all ions were linear.

4.2.9 Nutrient analysis of EOS and final harvest trees.

The nutrient content of leaves/needles from both harvests were examined. 1988 and 1989 year classes were chosen from Sitka spruce and for oak, 1F and 2F leaves. These samples were taken from all 20 EOS harvest seedlings (5 per treatment). At the final harvest, 5 Sitka spruce seedlings per treatment were used for analysis, with 1988, 1989 and 1990 year classes chosen. One-fifth of the leaf/needle dry weight was taken and ground in a ball-mill for five minutes.

0.2g of this ground material was acid-digested using a sulphuric acid - H_2O_2 digestion mixture (Allen, 1989). Blanks contained acid but no plant material. The digest was analysed for K^+ by flame emission and for Ca^{2+} , Mg^{2+} , Mn^{2+} and Zn^{2+} by atomic absorption (as in section 4.2.8.2.1). 400ppm LaCl_2 was again added for Ca^{2+} and Mg^{2+} analysis. The spectrophotometer was calibrated using a set of standard solutions prior to each analysis.

4.2.10 Wax content of Sitka spruce needles (using EOS trees).

Due to the senescence of oak leaves towards the end of the growing season, wax was only extracted from Sitka spruce needles. Samples of 50 needles from 1989 and 1988 year classes from all EOS harvest trees (5 per treatment) were analysed for total wax content. Needles were selected at random and carefully removed from the appropriate shoots using fine forceps.

The extraction method followed that of Watterson (1989). The needles were immersed for 20s in 25ml of chloroform in a clean glass container. The chloroform extract was filtered through chloroform-washed Whatman 42 filter paper, into a container previously weighed to 10⁻⁴g. Most of the chloroform evaporated at 60°C in a drying cabinet. The residual chloroform in the extract was removed in a vacuum dessicator containing activated charcoal. The dessicator had previously been flushed through with dry nitrogen. After 24h the container was removed and weighed immediately. The needles were placed in paper bags, oven-dried at 80°C for 3 days and then weighed. Needle wax content was expressed on a needle dry weight basis.

4.2.11 Water loss in Sitka (using EOS trees).

Estimations of the rate of water loss in Sitka spruce were made following the method of Neighbour *et al* (1989). A series of drying curves for both 1989 and 1988 year classes were constructed.

Before the harvest, all 20 seedlings (5 per treatment) were allowed to stand in water, in a controlled temperature (CT) room at 20°C, 24h before the start of the experiment. A 5cm length of 1 shoot from the appropriate year class was excised from each seedling and paraffin wax used to seal the cut end(s). Each shoot was weighed within one minute of being excised and then at set time intervals over that day and the following two days. The shoots were maintained in the CT room for the duration of the experiment.

4.2.12 Timetable of measurements made during the experiment.

A detailed timetable of all measurements made during the 1989 season and at both harvests is given in Table 4.03.

Table 4.03. Detailed sequence of measurements made during the 1989 season and at both 1989 and 1990 harvests.

Date	Measurements
1989	
10/5-12/5	Pre-treatment growth measurements.
24/5-26/5	First misting episode. Droplet collection on 26/5.
31/5-2/6	Non-destructive growth measurements.
15/6-17/6	Misting episode. Droplet collection on 17/6.
20/6-22/6	Misting episode. Droplet collection on 22/6.
6/7-8/7	Non-destructive growth measurements.
10/7-12/7	Misting episode. Droplet collection on 12/7.
18/7-20/7	Misting episode. Droplet collection on 20/7. LCA round on 20/7.
25/7-27/7	Non-destructive growth measurements.
31/7	LCA round.
1/8-3/8	Misting episode. Droplet collection on 3/8. LCA round on 3/8.
16/8-18/8	Misting episode. Droplet collection on 18/8.
22/8-24/8	Non-destructive growth measurements.

4/9-6/9	Final misting episode. Droplet collection on 6/9. LCA round on 6/9.
15/9	Evening LCA round.
23/9-3/10	1989 end of season (EOS) harvest. Measurements in chronological order: Wax content of Sitka spruce needles. Water loss in Sitka spruce. Non-destructive growth measurements of oak and Sitka spruce. Destructive harvests of oak and Sitka spruce. Nutrient analysis of oak and Sitka spruce foliage.
17/10-19/10	Final non-destructive growth measurements in 1989.
19/10-20/12	Monitoring of leaf fall in oak. Sitka spruce re-potted in November 1989. Seedlings maintained outside over 1989/1990 winter.
1990	
24/4-22/5	Monitoring of spring budburst in oak and Sitka spruce.
24/5-	1990 final harvest. Measurements in chronological order: Non-destructive growth measurements. Sitka spruce needles analysed for anti-oxidant enzyme levels (Mehlhorn <i>et al</i> , in press). Destructive growth measurements. Nutrient analysis of Sitka spruce foliage.

4.3 DATA ANALYSIS.

An ANOVA was used to analyse almost all measurements made throughout the experiment. From the randomised block design of the experiment, each H_2O_2 treatment consisted of two blocks, each containing 10 trees. It was therefore necessary not only to look for differences between the H_2O_2 treatments, but also to take into account any differences between the two blocks making up each treatment. For this reason, a two-way ANOVA was used and F ratios obtained for H_2O_2 treatment differences, differences between the two blocks (regardless of H_2O_2 treatment) and also any H_2O_2 treatment-block interaction.

The incorporation of four treatments in this dose-response study meant that treatment sum of squares were partitioned into linear and quadratic components in order to test for both linear and quadratic relationships between H_2O_2 and plant response. An example of the output from this ANOVA is given in Appendix 2. As before, in cases where significant F ratios arose, determination of significant differences between the means was achieved by calculation of the LSD.

The covariates used in the analysis of variance are presented in the following sections. The choice of which covariate to use in any one analysis was made by examining the variation contributed by the potential covariate to the parameter under consideration. An F ratio was obtained for the effect of the potential covariate. If significant (at $P < 0.05$) then the covariate was used in the ANOVA. Where covariates were used, the values subsequently presented will be adjusted means.

4.3.1 Non-destructive measurements.

4.3.1.1 Growth in the 1989 season.

All measurements made on one particular date were treated separately, so that the effect of H_2O_2 on cumulative growth could be assessed. For both species, pre-treatment measurements of height, basal diameter and leaf number (for oak) were used as covariates for subsequent measurements of these parameters. Pre-treatment measurements of shoot length were used as a covariate for changes in extension growth through the season. In Sitka spruce, pre-treatment shoot length consisted of pre-1989 growth. In oak, a second covariate was included for extension growth measurements. This was the 0 year's growth achieved before treatment began (pre-treatment 1989 growth). The reason for this is discussed in more detail in section 4.3.2.

For the analysis, all data were log transformed to ensure they were normally distributed.

4.3.1.2 Tree health in 1989.

A Chi-squared test was used to analyse any differences in the health of the seedlings as a result of H₂O₂ treatment.

4.3.1.3 Leaf loss in oak.

Differences in autumn leaf loss resulting from H₂O₂ treatment were determined by a two-way ANOVA. Leaf loss initially expressed as a percentage of the number of leaves on 22/8/89, was subjected to an arcsine transformation before analysis. No covariates were used in this analysis.

4.3.1.4 Spring budburst in 1990.

A Chi-squared test was used to analyse any treatment differences in spring budburst for both species.

4.3.2 Destructive harvests.

Pre-treatment measurements of height, basal diameter and leaf number (for oak) were used as covariates for measurements of these parameters made at both 1989 EOS and 1990 final harvests. Measurements of pre-1989 extension growth were used as covariates in the analysis of Sitka spruce data from both harvests.

Oak seedlings were randomly chosen for the EOS harvest on the basis of total pre-treatment shoot length and an ANOVA of this data had suggested no significant differences between the treatments (Table 4.04). However, at the time of the harvest, total pre-treatment extension growth was separated into pre-1989 shoot length and pre-treatment 1989 shoot length. There was no significant difference in pre-1989 extension growth between the treatments, but there was a significant linear trend in the data for pre-treatment 1989 shoot length, with a significant difference between 20uM and 100uM seedlings (Table 4.04). For this reason, two covariates (pre-1989 shoot length and pre-treatment 1989 shoot length) were used in the analysis of oak harvest data.

Leaf/needle and stem dry weight were combined to give a value for total above ground dry weight. Other parameters were also calculated:

Specific leaf area ratio (SLAR) = leaf area/ leaf dry weight.

Mean leaf area (MLA) = leaf area/ leaf number.

Mean leaf weight (MLW) = leaf dry weight/ leaf number.

Mean stem weight (MSW) = stem dry weight/ stem length.

Root-shoot ratio (RSR) = root dry weight/ above ground dry weight.

Table 4.04. Statistical analysis of pre-treatment extension growth in oak.

Extension growth (cm)	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
Total pre-treatment					
EOS harvest	T 0.69 L 0.54 Q 0.37	3.85	3.91	3.85	3.58
Pre-1989					
EOS harvest	T 0.69 L 0.54 Q 0.37	3.66	3.57	3.70	3.49
Pre-treatment 1989					
EOS harvest	T 2.68 L 5.17* Q 0.56	1.74 ab	2.70 b	1.32 ab	0.53 a

* indicates a significant F ratio at $P < 0.05$. All other F ratios are non-significant. The means with different letters are significantly different at $P < 0.05$. The degrees of freedom for the F ratios are:

T = treatment F ratio, df=3,16.

L = linear F ratio, df=1,16.

Q = quadratic F ratio, df=1,16.

4.3.3 Effects on photosynthesis and leaf conductance in oak.

A two-way ANOVA was used to analyse the data from the four gas exchange measurement rounds. Again, linear and quadratic trends and differences between blocks were assessed. LSD was used to calculate significant differences between means in cases of significant F ratios. Photon flux density and air temperature were tested as potential covariates for all gas exchange measurements and for each measurement date, the covariates chosen were: 20/7/89 and 6/9/89 - photon flux density; 31/7/89 and 3/8/89 - air temperature. For each date, the effect of the chosen covariate was significant.

For the measurements undertaken on the evening of the 15/9/89, a two-way ANOVA was again used to analyse the data, but with only two H_2O_2 treatments (0uM and 100uM) used in the investigation, the presence of linear and quadratic trends were not tested. Air temperature was used as a covariate in the analysis.

4.3.4 Effects on surface droplet chemistry.

For each H_2O_2 treatment in this study, there were only two values, each value representing one block. With the restriction placed on the analysis due to the low degrees of freedom ($n=8$), it was only possible to treat the data for differences between treatments. All data were log transformed to ensure they were normally distributed.

Initial analysis involved a one-way ANOVA of each collection date for differences between the treatments for both species. The time interval between the end of misting and the start of collection was used as a covariate. A correlation matrix of the relationship between the ions (across all treatments) at each date was also obtained. The mean ionic concentration collected for each treatment was calculated for both species. This represented the average collected throughout the season. This data was subjected to a one-way ANOVA to test for differences between the treatments. The presence of any correlation between the ions was also tested.

For Sitka spruce and oak, an ionic concentration averaged across all treatments was calculated at each collection date. The seasonal trend for each ion then became apparent.

On three separate occasions, high H^+ concentrations were seen in droplets collected from seedlings treated with H_2O_2 . For these dates, the data from both species were combined and a two-way ANOVA used to test for differences between treatments, for a linear trend between H_2O_2 treatment and droplet H^+ concentration, for differences between the two species and any treatment-species interaction. Analysis of the correlation between droplet H^+ and SO_4^{2-} concentration was undertaken.

The changes in mist composition between the start and the end (i.e. mist deposited in the control collector) of the misting episode were also analysed. The differences between treatments and between dates were negligible so that the data were combined and a one-way ANOVA used to test the effect of time (before or after misting) on mist composition.

4.3.5 Nutrient analysis of EOS and final harvest trees.

Nutrient concentration was calculated on a leaf/needle dry weight basis. The effects of H_2O_2 exposure and the presence of any linear or quadratic trends were assessed. No covariates were used in the analysis.

4.3.6 Wax content of Sitka spruce needles.

Total wax content was expressed as a percentage of needle dry weight. The data were analysed for the effects of H_2O_2 treatment, including linear and quadratic trends. No covariates were used.

The data from the two year classes (1988 and 1989) were combined and analysed as above.

4.3.7 Water loss in Sitka spruce.

The initial weight of the excised shoots were designated as 100% and all other values were expressed as a percentage of this initial value. This change in weight with time was presented as a series of drying curves.

The rate of water loss for each day of the three days of the experiment was calculated on a g day^{-1} basis. The data from both 1988 and 1989 year classes were combined and a two-way ANOVA used to analyse the results for any effects of H_2O_2 treatment, for differences between year classes and any treatment-year class interaction. Linear and quadratic trends between H_2O_2 treatment and plant response were also tested.

4.4 RESULTS

4.4.1 Mist application.

An assessment of the volume of mist applied to the individual blocks suggested very little difference between blocks or treatments (Table 4.05). The total volume of mist applied was approximately 48l over the 17 week period.

Table 4.05. A summary of mist application for both tree species.

Treatment uM H ₂ O ₂	Actual mean pH	Mean H ⁺ concentration (uM)	Total volume applied (l)
0	4.02	95 ± 17.8	47
	3.99	102 ± 18.3	50
20	3.98	105 ± 17.4	48
	3.99	102 ± 19.0	48
50	3.99	102 ± 18.1	49
	4.00	100 ± 19.1	46
100	4.00	100 ± 19.6	48
	3.99	102 ± 18.2	49

The figures represent the overall means for the experiment ± the standard deviation of that mean.

4.4.2 Environmental conditions.

4.4.2.1 Air pollution data for the 1989 season.

Fig.4.01 gives the mean daily O₃, NO and NO₂ concentrations at the field site from May to September 1989. O₃ was the dominant pollutant in terms of concentration.

4.4.2.2 Climatic data.

Table 4.06 provides a summary of the daily mean, maximum and minimum air temperatures for each month during the experiment. Total monthly rainfall is also given.

Fig.4.01. Mean daily pollutant concentrations for May - September 1989.

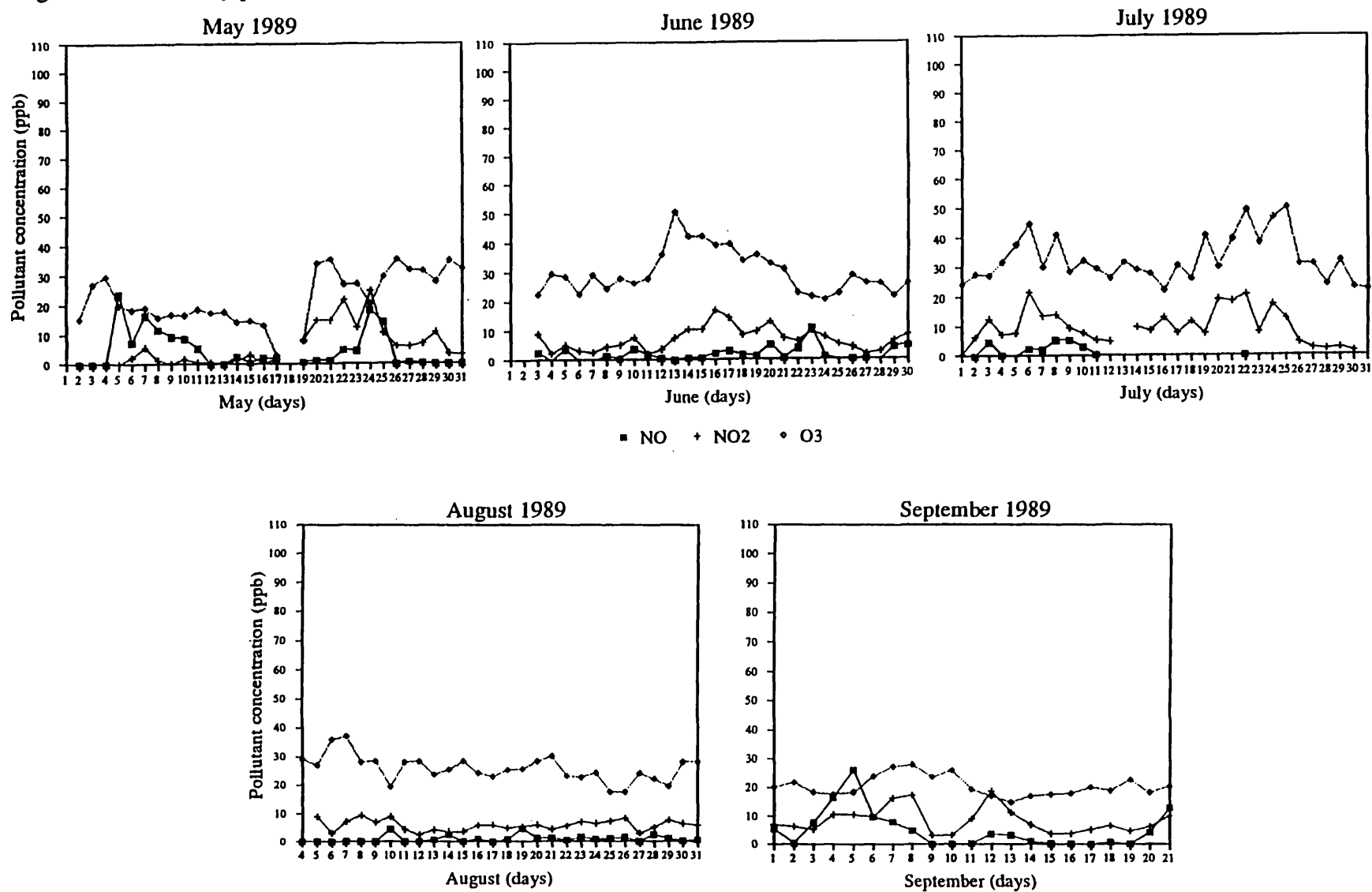


Table 4.06. Daily mean, minimum and maximum air temperatures and rainfall in the 1989 season.

Month	Air temperature (°C)			Rainfall total (mm)
	Mean	Minimum	Maximum	
May	14.3	7.8	20.8	21
June	16.3	8.9	21.0	24.5
July	19.3	12.4	24.4	55
August	17.0	10.9	22.9	54
September	15.3	10.1	19.7	27

4.4.3 Non-destructive and destructive growth measurements in oak.

Analysis of the 1989 pre-treatment growth measurements (taken on 3/5/89) suggested no significant differences between treatments or blocks in height, basal diameter or shoot length (Table 4.07).

4.4.3.1 Non-destructive growth measurements.

4.4.3.1.1 1989 measurements.

Growth measurements were made on five separate occasions after misting began. These are summarised in Table 4.08.

There was no significant effect of H₂O₂ treatment on height, basal diameter or leaf number at any time during the season. However, on the 28/7/89 (after 72h of misting), there was a significant treatment effect on 1989 extension growth (Table 4.09). This effect was seen for the remainder of the season, on 22/8/89 and 17/10/89 (Table 4.09). The extension growth in 1989 achieved by control seedlings was 32-37% greater than 20uM treated seedlings on these dates (Fig.4.02). Extension growth in the control was 26-31% greater than in the 100uM treatment on these dates but this was never significant (Fig.4.02). 1989 shoot length in the 50uM treatment was significantly greater than both 20uM and 100uM treatments by 36-42% and 30-37% respectively on the above dates (Fig.4.02).

When the data was split into the component flushes, first flush (1F) and third flush (3F) growth in 1989 were not significantly affected by H₂O₂ exposure, but there was a significant treatment effect on second flush (2F) growth (Table 4.09). 2F growth in control seedlings was 35-40% greater than in 20uM treated seedlings on 28/7/89, 22/8/89 and 17/10/89 (Fig.4.02). On the same days, 2F growth in the 50uM treatment was significantly greater than in the 20uM by 40-43% (Fig.4.02).

4.4.3.1.2 EOS and final harvest measurements.

Five seedlings per treatment had been chosen at random for the 1989 EOS harvest. Analysis of pre-treatment growth measurements for this sub-sample suggested no significant differences in height, basal diameter and pre-1989 shoot length between the treatments. However, there was a significant linear trend in the data for pre-treatment 1989 shoot length and leaf number, with a significant difference between 20uM and 100uM treatments in both cases (Table 4.10).

At the time of the harvest however, there was no significant effect of H₂O₂ treatment on height, basal diameter, leaf number or 1989 extension growth (Table 4.10).

Table 4.07. F ratios for pre-treatment non-destructive growth measurements in oak.

	F ratio				
	Treatment (df=3,72)	Linear (df=1,72)	Quadratic (df=1,72)	Block (df=1,72)	Treatment- block (df=3,72)
Height	0.42	0.43	0.07	0.36	0.73
Basal diameter	0.51	1.37	0.16	0.10	0.24
Leaf number	0.94	1.74	0.06	0.01	1.04
Pre-1989 shoot length	0.20	0.48	0.02	0.75	0.61
Pre- treatment 1989 shoot length	0.96	1.99	0.01	0.02	1.88

All F ratios were non-significant.

Table 4.08. Growth measurement dates.

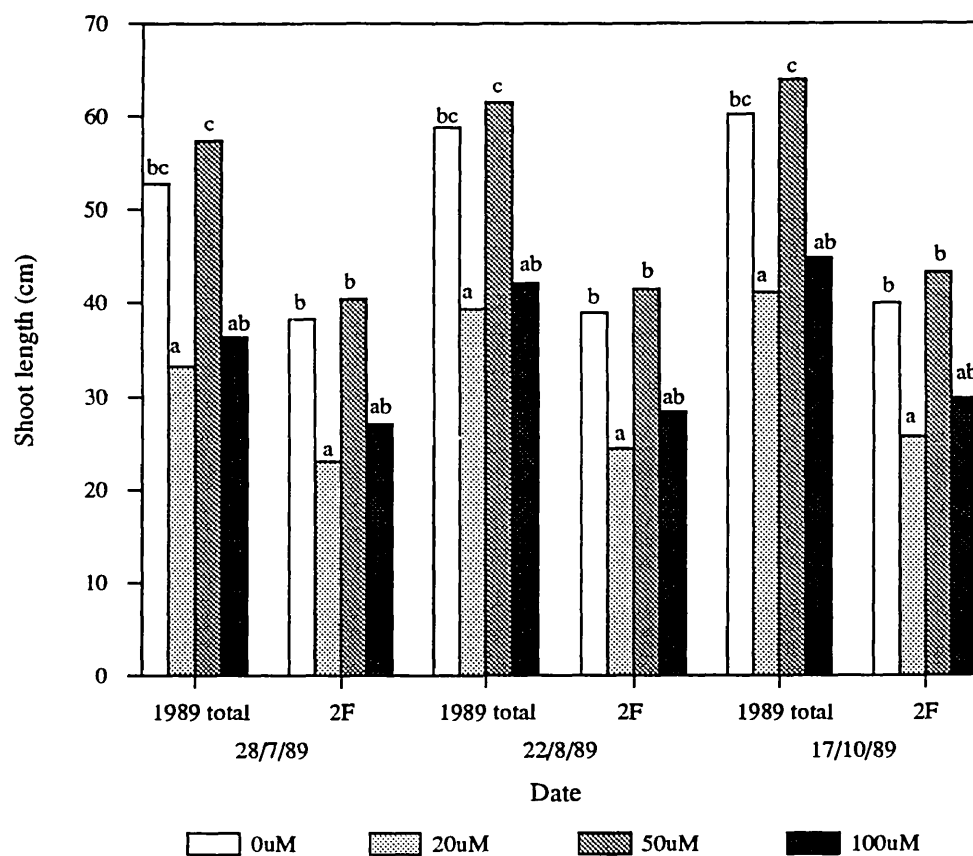
Date	Number of misting hours up to this date.
31/5/89	24
5/7/89	48
28/7/89	72
22/8/89	96
17/10/89	108

Table 4.09. 1989 extension growth in oak.

Date	Flush	F ratio		
		Treatment (df=3,71)	Linear (df=1,71)	Quadratic (df=1,71)
31/5/89	Total	1.74	2.61	0.48
5/7/89	Total	2.14	0.01	0.84
28/7/89	Total	4.21 *	0.88	1.61
	1F	1.74	1.47	1.27
	2F	3.87 *	0.01	0.46
	3F	1.22	3.37	0.02
22/8/89	Total	3.57 *	0.38	0.87
	1F	2.23	2.97	2.49
	2F	4.20 *	0.21	0.05
	3F	0.55	0.52	0.48
17/10/89	Total	3.38 *	0.24	0.73
	1F	1.83	0.67	1.59
	2F	3.13 *	0.08	0.09
	3F	0.63	1.06	0.08

* indicates a significant F ratio at $P < 0.05$. All other F ratios are non-significant. These results were obtained using pre-1989 and pre-treatment 1989 shoot length as covariates.

Fig.4.02. Changes in extension growth in oak during the 1989 season.



Within any one category, means with different letters are significantly different at $P < 0.05$.

Table 4.10. Non-destructive growth measurements for EOS harvest oak.

Parameter	F ratio		
	Treatment	Linear	Quadratic
Pre-treatment			
Height	2.15	3.34	2.42
Basal diameter	0.50	0.86	0.02
Leaf number	2.28	4.63 *	0.03
Pre-1989 shoot length	0.69	0.54	0.37
Pre-treatment 1989 shoot length	2.68	5.17 *	0.56
EOS harvest			
Height	0.67	1.87	0.31
Basal diameter	0.27	0.16	0.62
Total leaf number	0.80	0.02	0.62
1F leaf number	2.30	3.31	0.13
2F leaf number	1.02	1.06	0.55
3F leaf number	1.35	0.40	3.05
1989 shoot length	0.70	1.90	0.20
1F shoot length	2.16	2.46	0.75
2F shoot length	0.93	1.26	0.52
3F shoot length	1.22	0.23	2.11

* indicates a significant F ratio at $P < 0.05$. All other F ratios are non-significant. The EOS harvest results were obtained using pre-1989 and pre-treatment 1989 shoot length as covariates. The degrees of freedom for the F ratios are:
Treatment, $df=3,16$ (pre-treatment) or $3,14$ (harvest)
Linear and Quadratic, $df=1,16$ or $1,14$.

At the final harvest in 1990, there were also no significant differences in the pre-treatment measurements between the treatments for the seedlings that were maintained until this harvest (Table 4.11).

Measurements of final height, basal diameter and leaf number showed no significant effect of exposure to H_2O_2 mist (Table 4.11). However, there was a significant treatment effect on 1989 extension growth (Table 4.11), with control and 50uM seedlings achieving approximately 30% greater shoot length than 20uM seedlings and 17% greater shoot length than 100uM seedlings (Fig.4.03). When the individual flushes were considered, there was no significant effect on 1F or 3F growth (Table 4.11). There was a significant H_2O_2 treatment effect on 2F growth (Table 4.11), with 50uM treated seedlings achieving 36% greater extension growth than 20uM seedlings (Fig.4.03). This pattern of results was the same as that seen during the 1989 treatment season. There was no significant effect of H_2O_2 treatment on 1990 extension growth (Table 4.11 and Fig.4.03).

4.4.3.1.3 Tree health in 1989.

The health of the oak seedlings, expressed as the incidence of visible disease/pest damage symptoms, was not significantly affected by H_2O_2 treatment at any time during the 1989 season. For all values of χ^2 , $P > 0.05$.

The mean health scores for each treatment at different times in the season are given in Table 4.12. These scores represent the severity of the mildew outbreak, where a score of 1 = mildew occurring on 0-25% of leaves, 2 = mildew on 26-50% of leaves and so on (see section 4.2.6.1). There was little change in the frequency of infection through the season (Table 4.12) and no one tree was especially affected by the disease.

4.4.3.1.4 Autumn 1989 leaf loss and spring 1990 budburst.

The results of the ANOVA of autumn leaf loss in 1989 showed no significant effect of H_2O_2 treatment (Table 4.13). Budburst in spring 1990 was also unaffected by H_2O_2 treatment (for all values of χ^2 , $P > 0.05$).

4.4.3.2 Destructive growth measurements.

4.4.3.2.1 1989 EOS harvest.

There were no significant effects of H_2O_2 treatment on any of the destructive growth measurements made at the EOS harvest (Table 4.14). The data from the individual flushes were also non-significant (at $P < 0.05$).

Table 4.11. Non-destructive measurements for final harvest oak.

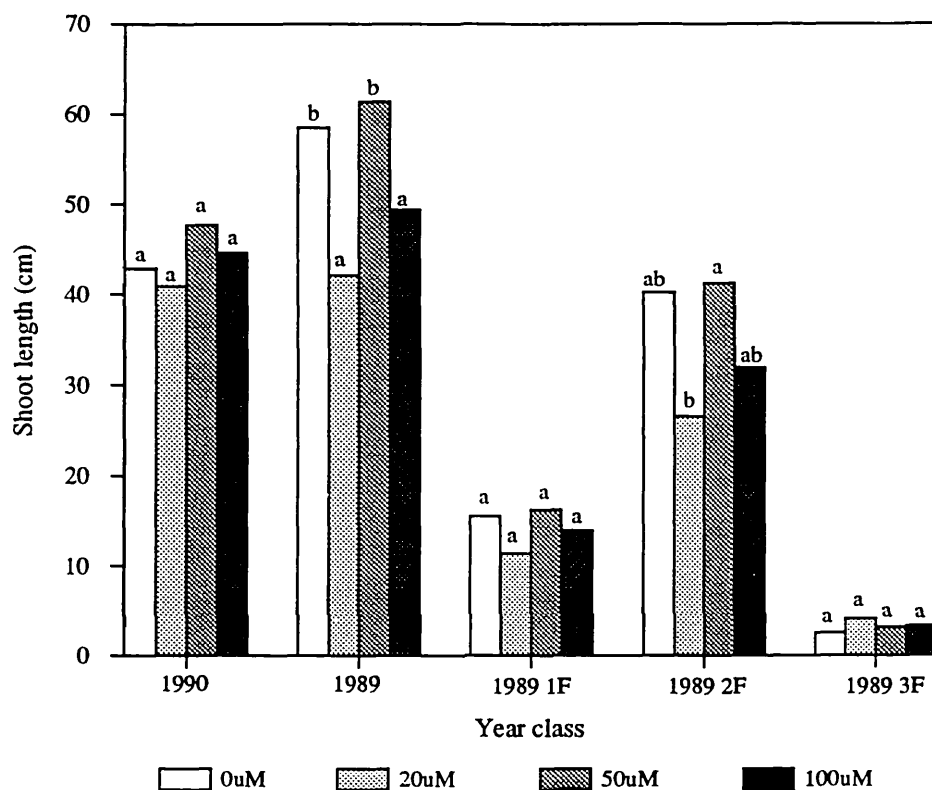
Parameter	F ratio		
	Treatment	Linear	Quadratic
Pre-treatment			
Height	0.53	0.29	0.92
Basal diameter	0.34	0.79	0.24
Pre-1989 shoot length	0.63	0.52	0.66
Pre-treatment 1989 shoot length	0.03	0.01	0.03
Final harvest			
Height	0.86	0.01	0.54
Basal diameter	0.86	1.96	0.23
Leaf number	1.14	0.13	0.14
1989 shoot length	5.35 *	2.04	0.01
1989 1F	1.09	0.69	0.09
1989 2F	3.41 *	0.54	0.08
1989 3F	0.12	0.23	0.01
1990 shoot length	0.94	0.10	2.01

* indicates a significant F ratio at $P < 0.05$. All other F ratios are non-significant. These results were obtained using pre-1989 and pre-treatment 1989 shoot length as covariates. The degrees of freedom for the F ratios are:

Treatment, $df=3,56$ (pre-treatment); 3,54 (harvest height and basal diameter); 3,54 (harvest shoot length).

Linear and quadratic, $df=1,56$ or 1,54.

Fig.4.03. Non-destructive measurements of extension growth in oak at the 1990 final harvest.



Within any one category, means with different letters are significantly different at $P < 0.05$.

Table 4.12. Mean scores for the occurrence of mildew on oak leaves in 1989.

Month	Health score [#]			
	0uM	20uM	50uM	100uM
May	1.95	2.15	2.25	2.13
June	1.84	1.89	1.93	2.00
July	1.68	1.83	1.75	1.92
August	1.76	1.80	1.82	1.80

* see text for details.

Table 4.13. F ratios for leaf loss from oak in autumn 1989.

Date	F ratio		
	Treatment (df=3,56)	Linear (df=3,56)	Quadratic (df=3,56)
17/10/89	0.32	0.01	0.01
27/10/89	0.31	0.37	0.56
3/11/89	0.24	0.39	0.22
10/11/89	0.21	0.14	0.18
17/11/89	0.34	0.02	0.40
24/11/89	0.19	0.02	0.45
1/12/89	0.67	0.06	1.22

All F ratios were non-significant at $P < 0.05$.

Table 4.14. Growth parameters for oak measured at the 1989 EOS harvest.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
Total leaf area (cm ²)	T 0.16 L 0.46 Q 0.01	6.54	6.53	6.47	6.39
Total SLAR (cm ² g ⁻¹)	T 0.68 L 0.78 Q 0.65	4.56	4.55	4.58	4.51
Total mean leaf area (cm ² leaf ⁻¹)	T 0.94 L 0.29 Q 0.63	1.95	2.12	1.70	1.92
Total mean leaf weight (g leaf ⁻¹)	T 0.89 L 0.23 Q 0.44	-2.64	-2.45	-2.89	-2.65
Total leaf dry weight (g)	T 0.68 L 0.62 Q 0.27	1.97	2.05	1.77	1.86
1989 stem dry weight (g)	T 0.62 L 1.21 Q 0.53	2.23	2.12	2.00	2.01
1989 mean stem weight (g cm ⁻¹)	T 1.17 L 1.48 Q 0.02	-3.17	-3.15	-3.36	-3.44
Total above ground dry weight (g)	T 0.57 L 0.96 Q 0.37	2.80	2.78	2.59	2.64
Root dry weight (g)	T 1.24 L 3.29 Q 0.27	3.43	3.32	3.19	3.11

Total plant dry weight (g)	T 1.02 L 2.53 Q 0.33	3.86	3.79	3.69	3.60
Root:shoot ratio (RSR)	T 0.57 L 0.99 Q 0.05	0.63	0.51	0.60	0.48

T = treatment F ratio, with df=3,14.

L = linear F ratio, with df=1,14.

Q = quadratic F ratio, with df=1,14.

All the means presented have been adjusted for pre-1989 and pre-treatment 1989 shoot length as covariates. All F ratios were non-significant at $P<0.05$.

4.4.3.2.2 1990 final harvest.

For all the destructive growth measurements made at the final harvest, there were no significant effects of H₂O₂ treatment (Table 4.15). The data from the individual flushes in 1989 were also non-significant.

At both EOS and final harvests, there was no effect of H₂O₂ treatment on soil pH (Table 4.16). The mean soil pH increased during the season of treatment and showed a further, but small, increase between both harvests. The increase in soil pH seen in 1989 may have been the result of watering the seedlings with tap water.

Table 4.16. Oak soil pH at all harvests.

Harvest date	Mean soil pH			
	0uM	20uM	50uM	100uM
Initial (5/89)	5.32			
EOS (9/89)	7.16	7.15	7.14	7.11
Final (5/90)	7.59	7.47	7.46	7.54

4.4.4 Non-destructive and destructive growth measurements in Sitka spruce.

As for oak, there were no significant differences between treatments or blocks for 1989 pre-treatment growth measurements of height, basal diameter and total shoot length (Table 4.17).

4.4.4.1 Non-destructive growth measurements.

4.4.4.1.1 1989 measurements.

The dates on which measurements were made are as described in Table 4.18. There were no significant effects of H₂O₂ treatment on height, basal diameter or shoot length at any time in the 1989 season. Table 4.18 gives the data for extension growth. Similarly, there were no significant differences between blocks or a treatment-block interaction for the above parameters.

Table 4.15. Growth parameters for oak measured at the 1990 final harvest.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
Total leaf area (cm ²)	T 1.75 L 0.04 Q 2.04	6.75	6.65	6.92	6.66
Total SLAR (cm ² g ⁻¹)	T 0.85 L 0.38 Q 0.45	5.05	5.00	5.03	5.02
Total mean leaf area (cm ² leaf ⁻¹)	T 0.61 L 0.01 Q 1.76	2.00	2.06	2.13	2.00
Total mean leaf weight (g leaf ⁻¹)	T 0.89 L 0.23 Q 0.37	-2.50	-2.45	-2.41	-2.53
Total leaf dry weight (g)	T 1.52 L 0.01 Q 2.84	1.67	1.65	1.88	1.64
1989 stem dry weight (g)	T 2.47 L 0.43 Q 0.01	2.92	2.77	2.96	2.87
1990 stem dry weight (g)	T 0.97 L 0.37 Q 1.62	-0.96	-0.96	-0.73	-0.90
1989 mean stem weight (g cm ⁻¹)	T 1.19 L 0.60 Q 2.34	-3.73	-3.70	-3.55	-3.67
1990 mean stem weight (g cm ⁻¹)	T 0.40 L 0.41 Q 0.15	-4.62	-4.64	-4.55	-4.58

Total above ground dry weight (g)	T 2.30 L 0.02 Q 2.69	2.53	2.46	2.70	2.48
Root dry weight (g)	T 1.06 L 0.01 Q 1.70	3.13	3.10	3.24	3.09
Total plant dry weight (g)	T 1.58 L 0.03 Q 2.15	3.58	3.53	3.70	3.53
Root:shoot ratio (RSR)	T 1.00 L 0.01 Q 0.49	0.59	0.64	0.54	0.61

T = treatment F ratio, with df=3,54.

L = linear F ratio, with df=1,54.

Q = quadratic F ratio, with df=1,54.

All the means presented have been adjusted for pre-1989 and pre-treatment 1989 shoot length as covariates. There were no significant differences between the means at $P < 0.05$.

Table 4.17. F ratios for pre-treatment non-destructive growth measurements in Sitka spruce.

	F ratio				
	Treatment (df=3,72)	Linear (df=1,72)	Quadratic (df=1,72)	Block (df=1,72)	Treatment- block (df=3,72)
Height	1.01	2.28	0.42	0.21	0.82
Basal diameter	1.25	1.68	1.48	0.25	0.84
Shoot length	0.36	0.03	0.24	0.29	0.40

All F ratios are non-significant at $P < 0.05$.

Table 4.18. Sitka spruce extension growth throughout the 1989 season.

Date	F ratio	Treatment means (log cm)			
		0uM	20uM	50uM	100uM
31/5/89	T 1.299 L 0.009 Q 3.887	5.582	5.664	5.710	5.603
5/7/89	T 1.267 L 2.922 Q 0.610	5.918	5.897	5.913	5.806
28/7/89	T 1.651 L 3.035 Q 1.114	6.001	5.971	6.013	5.885
22/8/89	T 0.349 L 0.754 Q 0.278	6.029	6.045	6.032	5.981
17/10/89	T 0.286 L 0.710 Q 0.078	6.210	6.200	6.059	6.024

T = treatment F ratio, with df=3,71.

L = linear F ratio, with df=1,71.

Q = quadratic F ratio, with df=1,71.

All the means presented have been adjusted for pre-treatment shoot length measurements as a covariate. There were no significant differences between the means at $P < 0.05$.

4.4.4.1.2 EOS and final harvest measurements.

Analysis of pre-treatment growth measurements of the seedlings chosen for the 1989 EOS harvest, suggested no significant differences between the treatments in height, basal diameter or shoot length (Table 4.19). No effects of H₂O₂ treatment were found on height, basal diameter or 1989 extension growth at this harvest (Table 4.19).

At the 1990 final harvest, there were again no significant differences in pre-treatment plant size with respect to height, basal diameter or shoot length. Final height, basal diameter and 1989 and 1990 extension growth were unaffected by H₂O₂ treatment (Table 4.20).

4.4.4.1.3 Tree health in 1989 and 1990 spring budburst.

There was no significant effect of H₂O₂ treatment on seedling health in 1989 nor on spring budburst in 1990. All values of chi² were non-significant ($P > 0.05$).

4.4.4.2 Destructive growth measurements.

4.4.4.2.1 1989 EOS harvest.

Exposure to H₂O₂ during the 1989 season did not significantly affect the majority of parameters assessed at the EOS harvest (Table 4.21). However for 1989 needle dry weight and total needle dry weight there were significant linear trends in the data (Table 4.21). In both cases, there was a significant difference between control and 100uM treatments (Fig.4.04). 1989 needle dry weight and total needle dry weight in control seedlings were 29% and 27% greater respectively than in 100uM treated seedlings (Fig.4.04). The significant effect on total needle dry weight was the result of the effect on 1989 needles, as these made the greatest contribution to total needle dry weight. There were no significant effects of H₂O₂ treatment on specific needle area, mean needle weight or mean stem weight.

4.4.4.2.2 1990 final harvest.

The results of the analysis of the final harvest data are presented in Table 4.22. The parameters that were significantly affected by H₂O₂ showed a quadratic response to H₂O₂ treatment (Table 4.22). 1990 and total needle dry weight in the 50uM treated seedlings were 14% and 12% greater, respectively, than in the 100uM treated seedlings (Fig.4.05). 1989, 1990 and total stem dry weight was significantly greater in the 50uM treatment than in the control (Fig.4.05). When total above ground dry weight was considered, this also showed a quadratic response to H₂O₂, with the 50uM seedlings having 11% greater above ground dry weight than both control and 100uM seedlings (Table 4.22 and Fig.4.05). There was no significant effect of H₂O₂ treatment on specific needle area or mean needle weight.

Table 4.19. Non-destructive growth measurements for EOS harvest Sitka spruce.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
Pre-treatment					
Height (cm)	T 0.31 L 0.09 Q 0.11	3.41	3.50	3.44	3.47
Basal diameter (mm)	T 0.13 L 0.01 Q 0.14	1.90	1.93	1.92	1.91
Pre-1989 shoot length (cm)	T 1.14 L 1.64 Q 1.71	4.69	4.76	4.74	4.69
EOS harvest					
Height (cm)	T 0.87 L 0.05 Q 0.01	3.78	3.92	3.78	3.85
Basal diameter (mm)	T 1.54 L 4.25 Q 0.10	2.59	2.53	2.52	2.47
1989 shoot length (cm)	T 1.93 L 4.35 Q 0.79	6.26	6.29	6.03	6.02

T = treatment F ratio with degrees of freedom (df)=3,16 for pre-treatment measurements and 3,15 for harvest data.

L = linear F ratio, df=1,16 or 1,15 (as for T).

Q = quadratic F ratio, df=1,16 or 1,15.

All F ratios were non-significant at $P < 0.05$. The harvest means have been adjusted for pre-treatment as covariate.

Table 4.20. Non-destructive growth measurements for final harvest Sitka spruce.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
Pre-treatment					
Height (cm)	T 1.78 L 2.58 Q 0.92	3.45	3.39	3.45	3.50
Basal diameter (mm)	T 1.65 L 2.17 Q 1.31	1.91	1.90	1.93	1.89
Pre-1989 shoot length (cm)	T 0.27 L 0.12 Q 0.06	4.70	4.74	4.70	4.74
Final harvest					
Height (cm)	T 0.26 L 0.65 Q 0.13	3.92	3.91	3.91	3.88
Basal diameter (mm)	T 1.02 L 1.57 Q 0.73	2.72	2.70	2.72	2.67
1990 shoot length (cm)	T 1.46 L 0.30 Q 4.02	6.88	6.99	7.10	6.95
1989 shoot length	T 0.66 L 0.12 Q 1.83	5.96	6.02	6.08	5.99

T = treatment F ratio with degrees of freedom (df)=3,56 for pre-treatment measurements and 3,55 for harvest data.

L = linear F ratio, df=1,56 or 1,55 (as for T).

Q = quadratic F ratio, df=1,56 or 1,55.

All F ratios were non-significant at $P < 0.05$. The harvest means have been adjusted for pre-treatment shoot length as covariate.

Table 4.21. Growth parameters for Sitka spruce at the 1989 EOS harvest.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
1989 needle area (mm ²)	T 1.81 L 2.78 Q 2.55	2.80	2.81	2.82	2.75
1988 needle area (mm ²)	T 0.64 L 0.23 Q 1.65	2.81	2.82	2.83	2.81
1989 needle dry weight (g)	T 1.98 L 4.82* Q 0.07	2.65	2.51	2.55	2.32
1988 needle dry weight (g)	T 1.81 L 0.65 Q 0.65	0.96	0.92	1.05	0.87
Total needle dry weight (g)	T 2.19 L 5.01* Q 0.18	2.82	2.70	2.75	2.55
1989 stem dry weight (g)	T 0.37 L 1.03 Q 0.02	1.76	1.69	1.69	1.54
1988 stem dry weight (g)	T 0.96 L 0.15 Q 1.24	2.33	2.17	2.26	2.34
Total stem dry weight (g)	T 0.15 L 0.45 Q 0.01	2.89	2.87	2.84	2.80
Above ground dry weight (g)	T 0.81 L 2.13 Q 0.03	3.55	3.49	3.49	3.37

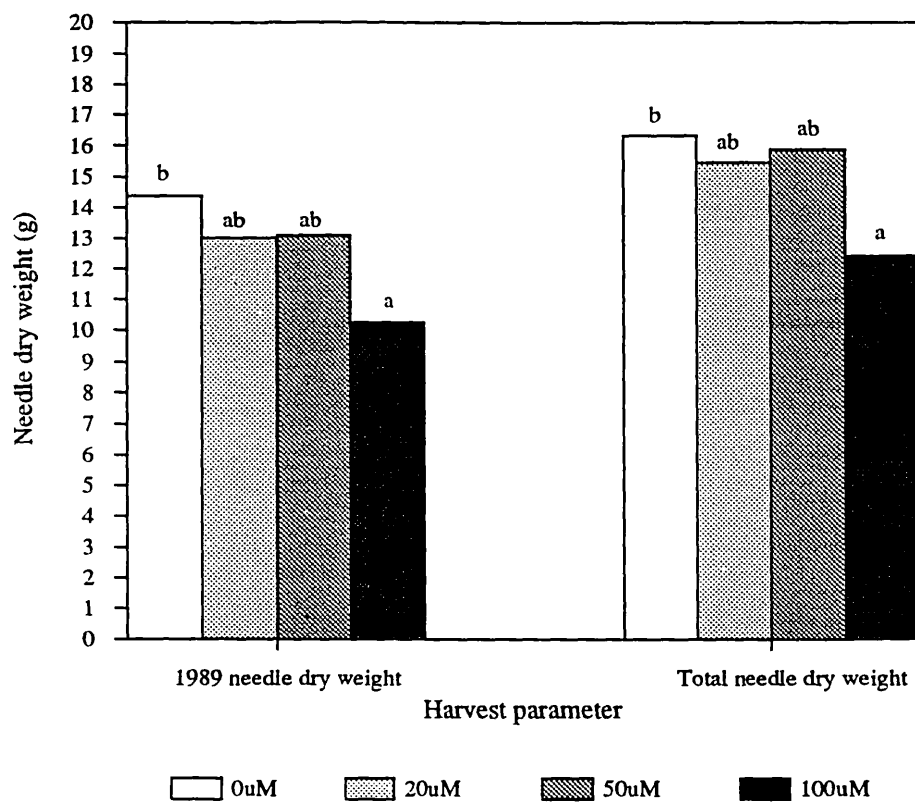
Root dry weight (g)	T 0.16 L 0.48 Q 0.01	3.64	3.62	3.57	3.47
Total plant dry weight (g)	T 0.39 L 1.12 Q 0.02	4.30	4.26	4.24	4.12
Root:shoot ratio (RSR)	T 0.02 L 0.01 Q 0.01	-0.09	-0.13	-0.08	-0.09

* indicates a significant F ratio at $P < 0.05$ and significant differences between the means are presented in Fig.4.04. In all other cases there were no significant differences between the means at $P < 0.05$. The means presented have been adjusted for pre-treatment shoot length as covariate. The degrees of freedom are:

Treatment (T) $df=3,15$.

Linear (L) and quadratic (Q) $df=1,15$.

Fig.4.04. Effect of H₂O₂ on needle dry weight in Sitka spruce at the 1989 EOS harvest.



For any one parameter, means with different letters are significantly different at $P < 0.05$.

Table 4.22. Growth parameters for Sitka spruce at the 1990 final harvest.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
1990 needle area (mm ²)	T 0.32 L 0.65 Q 0.02	6.26	6.28	6.24	6.23
1989 needle area (mm ²)	T 0.02 L 0.02 Q 0.03	6.44	6.45	6.46	6.44
1990 needle dry weight (g)	T 2.28 L 1.24 Q 5.47*	3.27	3.38	3.38	3.22
1989 needle dry weight (g)	T 1.00 L 0.19 Q 1.66	2.49	2.49	2.60	2.50
1988 needle dry weight (g)	T 0.49 L 0.01 Q 0.12	0.90	0.84	0.93	0.87
Total needle dry weight (g)	T 2.15 L 0.65 Q 5.77*	3.72	3.78	3.82	3.69
1990 stem dry weight (g)	T 1.70 L 0.40 Q 4.53*	1.46	1.56	1.68	1.53
1989 stem dry weight (g)	T 2.08 L 0.03 Q 5.01*	2.06	2.14	2.29	2.14
1988 stem dry weight (g)	T 2.01 L 0.03 Q 0.04	2.69	2.57	2.70	2.65

Total stem dry weight (g)	T 2.78 L 0.52 Q 5.05*	3.39	3.39	3.53	3.41
Above ground dry weight (g)	T 2.90* L 0.01 Q 7.27**	4.27	4.29	4.39	4.26
Root dry weight (g)	T 0.76 L 0.03 Q 1.26	4.23	4.21	4.35	4.19
Total plant dry weight (g)	T 1.43 L 0.02 Q 2.91	4.95	4.95	5.07	4.93
Root:shoot ratio (RSR)	T 0.12 L 0.23 Q 0.03	-0.03	-0.09	-0.05	-0.07

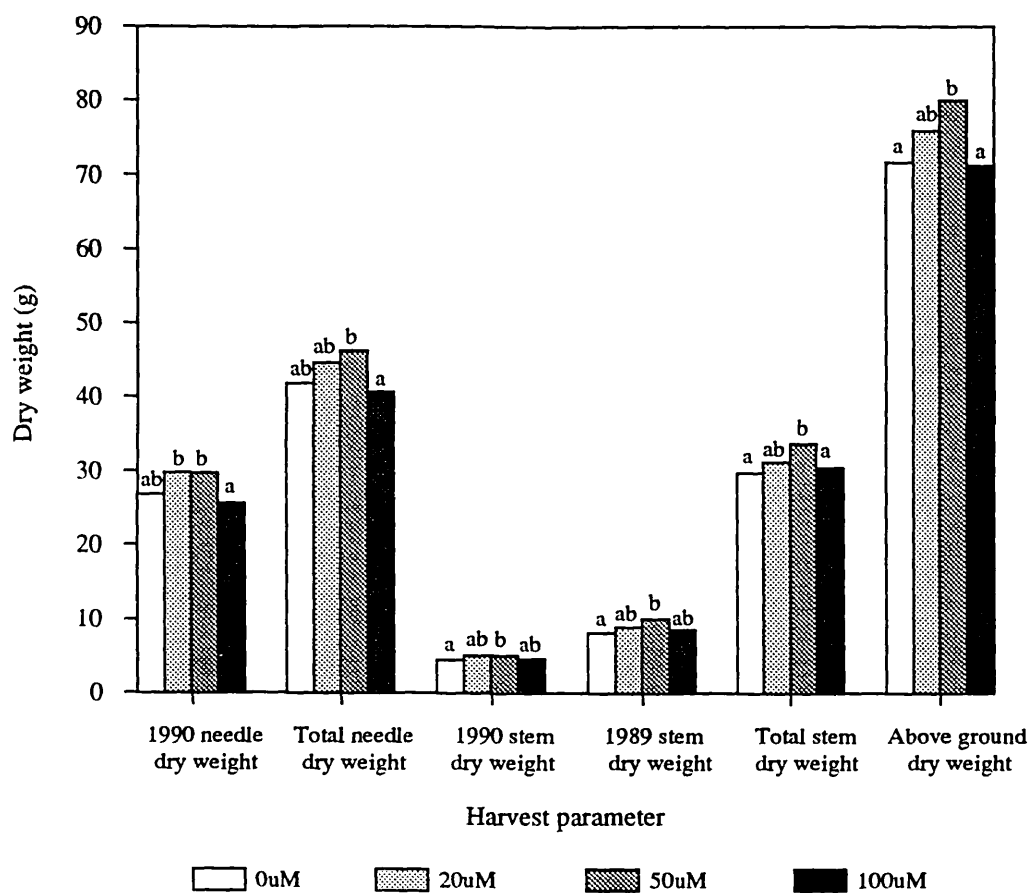
* and ** indicate significant F ratios at $P < 0.05$ and $P < 0.01$ respectively and significant differences between the means are presented in Fig.4.05. All other F ratios are non-significant. These results were obtained using pre-treatment shoot length as a covariate.

The degrees of freedom are:

Treatment (T) $df=3,56$.

Linear (L) and quadratic (Q) $df=1,56$.

Fig.4.05. Effect of H₂O₂ on needle and stem dry weight at the 1990 final harvest



For any one parameter, means with different letters are significantly different at $P < 0.05$.

At both EOS and final harvests, there was no effect of H₂O₂ treatment on soil pH (Table 4.23). Soil pH increased during 1989 for all treatments as seen for oak. Watering with tap water during 1989 may have accounted for the change in pH.

Table 4.23. Sitka spruce soil pH at all harvests.

Harvest date	Mean soil pH			
	0uM	20uM	50uM	100uM
Initial (5/89)	5.30			
EOS (9/89)	7.01	7.00	6.99	6.97
Final (5/90)	6.84	6.86	6.80	6.80

4.4.5 Effects on gas exchange in oak.

For the four gas exchange measurement rounds made during the season, there was no significant effect of H₂O₂ treatment on net photosynthesis (Table 4.24) or on internal CO₂ concentration. However, all H₂O₂ treated trees consistently had higher values for leaf conductance (gl) than the controls (Fig.4.06).

This increase in gl was significant on two separate occasions (Table 4.24). On 20/7/89 (post-misting), a significant linear trend was found in the data so that increasing H₂O₂ concentration led to an increase in gl. For this date gl in the 100uM treatment was 30% greater than in 0 and 50uM treatments (Fig.4.06). A significant linear trend was also found on 31/7/89 (pre-misting), with a significant difference (19%) between control and 100uM means only (Fig.4.06). The means showed the same trend three days later, on 3/8/89 (post-misting), but this was no longer significant (Table 4.24 and Fig.4.06).

The climatic conditions on the four measurement days are given in Table 4.25. Photon flux density was generally high, but for the first 3 dates the ranges were wide.

For the measurements made on the night of 15/9/89, the 100uM treated trees again had a higher gl than the controls, but this was non-significant (Table 4.26).

Table 4.24. Net photosynthesis (P_n) and leaf conductance (gl) in oak in 1989.

Parameter	F ratio	Treatment means (log)			
		0uM	20uM	50uM	100uM
P_n ($\mu\text{molm}^{-2}\text{s}^{-1}$)					
20/7/89	T 0.89 L 0.40 Q 1.82	4.62	4.83	5.45	4.91
31/7/89	T 1.10 L 2.29 Q 0.04	6.17	6.91	6.65	7.26
3/8/89	T 2.06 L 2.35 Q 0.91	3.10	3.97	2.90	4.53
6/9/89	T 0.63 L 0.74 Q 0.41	6/46	7.25	7.04	7.21
gl ($\text{molm}^{-2}\text{s}^{-1}$)					
20/7/89	T 2.33 L 4.54* Q 0.75	0.080	0.095	0.082	0.115
31/7/89	T 2.97* L 7.14** Q 0.18	0.139	0.163	0.157	0.173
3/8/89	T 1.63 L 2.51 Q 2.32	0.136	0.157	0.169	0.161

6/9/89	T 1.54 L 0.21 Q 3.88	0.108	0.115	0.134	0.116
--------	----------------------------	-------	-------	-------	-------

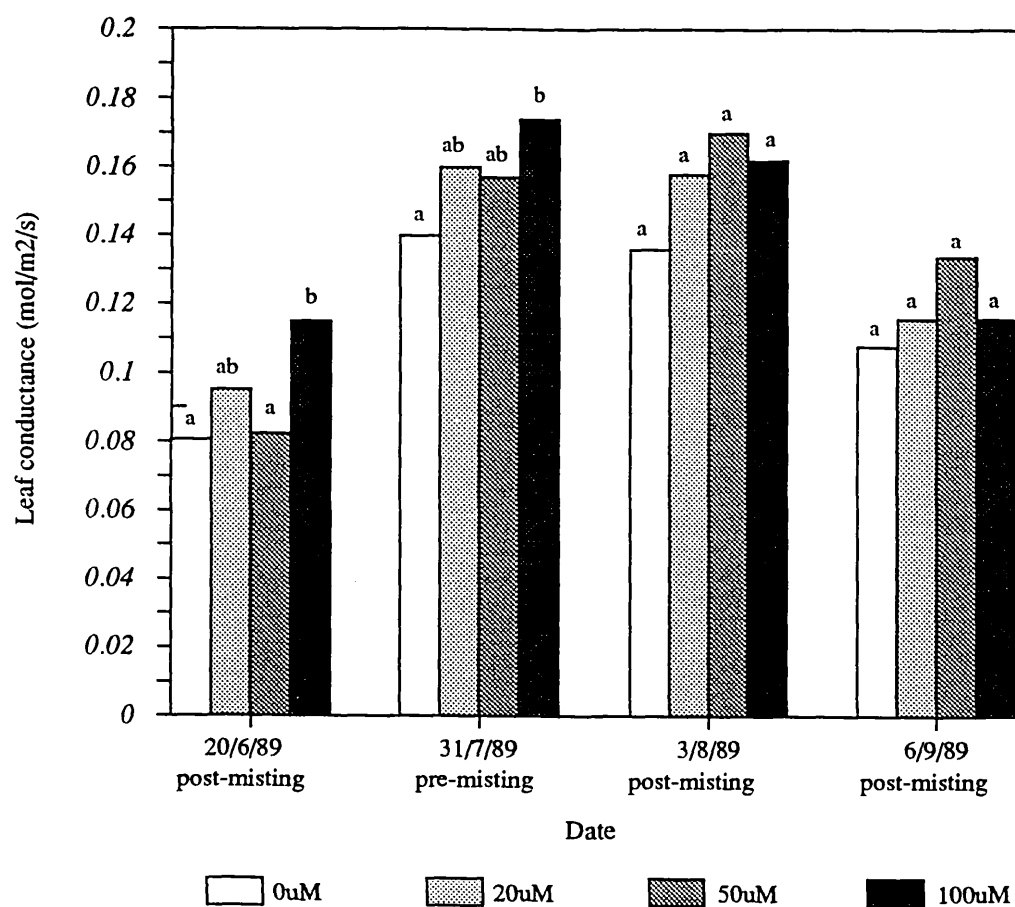
* and ** indicate significant F ratios at $P < 0.05$ and $P < 0.01$ respectively and significant differences between the means are presented in Fig.4.06. All other F ratios are non-significant. The results were obtained using photon flux density (20/7/89 and 6/9/89) and air temperature (31/7/89 and 3/8/89) as covariates. The degrees of freedom are:

Treatment (T) $df=3,71$.

Linear (L) and quadratic (Q) $df=1,71$.

The significant differences between the gl means are shown in Fig.4.06.

Fig.4.06. Effect of H₂O₂ on leaf conductance in oak throughout the 1989 season.



For each date means with different letters are significantly different at $P < 0.05$.

Table 4.25. Photon flux density and air temperature values for the gas exchange measurement days.

Date	Photon flux density ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Temperature °C
20/7/89	1678 500-1950	32.6 30.2-33.8
31/7/89	875 290-1840	22.1 21.0-23.1
3/8/89	883 420-2150	28.5 27.3-30.3
6/9/89	1532 1160-1750	29.8 26.8-31.2

Table 4.26. Leaf conductance in oak on the evening of 15/9/89.

F ratio	Treatment means (mol m ⁻² s ⁻¹)	
Treatment (df=1,35)	0uM	100uM
2.86 ns	0.077	0.090

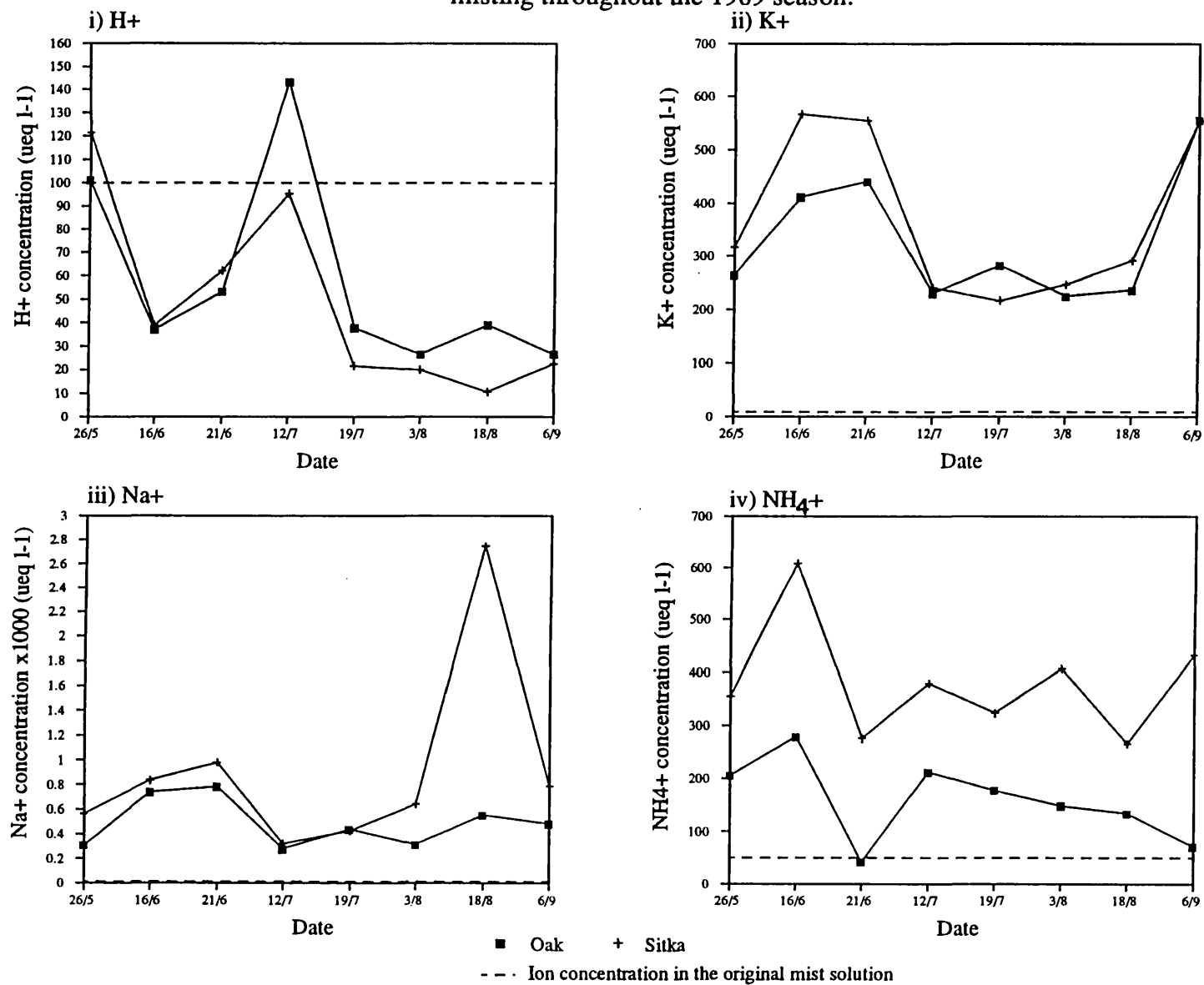
ns denotes non-significance. The means presented have been adjusted for the covariate (air temperature).

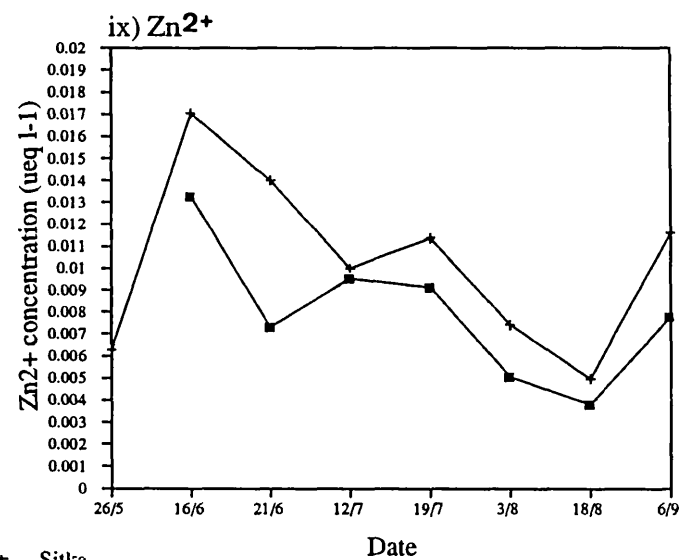
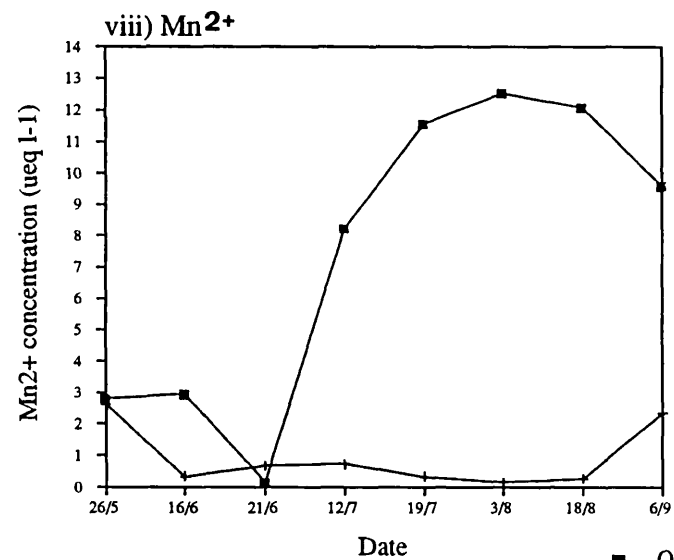
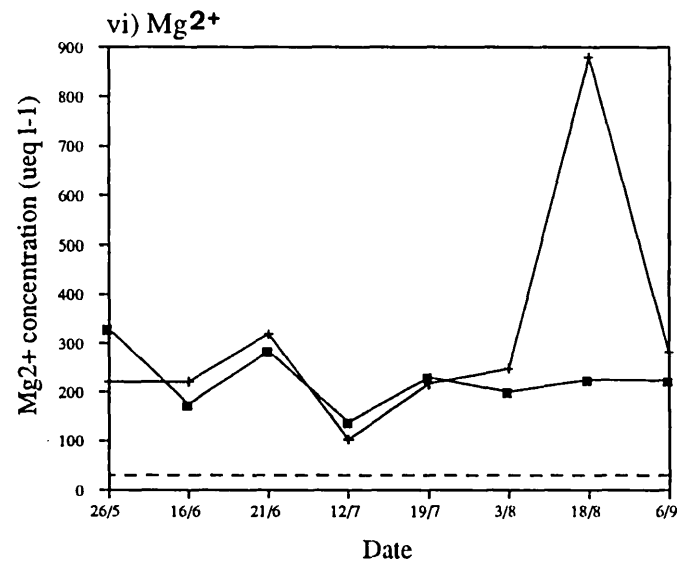
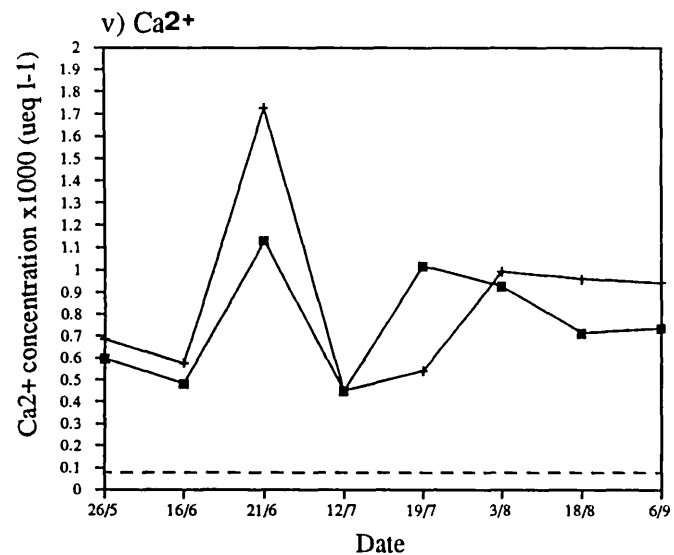
4.4.6 Surface droplet chemistry.

There was very little change in the composition of the mist solution between the start and the end of the misting episode. There were no significant differences between the H₂O₂ treatments or between dates for the ions analysed.

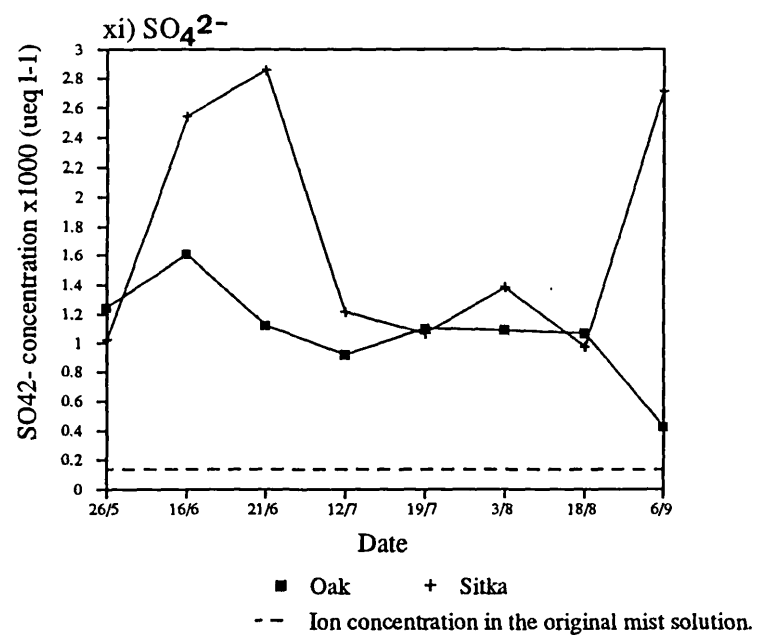
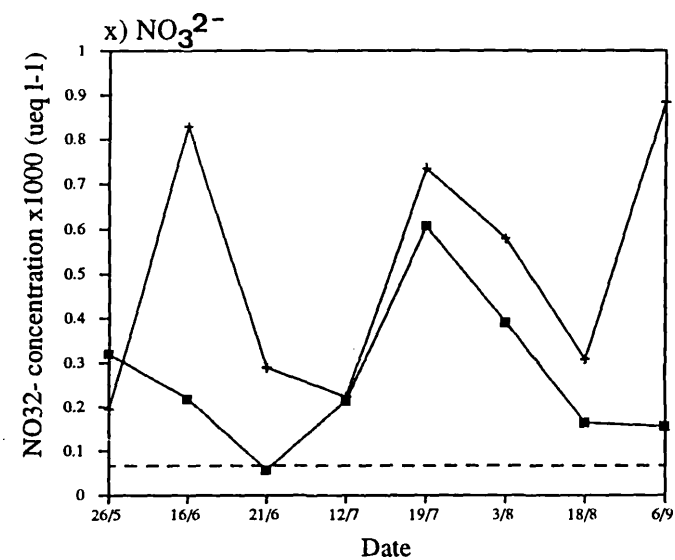
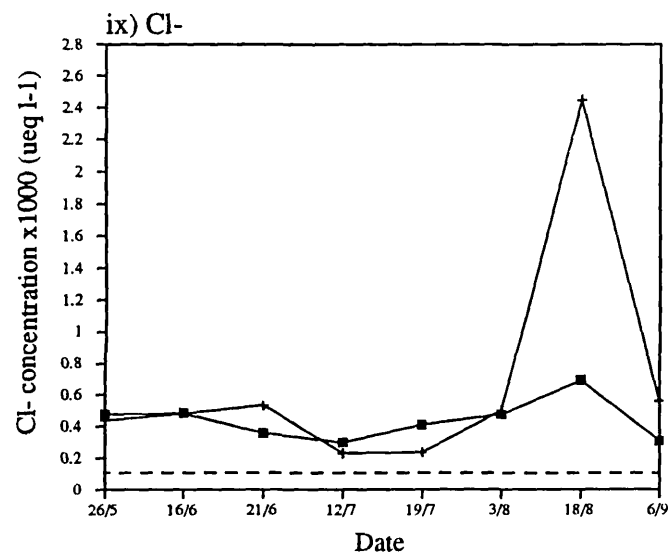
Fig.4.07 i-xi shows the droplet ion concentration (averaged across all treatments) for both species, together with the ion concentration in the original mist solution. H⁺ droplet concentration (Fig.4.07i) was higher at the beginning of the season and generally decreased with time. On two separate occasions early on in the season, 26/5/89 and 12/7/89, the levels of H⁺ in collected droplets were greater than the original mist concentration. H⁺ concentration from the middle to the end of the season was greater in droplets collected from oak than Sitka spruce. K⁺ droplet content (Fig.4.07ii) was high early in the season and increased again at the end of the season. Droplet Na⁺ content (Fig.4.07iii) was reasonably consistent throughout the season, except for a large peak seen for Sitka spruce on 18/8/89, where the concentration was threefold greater than at any other time. Levels of NH₄⁺ in droplets (Fig.4.07iv) varied with time, although the concentration in droplets collected from oak declined during the season. On 21/6/89 in oak, NH₄⁺ droplet content was less than that in the original mist solution. K⁺ droplet content (Fig.4.07iv) was high early in the season and increased again at the end of the season.

Fig.4.07. Ion content of droplets collected from oak and Sitka spruce after misting throughout the 1989 season.





■ Oak + Sitka
 -- Ion concentration in the original mist solution.
 There was no Mn^{2+} and Zn^{2+} in the original mist solution.



Droplet Ca^{2+} content (Fig.4.07v) was fairly constant with time, with a peak on 21/6/89 for both species. Levels of Mg^{2+} in droplets (Fig.4.07vi) showed no obvious change through the season, except for a large peak on 18/8/89 for Sitka spruce, which was approximately three times greater than at any other time. Mn^{2+} droplet concentration (Fig.4.07vii) in Sitka spruce was high at the start and the end of the season. For oak, there was a large increase in Mn^{2+} droplet content from 21/6/89, which was maintained for the remainder of the season. Droplet Zn^{2+} content (Fig.4.07viii) was low, but showed peaks at the start and at the end of the season.

Levels of Cl^- in droplets (Fig.4.07ix) were generally constant through the season, except for the 18/8/89, when in Sitka spruce, the Cl^- concentration was approximately six times greater than at any other time. NO_3^{2-} droplet concentration (Fig.4.07x) in Sitka spruce peaked three times during the season, 16/6/89, 19/7/89 and 6/9/89. Oak droplets showed only one large peak on 19/7/89, but on the 21/6/89, the concentration was less than that in the original mist solution. Droplet SO_4^{2-} content (Fig.4.07xi) in Sitka spruce peaked early in the season and again at the end of the season. SO_4^{2-} levels in oak droplets showed a small peak at the start of the season, but declined with time.

Generally, the concentrations of ions in the collected droplets were much greater than that in the original mist solution. The only exceptions were H^+ and on one occasion, NO_3^{2-} and NH_4^+ in oak on 21/6/89, as discussed above. Levels of NH_4^+ , Mn^{2+} and NO_3^{2-} (Fig.4.07iv,viii,x) were for the most part greater in droplets collected from oak than from Sitka spruce. It was also noted that on 18/8/89, Na^+ , Mg^{2+} and Cl^- concentrations (Fig.4.07iii,vi,ix) in Sitka spruce droplets were almost three-fold greater than on any other occasion.

Analysis of each collection date yielded few significant results throughout the season for both species. The only significant effects of H_2O_2 treatment were for H^+ droplet content for both oak and Sitka spruce, but the effects were seen on different days (Table 4.27). On 21/6/89, H^+ concentration in droplets collected from oak seedlings increased with increasing H_2O_2 concentration, with 100uM droplets having 84% greater H^+ content than the controls (Fig.4.08). For Sitka spruce, the effect of exposure to H_2O_2 on H^+ droplet content was significant on 12/7/89. Levels of H^+ in droplets collected from 20uM, 50uM and 100uM H_2O_2 treated seedlings were significantly greater (110%, 153% and 205% respectively) than the control (Fig.4.08). This increase in H^+ droplet content with increasing H_2O_2 concentration was also seen on 26/5/89, but was non-significant for both species (Table 4.27 and Fig.4.08).

Table 4.27. F ratios for the effect of H₂O₂ concentration on H⁺ droplet content for oak and Sitka spruce.

Date	F ratio (df=3,3)	
	Oak	Sitka spruce
25/6/89	7.48 ns	6.95 ns
21/6/89	23.75 *	6.81 ns
12/7/89	5.81 ns	90.58 **

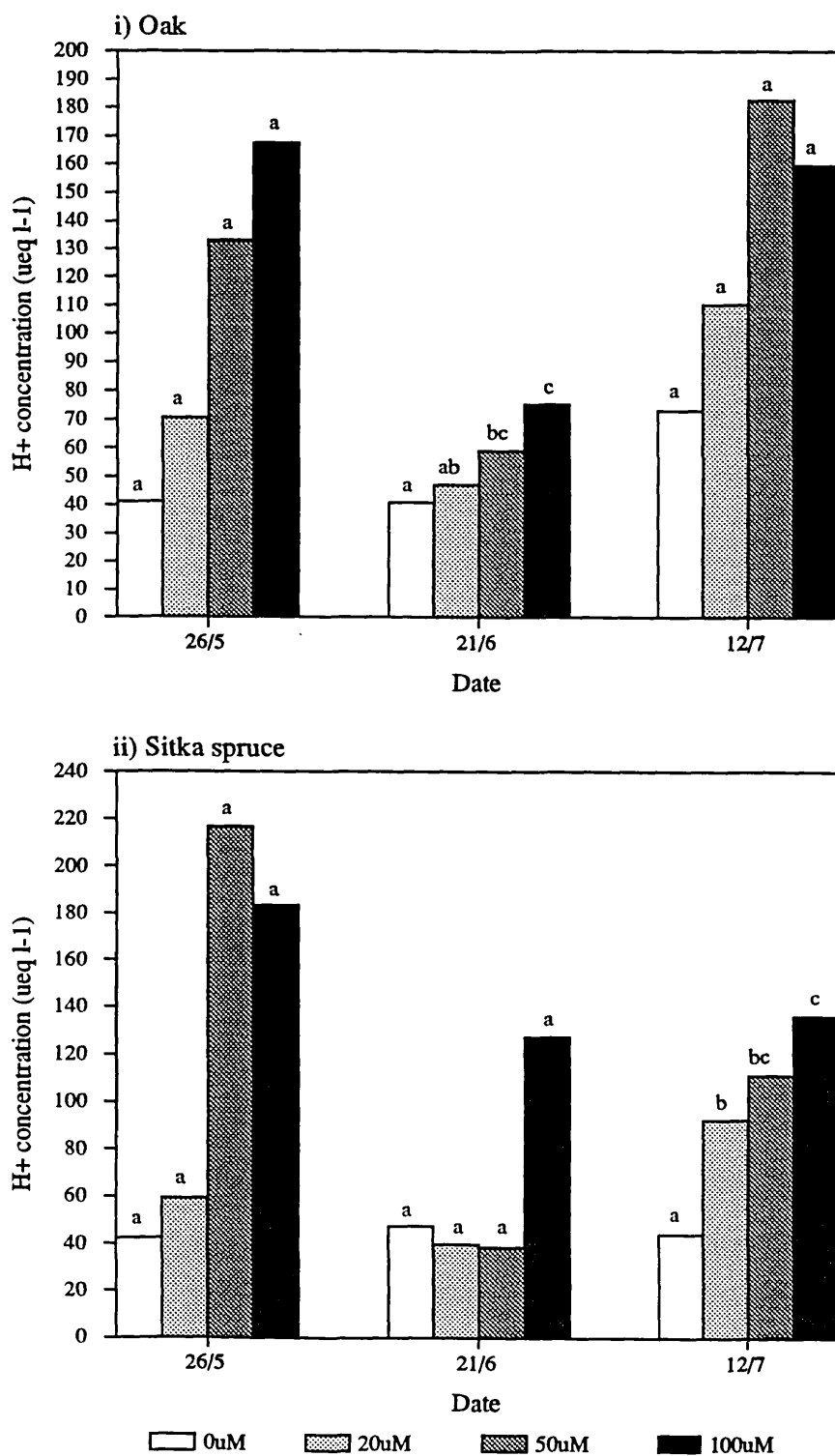
* and ** indicate significant F ratios at P<0.05 and P<0.01 respectively. ns indicates non-significance at P<0.05. The numbers in brackets represent the degrees of freedom for all F ratios.

Table 4.28. Effect of H₂O₂ on combined oak and Sitka spruce H⁺ droplet content.

Date	F ratio			
	Treatment (df=3,8)	Linear (df=1,8)	Species (df=1,8)	Treatment- species (df=3,8)
26/5/89	7.11 *	17.14 **	0.40 ns	0.17 ns
21/6/89	7.34 *	14.71 **	0.49 ns	0.93 ns
12/7/89	13.35 **	24.46 **	4.39 ns	0.69 ns

* and ** indicate significant F ratios at P<0.05 and P<0.01 respectively. ns indicates non-significance. The numbers in brackets represent the degrees of freedom for the appropriate F ratio.

Fig.4.08. Changes in H⁺ droplet content with H₂O₂ concentration for oak and Sitka spruce.



For any one date, means with different letters are significantly different at $P < 0.05$.

On two of these three dates, H^+ droplet content was significantly correlated with only one other ion. On 26/5/89 and 21/6/89, only in droplets collected from Sitka spruce seedlings, H^+ and SO_4^{2-} concentration were significantly positively correlated (Fig.4.09i and ii). The correlation between these two ions was not significant on 12/7/89, but the same trend was observed, of an increase in H_2O_2 concentration leading to an increase in droplet H^+ and SO_4^{2-} concentration (Fig.4.09iii).

When the data from both species were combined, the effect of H_2O_2 on H^+ droplet content was significant on all three dates (Table 4.28). There were also significant linear trends in the data on all three occasions. On 26/5/89, H^+ concentration in 50uM and 100uM treatments was 320% and 169% greater than in the 0uM and 20uM treatments respectively (Fig.4.10i). On 21/6/89, the 100uM droplets had 105-129% greater H^+ than the other treatments and on 12/7/89, levels of H^+ in the 50uM and 100uM treatments were 150% greater than in the control (Fig.4.10i). There were no significant differences between the species or any treatment-species interaction (Table 4.28).

In the analysis of this combined data, H^+ was again positively correlated with SO_4^{2-} , but only on 21/6/89 (Fig.4.10ii).

There was little effect of H_2O_2 treatment on the average ion droplet concentration over the experimental period of oak or Sitka spruce (Table 4.29). Exposure to H_2O_2 significantly increased the Zn^{2+} content of oak droplets and the H^+ content of Sitka spruce droplets (Table 4.29). There were no other significant effects of H_2O_2 treatment.

In oak, seedlings treated with 100uM H_2O_2 had on average significantly greater Zn^{2+} (51%) in droplets than control seedlings (Table 4.30). H^+ droplet content in Sitka spruce (Table 4.30) exposed to 50uM and 100uM H_2O_2 was significantly greater than in control (124% and 159%, respectively) and 20uM seedlings (75% and 96%, respectively).

This mean H^+ droplet concentration in Sitka spruce was significantly positively correlated with SO_4^{2-} concentration (Fig.4.11). Zn^{2+} concentration in oak was not significantly correlated with that of any ion.

Cation:anion ratios were calculated from these average concentrations and for both species all ratios were approximately 1:1 (Table 4.31).

The enrichment factors for the ions in collected droplets relative to the concentration in the original mist solution were also calculated from these mean concentrations for both species (Table 4.32). All ions except H^+ were found at a greater concentration in the droplets and H^+

Fig.4.09. Regression analysis of H^+ and SO_4^{2-} droplet content for Sitka spruce on three dates in 1989.

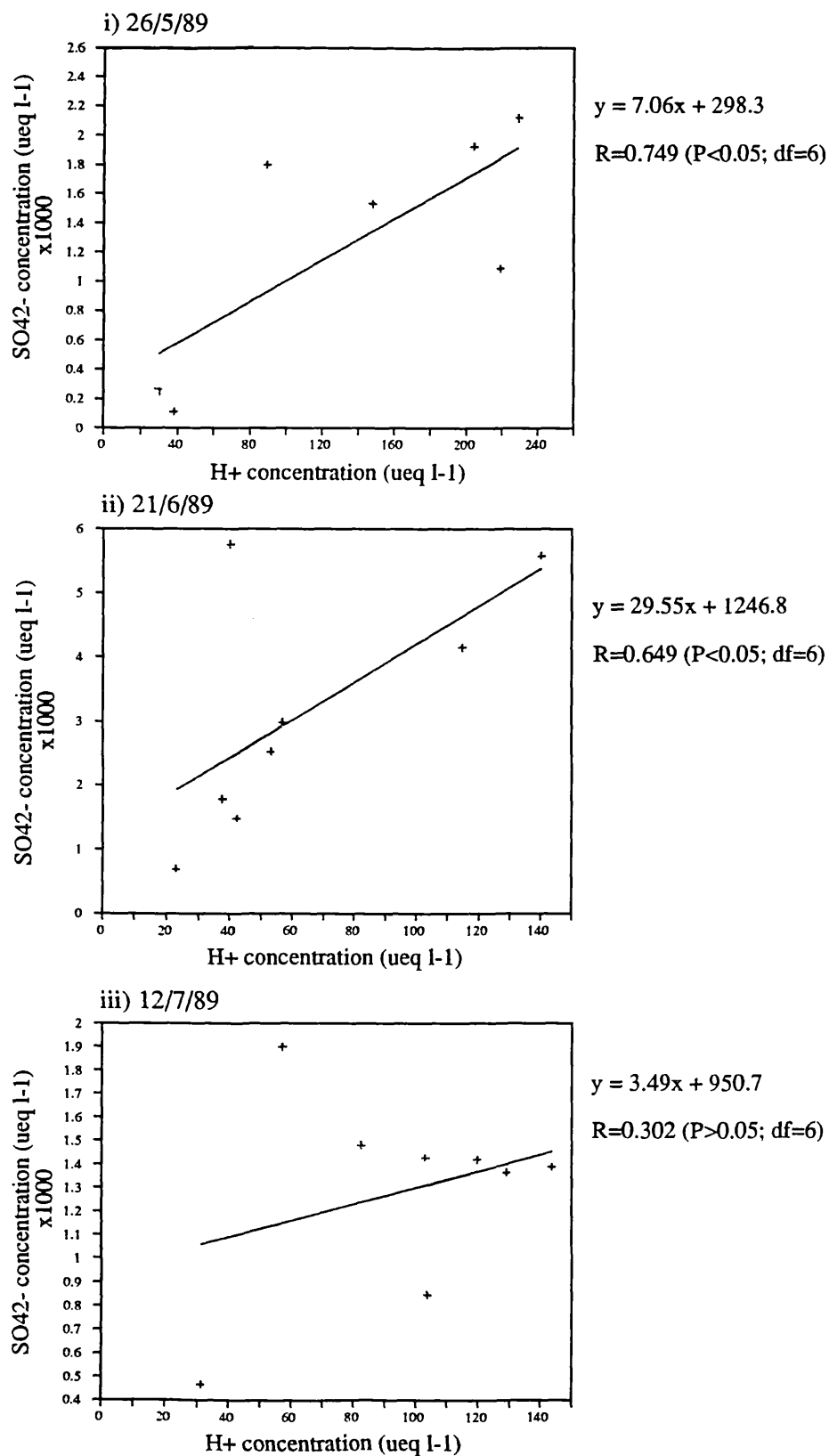
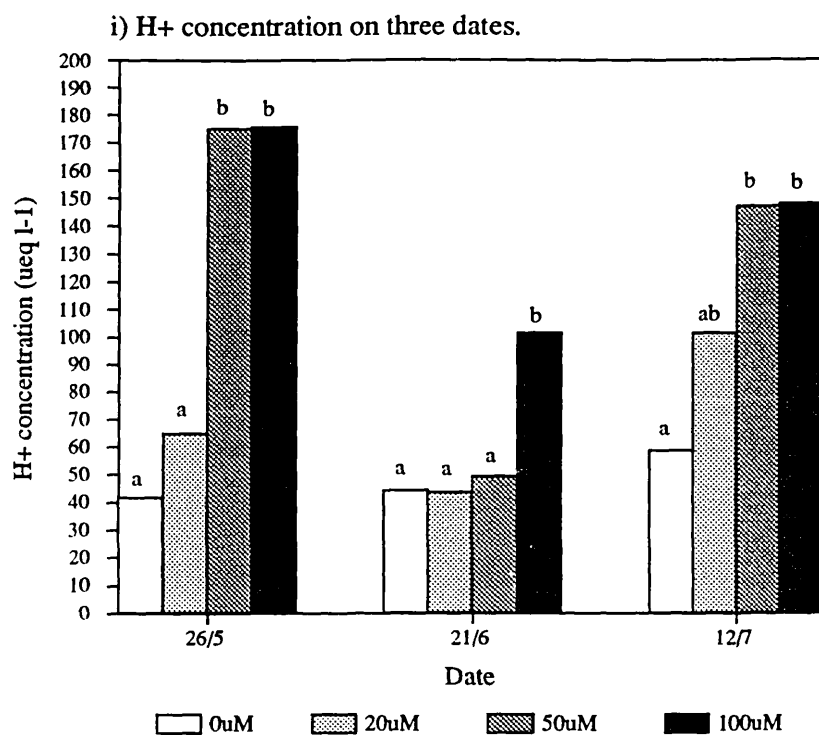


Fig.4.10. H⁺ droplet content for combined oak and Sitka spruce data.



For any one date, means with different letters are significantly different at $P < 0.05$.

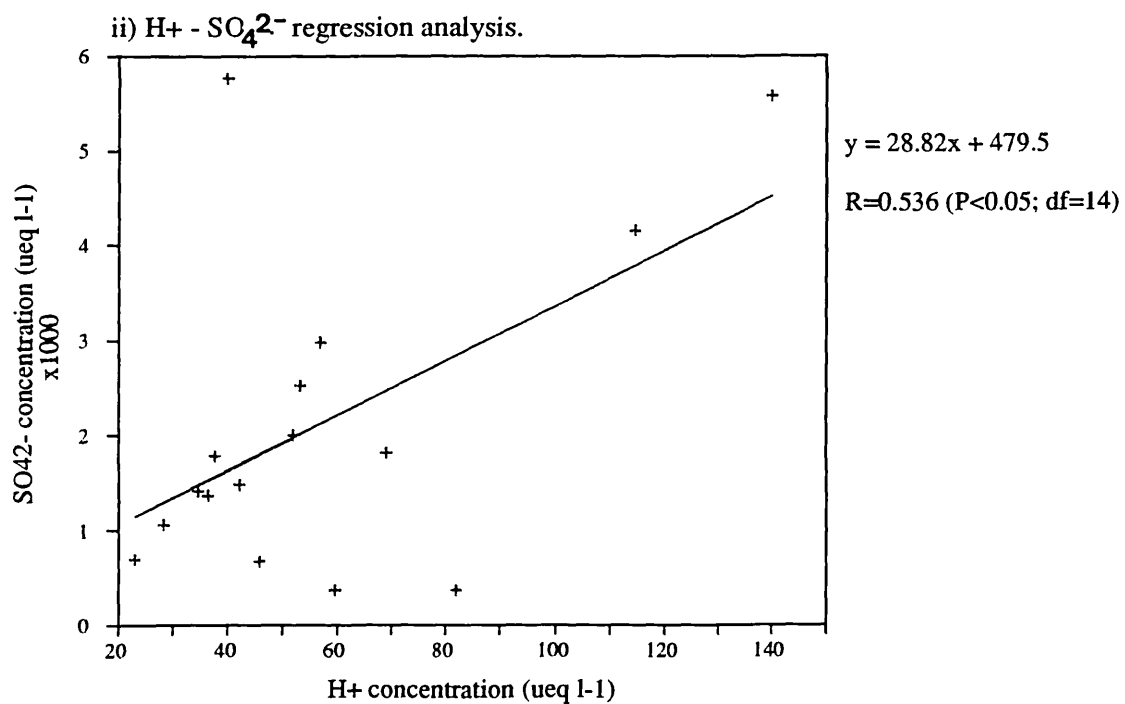


Table 4.29. F ratios for the effect of H₂O₂ on mean droplet concentration in oak and Sitka spruce.

Ion	F ratio (df=3,4)	
	Oak	Sitka spruce
H ⁺	5.22	12.44 *
Na ⁺	0.31	0.56
NH ₄ ⁺	0.73	1.28
K ⁺	0.70	0.07
Ca ²⁺	1.91	0.52
Mg ²⁺	2.19	0.99
Mn ²⁺	0.38	0.57
Zn ²⁺	12.85 *	1.42
Cl ⁻	2.82	5.48
NO ₃ ²⁻	0.56	0.21
SO ₄ ²⁻	0.50	1.28

* indicates a significant F ratio at P<0.05. All other F ratios are non-significant.

Table 4.30. Effect of H₂O₂ treatment on mean droplet concentrations of Zn²⁺ in oak and H⁺ in Sitka spruce.

Ion	Ion concentration (ueq l ⁻¹)			
	0uM	20uM	50uM	100uM
Zn ²⁺ Oak	0.0061 a	0.0079 bc	0.0064 ac	0.0092 b
H ⁺ Sitka spruce	30.02 a	38.44 a	67.30 b	75.44 b

For each ion the means with different letters are significantly different at P<0.05.

Fig.4.11. Regression analysis of mean H⁺ and SO₄²⁻ droplet content for Sitka spruce collected throughout the 1989 season.

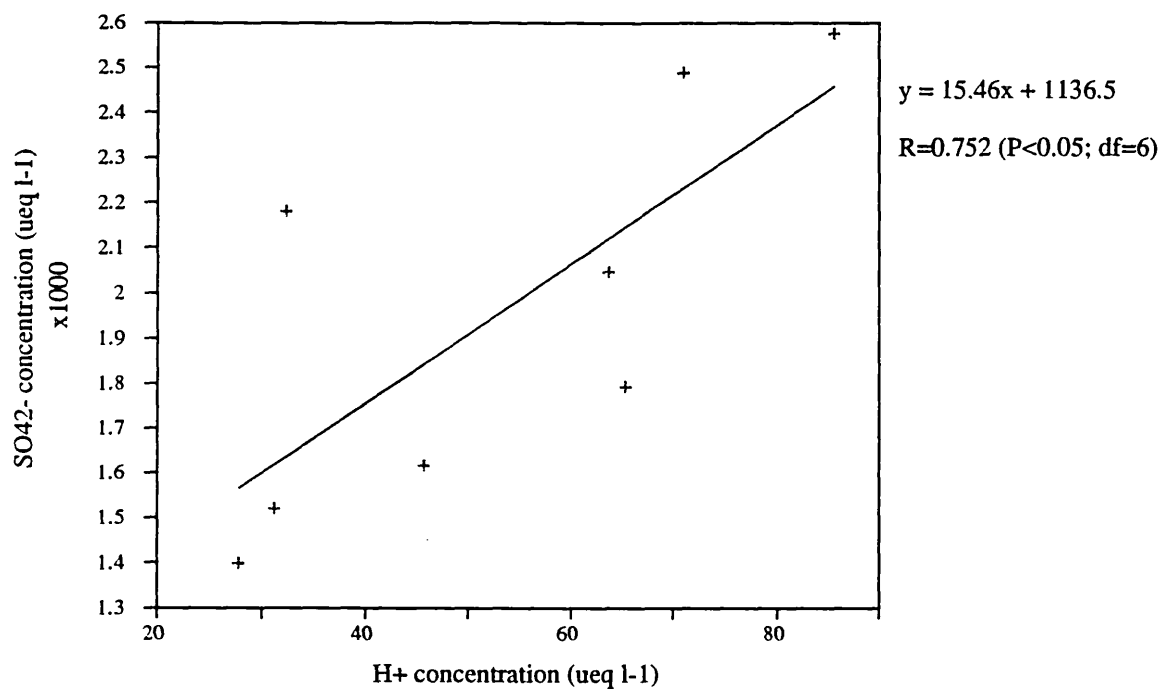


Table 4.31. Cation:anion ratios in surface droplets collected from oak and Sitka spruce.

Species	Cation:anion ratio			
	0uM	20uM	50uM	100uM
Oak	1.03	1.11	1.10	1.14
Sitka spruce	0.99	1.03	0.99	1.00

Table 4.32. Enrichment factors for ions in collected droplets for oak and Sitka spruce.

Ion	Enrichment factor [#]	
	Oak	Sitka spruce
H ⁺	0.56	0.53
Na ⁺	20	35
NH ₄ ⁺	4	7
K ⁺	35	38
Ca ²⁺	10	12
Mg ²⁺	9	12
Cl ⁻	5	8
NO ₃ ²⁻	4	7
SO ₄ ²⁻	8	14

[#] the enrichment factors were calculated relative to the ion concentration in the original mist solution.

droplet concentration was approximately halved in both species. Na^+ and K^+ showed greatest enrichment and the enrichment of all ions (except H^+) was greater in droplets collected from Sitka spruce than from oak.

4.4.7 Nutrient analysis of 1989 and 1990 harvest leaves and needles.

4.4.7.1 1989 EOS harvest oak leaves.

Generally, there was a lower concentration of ions, except for K^+ , in 2F leaves when compared with 1F leaves. This was true for all treatments. There were no significant effects of H_2O_2 treatment on the nutrient status of 1F or 2F leaves for any element, Table 4.33. The apparent linear decrease in Mn concentration and the increase in Zn content with increasing H_2O_2 in 2F leaves, were non-significant.

4.4.7.2 EOS and final harvest Sitka spruce needles.

For Sitka spruce at the EOS harvest, there was a higher concentration of Mg^{2+} and K^+ in 1989 needles than in 1988 needles. For Ca^{2+} and Mn^{2+} the situation was reversed, with higher levels in 1988 needles (Table 4.34).

The only significant effect of H_2O_2 treatment on the nutrient status of these seedlings was on the Zn^{2+} content of 1988 needles, so that with an increased H_2O_2 concentration, there was an increase in Zn^{2+} concentration. A significant treatment effect was found in the data ($F = 4.413$; $df=3,15$) with the 0uM and 20uM treated needles having 50-58% less Zn^{2+} than the 50uM and 100uM treatments (Table 4.34). This increase in Zn^{2+} needle content was also seen in 1989 needles (Table 4.34) but this was non-significant.

At the final harvest there was a lower concentration of ions in 1990 needles for all elements other than K^+ , when compared to 1989 and 1988 needles. This was true of all treatments (Table 4.35).

The only significant effect of H_2O_2 treatment was again on Zn^{2+} content. An increase in H_2O_2 concentration led to a significant decrease in needle Zn^{2+} content for both 1989 and 1990 year classes (Table 4.35). There was a significant treatment effect in both cases ($F=3.261$ for 1989; $F=4.17$ for 1990; $df=3,16$), with LSD analysis indicating a significant difference between the control and the means of all H_2O_2 -treated seedlings (Table 4.35). In 1989 needles, the H_2O_2 treatments had 57-75% less Zn^{2+} than the controls, while in 1990 needles there was 69-80% less Zn^{2+} . The apparent increase in Mn^{2+} concentration with increasing H_2O_2 concentration for both 1990 and 1988 needles was non-significant.

Table 4.33. Nutrient content of 1F and 2F oak leaves at the 1989 EOS harvest.

H ₂ O ₂ treatment	Ion concentration				
(uM)	Ca ²⁺ mg g ⁻¹	Mg ²⁺ mg g ⁻¹	K ⁺ mg g ⁻¹	Mn ²⁺ ug g ⁻¹	Zn ²⁺ ug g ⁻¹
1F: 0	9.16	4.48	9.83	436	187
20	7.36	4.29	10.05	415	253
50	8.26	3.71	9.48	464	196
100	9.21	4.63	8.79	480	120
2F: 0	7.53	3.46	12.79	252	90
20	7.24	3.53	13.69	181	115
50	6.64	2.97	13.94	146	110
100	6.53	3.41	14.22	112	247

For any one ion, there were no significant differences between the means at $P < 0.05$.

Table 4.34. Nutrient content of 1988 and 1989 Sitka spruce needles at the 1989 EOS harvest.

Treatment	Concentration				
	Ca ²⁺ mg g ⁻¹	Mg ²⁺ mg g ⁻¹	K ⁺ mg g ⁻¹	Mn ²⁺ ug g ⁻¹	Zn ²⁺ ug g ⁻¹
1989: 0	7.40	2.61	10.64	98	9.10
20	8.59	3.01	10.43	133	25.60
50	6.37	2.15	9.94	90	30.25
100	6.90	2.31	10.95	107	30.75
1988: 0	7.40	1.42	7.76	202	19.75a
20	10.41	1.82	8.06	239	17.25a
50	7.27	1.42	8.79	203	39.50b
100	8.00	1.21	8.39	155	40.75b

For any one ion there were no significant differences between the means, except for 1988 needle Zn²⁺, where means with different letters are significantly different at P<0.05.

Table 4.35. Nutrient content of 1988,1989 and 1990 Sitka spruce needles at 1990 final harvest.

Treatment	Concentration				
	Ca ²⁺ mg g ⁻¹	Mg ²⁺ mg g ⁻¹	K ⁺ mg g ⁻¹	Mn ²⁺ ug g ⁻¹	Zn ²⁺ ug g ⁻¹
1990: 0	2.49	1.55	14.03	36	43.0b
20	3.28	1.65	15.25	57	9.0a
50	3.68	1.79	14.60	58	8.5a
100	2.85	1.85	13.28	67	13.5a
1989: 0	13.58	2.69	6.26	123	28.0b
20	14.83	3.13	4.39	131	10.0a
50	15.90	2.75	6.37	133	7.0a
100	15.05	2.74	5.78	123	12.0a
1988: 0	13.53	1.99	5.03	153	33.0
20	13.69	2.28	3.53	186	21.5
50	15.90	2.44	6.14	213	-
100	14.17	1.99	5.04	236	29.5

- represents a missing value. For any one ion there were no significant differences between the means, except for 1989 and 1990 needle Zn²⁺. In these cases, means with different letters are significantly different at P<0.05.

When the values obtained at both harvests were compared, it was seen that for 1988 and 1989 needles, there was less Ca^{2+} and Mg^{2+} in both year classes at the EOS harvest, but higher levels of K^{+} . For Mn^{2+} and Zn^{2+} , the situation was more variable. This was especially true for Zn^{2+} , where the trend for an increase in Zn^{2+} concentration with increasing H_2O_2 concentration seen at the EOS harvest was reversed at the final harvest, where an increase in H_2O_2 concentration led to a decrease in Zn^{2+} needle content.

4.4.8 Wax content of Sitka spruce needles at the 1989 EOS harvest.

There was no significant effect of H_2O_2 treatment on the wax content of 1988 or 1989 needles, expressed on % needle dry weight basis. However, for both year classes, needle wax content in the 50uM and 100uM treatments was less than in the 0uM and 20uM treatments (Table 4.36). When the data from both year classes were combined, there were again no significant effects of exposure to H_2O_2 (Table 4.36).

Table 4.36. The effect of H_2O_2 concentration on the wax content of Sitka spruce needles.

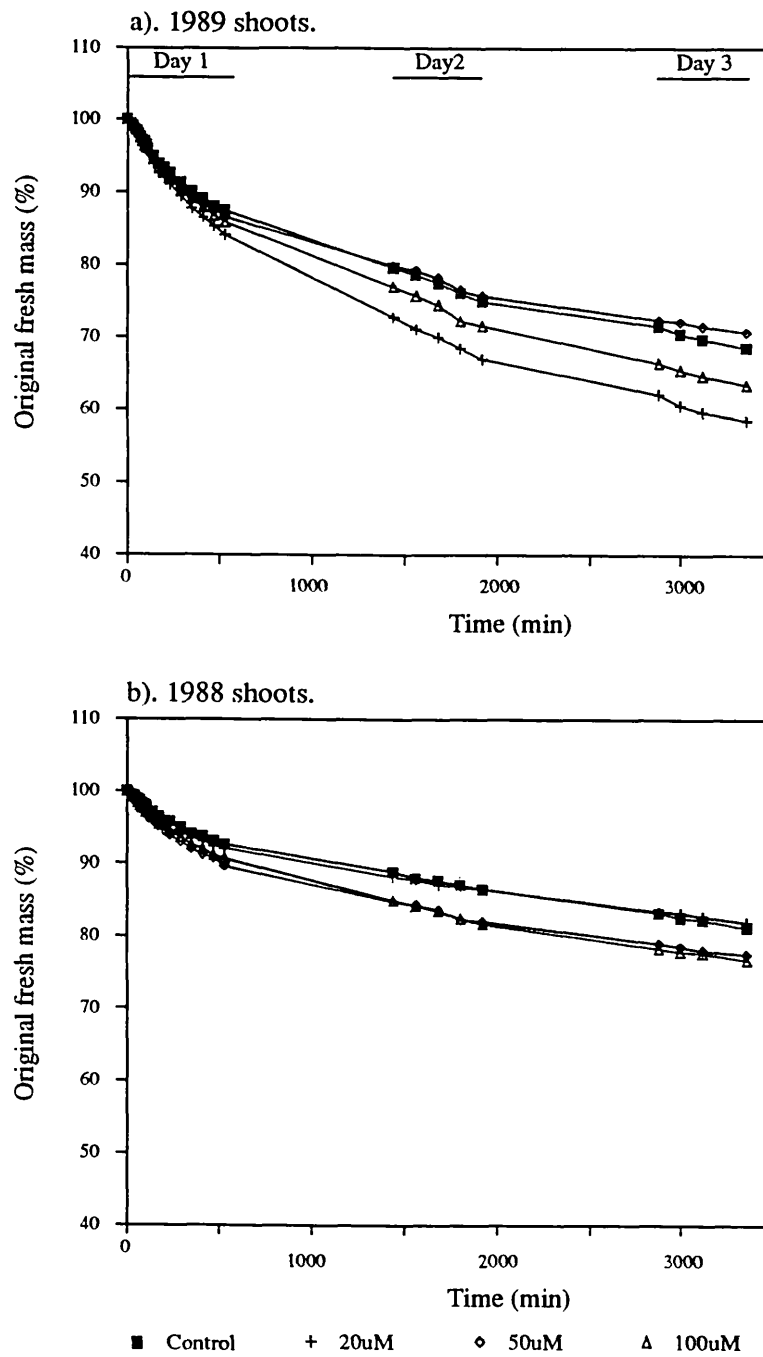
Year class	Wax content (% needle dry weight)			
	0uM	20uM	50uM	100uM
1989 needles	1.94	1.90	1.75	1.64
1988 needles	2.11	2.17	1.49	1.73
1988 + 1989	2.02	2.04	1.62	1.69

There was no significant difference between the means at $P < 0.05$.

4.4.9 Water loss in Sitka spruce at the 1989 EOS harvest.

Fig.4.12 shows the series of drying curves constructed for the change in weight (as a percentage of the initial weight) for the excised Sitka spruce shoots. The change in weight and therefore estimated water lost by the shoot, was greater in 1989 shoots (Fig.4.12i) than in 1988 shoots (Fig.4.12ii), for all treatments. This difference became obvious some three hours after excision. The high initial rate of water loss seen in Fig.4.12 was thought to be a

Fig.4.12. The change in weight with time of excised Sitka spruce shoots after exposure to H₂O₂.



combination of both stomatal and cuticular transpiration, the then steady fall was probably the result of cuticular transpiration alone or combined with incomplete stomatal closure (uncontrolled water loss).

The results from the ANOVA of the mean rate of water loss per day for each day of the three day experiment suggested no significant effect of exposure to H_2O_2 (Table 4.37). Similarly there were no linear or quadratic trends between H_2O_2 treatment and plant response.

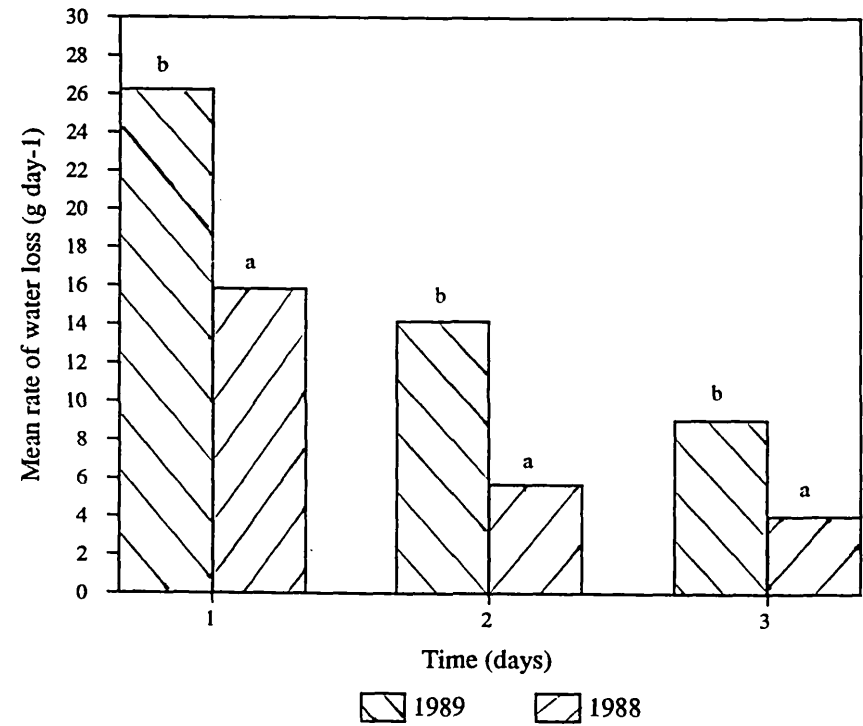
However, on each day there was a significant difference between the two year classes, with the 1989 shoots showing a significantly greater rate of water loss than 1988 shoots (Table 4.37 and Fig.4.13). The rate of water loss for 1989 shoots (all treatments) was 65% greater than 1988 shoots during day 1. This difference increased during day 2 and day 3 to 149% and 123% respectively. There were no H_2O_2 treatment-year class interactions (Table 4.37).

Table 4.37. F ratios for the mean rate of water loss per day for excised Sitka spruce shoots.

Day	F ratio				
	Treatment (df=3,32)	Linear (df=1,32)	Quadratic (df=1,32)	Year class (df=1,32)	Treatment- year class (df=3,32)
1	1.35	1.42	0.71	51.20 ***	1.15
2	0.49	0.41	0.37	18.31 ***	0.69
3	2.61	0.32	2.97	23.27 ***	2.47

*** indicates a significant F ratio at $P < 0.001$. All other F ratios are non-significant. The numbers in brackets represent the degrees of freedom for the associated F ratio.

Fig.4.13. Effect of year class on the mean rate of water loss from excised Sitka spruce shoots



For any one day, means with different letters are significantly different at $P < 0.05$.

4.5 DISCUSSION.

4.5.1 Effects on growth in oak.

No visible symptoms of foliar injury as a result of H_2O_2 treatment were seen at any time during the 1989 season. This lack of visible damage was consistent with the findings of other experiments involving the exposure of tree seedlings to H_2O_2 (Mallant *et al*, 1986, 1988; Masuch *et al*, 1986, 1989; Hanson and McLaughlin, 1989).

The health of oak leaves throughout the 1989 season, assessed for the most part by the incidence of mildew, was unrelated to treatment. The results of the χ^2 analysis suggested no significant difference in health between treatments. The assessment of the overall level of disease, using the arbitrary scale developed, indicated that on average, up to 50% of leaves on each tree showed some degree of infection. The scoring system did not take into account the area of the leaf showing disease symptoms. All seedlings were infected but no one tree was badly affected.

An investigation of the powdery mildew infection of *Quercus robur* leaves by Hewitt and Ayres (1976) showed that levels of total leaf carbohydrate varies as first the rate of net photosynthesis declined and then an abnormal pattern of translocation was established. Infected *Q. robur* leaves exported a smaller proportion of their photosynthate than did healthy leaves and imported a greater proportion of the photosynthate of healthy leaves on the same plant than did healthy leaves on healthy plants. Measurements of net photosynthesis in this study were made on healthy oak (*Q. petraea*) leaves that showed no signs of mildew infection and were not significantly affected by H_2O_2 treatment. It was possible that the photosynthetic rate of diseased leaves may have been more susceptible to the combined effects of H_2O_2 and mildew.

However, the results suggested that exposure to H_2O_2 did not alter the level of mildew infection in the season of treatment and there was an indication that there was a small reduction in the incidence of the disease with time. The scoring system used to assess the level of disease was possibly not sufficiently sensitive to detect subtle differences in the degree of infection e.g. differences in the percentage area of leaf infected or differences between flushes. The seedlings were maintained for only a few weeks in the subsequent season and the lack of any disease outbreak in this time, meant that no assessment of disease susceptibility in the season following treatment was possible.

The fungicide used to combat the mildew outbreaks was Benlate in which the active agent is Benomyl. Benomyl has been shown to provide protection against ozone (O_3) injury in grapevines, *Vitis labrusca* (Kender *et al*, 1973) and in the turfgrasses, *Poa annua*, *P. pratensis* and *Agrostis palustris* (Papple and Omrod, 1977). It is not known whether benomyl would provide protection against other oxidants like H_2O_2 .

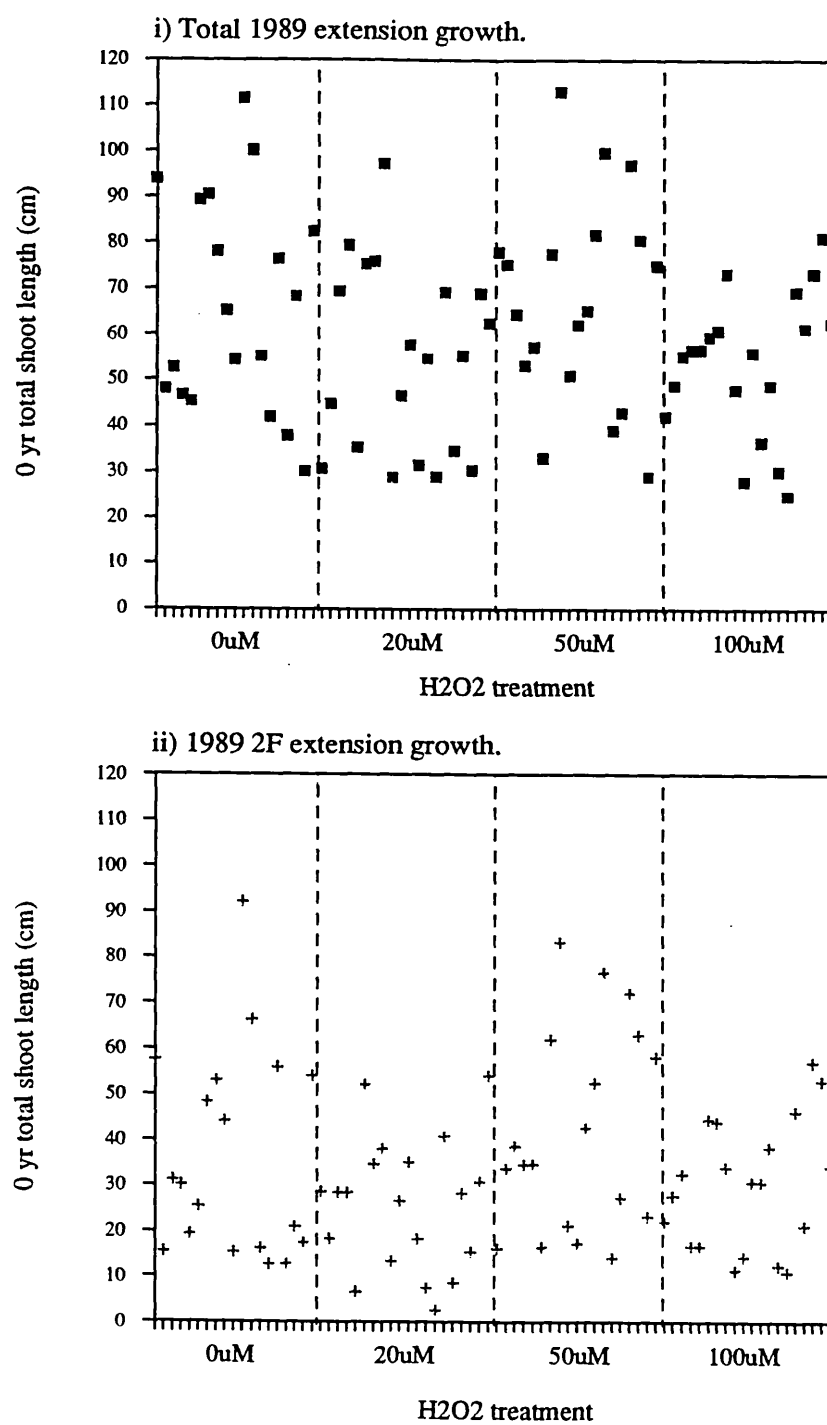
There were no H_2O_2 treatment differences in height, basal diameter or leaf number in 1989 or 1990. However, 1989 extension growth was affected by exposure to H_2O_2 . A significant treatment effect was seen on 28/7/89 after 72h of misting and continued to be significant for the remainder of the season. Seedlings exposed to 0uM and 50uM H_2O_2 had achieved greater shoot length than the 20uM and 100uM treated seedlings. When each individual flush was considered, only 2F shoot length was significantly affected, with the 20uM seedlings achieving less growth than 0uM and 50uM treatments. This effect was seen in 1989 for all seedlings and for those maintained until the 1990 final harvest. The pattern for 3F extension growth was in contrast to that seen at the first two flushes, with the 20uM seedlings, in this case, achieving greatest growth. However, the contribution of 3F growth to total extension growth was negligible when compared to 1F and 2F.

The potential for growth would have been determined in the previous year. Budburst in 1989 commenced in April, so that by the first mist application on 24/5/89, all 1F leaves were almost fully developed but 1F extension growth was not complete. It was likely that the treatments started too late to have any great influence on 1F shoot growth. However, the growth of further flushes could have been affected by H_2O_2 treatment.

Mallant *et al* (1986) and Masuch *et al* (1986) suggested that developing tissue in the form of current year Norway spruce (*Picea abies*) needles and young beech (*Fagus sylvatica*) leaves were more susceptible to the adverse effects of a 118uM H_2O_2 mist than mature tissue. There are also a number of experiments suggesting greater sensitivity of developing tissues than mature tissue to acid rain, without H_2O_2 (Wood and Bormann, 1974; Haines *et al*, 1980; Forsline *et al*, 1983). In this study only growth achieved in the season of treatment showed any response to H_2O_2 , but the response was variable.

The poor growth of the 20uM seedlings is somewhat puzzling. The results obtained for the 0uM and 50uM seedlings showed no significant difference between these two treatments, so that the negative response at 20uM H_2O_2 may have been an artefact. Fig. 4.14 shows the measurements of extension growth made at the end of the 1989 season. The poor 2F extension growth in the 20uM treatment was especially noticeable, with four trees achieving less 2F growth than the minimum in the other three treatments. However, it should also be

Fig.4.14. Extension growth in oak at the end of the 1989 season.



noted that there were seedlings in the 0uM and 50uM treatment which produced large 2F growth. A test for statistical outliers (after Sokal and Rohlf, 1981) on all seedlings showed that no one tree from any of the treatments was a significant outlier.

These results for 1989 extension growth were not translated into significant effects on the dry weight parameters measured at harvest. At both 1989 EOS and 1990 final harvests there were no significant effects of H₂O₂ treatment on any of the parameters measured. This was in contrast to the results obtained by Hanson and McLaughlin (1989) for red spruce (*Picea rubens*), where exposure to 235uM H₂O₂ for 9h three times a week for 16 weeks significantly increased root and current year shoot dry weight when compared to 0uM and 15uM treatments.

There are a number of reports of species differences in response to acidity (without H₂O₂) and gaseous pollutants (Lee and Weber, 1979; Haines *et al*, 1980; Percy, 1986; Ashenden and Bell, 1988; Ashmore *et al*, 1988, 1989) and this may be sufficient to explain the differences between the oaks in this study and the red spruce of Hanson and McLaughlin (1989). However, factors such as growing conditions, mist composition and frequency of application may also have contributed to the differences.

Autumn 1989 leaf loss was unaffected by treatment, so that exposure to H₂O₂ did not lead to early leaf senescence. Similarly, there was no effect of H₂O₂ on spring budburst in 1990. No buds failed to burst and all seedlings developed at the same rate. There are no other reports of H₂O₂ effects on these parameters.

4.5.2 Effects on growth in Sitka spruce.

As for oak, no foliar damage as a result of H₂O₂ treatment was seen. This was again consistent with the results from other work involving exposure of tree seedlings to H₂O₂ (Mallant *et al*, 1986, 1988; Masuch *et al*, 1986, 1989; Hanson and McLaughlin, 1989).

Generally, the Sitka spruce seedlings were not subjected to any pests or diseases during the exposure period from the end of May to September 1989. In April and May 1989 there were aphid (*Elatobium abietinum*) infestations, but these were treated with insecticide before H₂O₂ exposure began. Assessment of the health of the seedlings, based on the degree of infestation at these times, had showed no significant difference between the seedlings assigned to each treatment group. There were no further outbreaks during the season, so that in general the health of the seedlings (with respect to aphid damage) was good and there were no significant differences between the H₂O₂ treatments.

Measurements of height and basal diameter in Sitka spruce in 1989 or 1990 were not significantly affected by exposure to H_2O_2 . Hanson and McLaughlin (1989) also reported no effects of a range of H_2O_2 concentrations (up to 235uM) on height and basal diameter in red spruce (*Picea rubens*). Extension growth of Sitka spruce during the 1989 season and at both harvests showed no significant effect of H_2O_2 .

It was apparent from the results at both 1989 EOS and 1990 final harvests that 100uM H_2O_2 had adverse effects on needle growth. The significant decrease in 1989 needle dry weight seen at the EOS harvest (with respect to the control) was continued in 1990 needle dry weight (this time compared to 50uM treatment). However, for the seedlings maintained until the 1990 final harvest, 1989 needle dry weight was unaffected by H_2O_2 treatment. Needle area (of 50 needles), specific needle area and mean needle weight were not significantly affected by H_2O_2 exposure at either harvest.

It was possible that there was a reduction in the number of needles produced in the 100uM seedlings. However, at the 1989 EOS harvest, 1989 extension growth in the 100uM treatment was 18% less than that achieved by the control seedlings. Although this was non-significant, it may have contributed to the decrease in 1989 needle dry weight in the 100uM treatment (by 29% compared with the control). At the 1990 final harvest, there was a 14% reduction in needle dry weight in the 100uM seedlings compared with the 50uM treatment, whereas the decrease in 1990 shoot length was 16%.

There are other reports of H_2O_2 having an effect on needle growth. Mallant *et al* (1986) and Masuch *et al* (1986) saw decreases in the dry weight of current year needles of Norway spruce (*Picea abies*) after exposure to 118uM H_2O_2 for 3h daily for six weeks. Masuch *et al* (1989) observed a reduction in the length and volume of current year needles of the same species exposed to 25uM H_2O_2 for 3h, four times a week for 8 weeks. In this study with Sitka spruce at approximately the same concentration of 20uM, there was no significant effect of H_2O_2 on needle dry weight. Hanson and McLaughlin (1989) saw no effects of H_2O_2 (up to 235uM) on the dry weight of red spruce needles.

In this study at the final harvest, quadratic responses were seen in the data for 1989, 1990 and total stem dry weight, with the 50uM treatment significantly greater than the control. Stem weight per unit length of stem (mean stem weight) was not significantly affected. Not surprisingly, the response of total above ground dry weight to H_2O_2 was also quadratic, with the 50uM treatment being significantly greater than both 0uM and 100uM. These parameters

had shown no significant response to H_2O_2 at the EOS harvest. Root:shoot ratio (RSR) showed no effect of H_2O_2 treatment at both harvests. Therefore, H_2O_2 had not altered the allocation of dry matter between roots and shoots.

In the only other report of the effects of H_2O_2 on tree seedling growth, Hanson and McLaughlin (1989) observed a significant increase in root and current year shoot dry weight in red spruce after exposure to 235uM H_2O_2 . In addition, the mean relative growth rate of red spruce seedlings exposed to both 59uM and 235uM was significantly greater than 0uM and 15uM treatments. In this study the results for Sitka spruce stem dry weight at the final harvest also suggested a stimulation of growth at 50uM H_2O_2 when compared to the control. However, there was no such stimulation at the highest H_2O_2 concentration (100uM) in contrast to the results of Hanson and McLaughlin (1989). There are a number of reasons for these differences, namely different species, mist chemistry, soil composition and experimental conditions (the red spruce seedlings were maintained in a greenhouse). Both oak and Sitka spruce seedlings were maintained outside for the duration of the experiment and as such would have been exposed to ambient air pollution (mostly O_3) and to the natural weathering actions of wind and rain.

Stimulation at intermediate treatment concentrations, as seen here for 50uM H_2O_2 , has been observed in a number of acid dose-response studies (not including H_2O_2) with other species (Lee and Weber, 1979; Lee *et al*, 1981; Forsline *et al*, 1983; Ashenden and Bell, 1988; Ashmore *et al*, 1988, 1989).

Mallant *et al* (1986, 1988) and Masuch *et al* (1986) suggested that developing tissue in the form of current year Norway spruce (*Picea abies*) and needles and young beech (*Fagus sylvatica*) leaves were more susceptible to the adverse effects of H_2O_2 . There are a number of experiments suggesting greater sensitivity of developing tissues than mature tissue to acid rain (without H_2O_2) (Wood and Bormann, 1974; Haines *et al*, 1980; Forsline *et al*, 1983). However, Hanson and McLaughlin (1989) observed an effect on current year shoot dry weight, but not the previous year, in red spruce after exposure to H_2O_2 , although the effect here was a stimulation at the highest H_2O_2 concentration (235uM). In this study with Sitka spruce, it was not possible to reach such a conclusion from the parameters measured.

1989 budburst in Sitka spruce began in late April, so that by the first misting event (24/5/89) the seedlings had full length needles emerging from the bud, but little extension growth had occurred. The potential for growth in conifers is largely pre-determined in the previous season, but it is likely that the treatments were not started too late to influence shoot growth.

1990 budburst was unaffected by H_2O_2 treatment, with almost all buds bursting on all seedlings. The winter of 1989/1990 was typically mild, so that it was unlikely that the seedlings would have suffered frost damage.

It was observed that the decrease in total plant dry weight for both herbaceous and tree species at the highest H_2O_2 concentration used in each experiment was comparable. For broad bean (Expt.A) and pea (Expt.B) there was a significant reduction in total plant dry weight in the 50uM treatment by 27% and 15%, respectively, compared with the control. For both tree species at the 1989 EOS harvest, the decrease in total plant dry weight at 100uM H_2O_2 was 19% and 18% for oak and Sitka spruce respectively, compared with the control and was non-significant. Similarly, at the 1990 final harvest, total plant dry weight at 100uM H_2O_2 was 18% and 14% less than in the 50uM treatment for oak and Sitka spruce, respectively. This may reflect the greater variability in the growth of tree seedlings.

4.5.3 Gas exchange in oak.

Seedlings treated with H_2O_2 , at all concentrations, in 1989 showed an increase in leaf conductance (gl) when compared to the controls, with a significant difference between 0 and 100uM treatments on two out of four occasions. The relationship between gl and H_2O_2 concentration was generally linear. This effect was consistent, irrespective of whether measurements were made pre-misting or post-misting. This was in contrast to the results of Hanson and McLaughlin (1989), who found no effect of H_2O_2 treatment (at concentrations up to 235uM) on gl in red spruce, at any time during a 16 week exposure period. In this study with oak, the increases in gl seen for the 100uM treatment were 30% and 19% for the 20/7/89 and 31/7/89 measurements respectively, when compared to the controls. These percentages were similar to the value suggested by Taylor and Dobson (1989) of the 20% mean change in stomatal conductance needed to detect a significant effect ($P < 0.05$) of air quality. This reflected both the variability between seedlings and the difficulty of working under field conditions where temperature and light could vary considerably within a single measurement period.

The measurement made on the night of 15/9/89 was an attempt to investigate the possibility of treatment differences at a time when the stomatal opening would normally have been at a minimum. If permanent damage had been caused to the stomata by H_2O_2 treatment, the difference from the control plants would have been more pronounced than during the day. Although the results had again indicated an increase in conductance in 100uM treated seedlings when compared with the controls, this was non-significant. The mechanism by

which H_2O_2 was affecting g_l was therefore unclear. Even at night, it would not have been possible to distinguish between stomatal and cuticular components; any increase in g_l could either have been due to residual stomatal opening or to increased cuticular water loss.

It was observed that the night-time g_l values were high compared with daytime values. These night-time values were made in mid-September when the leaves were senescing and it was likely that stomatal control was being lost.

There are reports of H_2O_2 having significant effects on stomata. Slanina *et al* (1985), Mallant *et al* (1986) and Masuch *et al* (1986) observed an increase in the stomatal density of current and previous year needles of Norway spruce (*Picea abies*) and developing beech (*Fagus sylvatica*) leaves after exposure to 118 μM H_2O_2 . In another experiment involving beech, Masuch *et al* (1989) again saw an increase in the number of stomata of developing leaves after exposure to 21 μM and 147 μM H_2O_2 , but an increase in the degree of opening of each stoma was only significant at 147 μM . In this study with oak seedlings, it was possible that the increase in leaf conductance seen in H_2O_2 treated seedlings was the result of a combination of an increase in stomatal density and increased stomatal opening.

The increase in g_l seen in H_2O_2 seedlings suggested that there was a possibility that water stress could have developed. The trees were well watered, but on hot sunny days it was possible that the seedlings may have been subjected to temporary water stress.

Generally H_2O_2 treated seedlings had higher values for net photosynthesis than the controls, but these were non-significant. Hanson and McLaughlin (1989) found no effect on light-saturated photosynthesis in red spruce (*Picea rubens*) after 16 weeks exposure to 15, 59 or 235 μM H_2O_2 . In this investigation with oak, the photon flux density range on the measurement dates were large, so that light saturation may not have been attained for each measurement.

In this study with oak, 1989 extension growth in the 100 μM treatment was significantly reduced compared with the 50 μM seedlings, but both harvest dry weights were unaffected. High values of g_l in the H_2O_2 treatments were correlated with high values of net photosynthesis, but the latter were non-significant. It is rare to find a correlation between leaf conductance and net photosynthesis and effects on growth (Reich *et al*, 1986, 1987). Plant growth or dry weight is a measure of total net carbon gained over the entire growing season, whereas net photosynthesis at a certain time is an instantaneous measure of the production capacity of the foliage at that time. Carbon is lost through the processes of maintenance and construction respiration. More significantly, the gain in dry matter with time is greatly

dependent on the amount of carbon allocated to leaf or to non-photosynthetic tissue (Mooney and Winner, 1988). Growth in any one season is also affected by the growth in previous years and plant nutritional status (Kozłowski *et al.*, 1991).

The mechanism for the consistent increase in daytime leaf conductance as a result of H_2O_2 exposure is uncertain. There are a number of possible hypotheses including, disruption of the control of stomatal opening by H_2O_2 ; permanent cuticular injury leading to an increase in cuticular transpiration; an increase in stomatal density and stomatal opening; a stimulation of the photosynthetic rate by H_2O_2 leading to an increase in leaf conductance. There are also reports of externally applied H^+ , at concentrations greater than 100 μM , stimulating the opening of stomata in *Vicia faba* (Dittrich *et al.*, 1979; Bittisnich *et al.*, 1987). In view of the high H^+ concentrations (up to 200 μM) occasionally measured in the surface droplets collected from oak (and Sitka spruce) treated with H_2O_2 , this mechanism may have influenced the increase in leaf conductance observed. However, the increase in leaf conductance was seen both pre- and post-misting, suggesting a more permanent effect of H_2O_2 .

4.5.4 Surface droplet chemistry

There were few effects of H_2O_2 treatment on droplet ion concentration throughout the 1989 season. In the first half of the season (May - July) H^+ droplet content generally increased with increasing H_2O_2 concentration, for both species. This increase in H^+ was occasionally sufficient to acidify droplets above that in the mist, but only in H_2O_2 treatments. Droplet acidification above the H^+ concentration in the original mist solution was never seen in the control. In the second half of the season (July-September), the droplets collected from both species, were always less acidic than the applied mist, and this was independent of H_2O_2 concentration. For Sitka spruce, the mean H^+ droplet content collected through the season significantly increased with increasing H_2O_2 , but this average concentration was less than that in the original mist solution for all treatments.

It is known that there are species differences in the ability to neutralise H^+ in leaf surface droplets. Adams and Hutchinson (1984) observed that *Artemisia tilesii* increased the pH of droplets from 3 to 3.9 after 75 min contact, while droplets on *Spinacea oleracea* leaves were acidified to pH 2.8 before drying. Craker and Bernstein (1984) saw that red kidney bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) gave the greatest pH change of pH 4 droplets whereas corn (*Zea mays*) and timothy (*Phleum pratense*) had little effect over 4h. In another study, droplets of pH 3, 3.8 and 4 were neutralised in approximately 150 min to 4.4, 5 and 5.5 by sunflower (*Helianthus annuus*), radish (*Raphanus sativus*) and *A. tilesii* but

neutralisation by beet was significantly less (Hutchinson and Adams, 1987). However, in this study oak and Sitka spruce exposed to H_2O_2 possessed the ability to neutralise H^+ but this was not consistent until July.

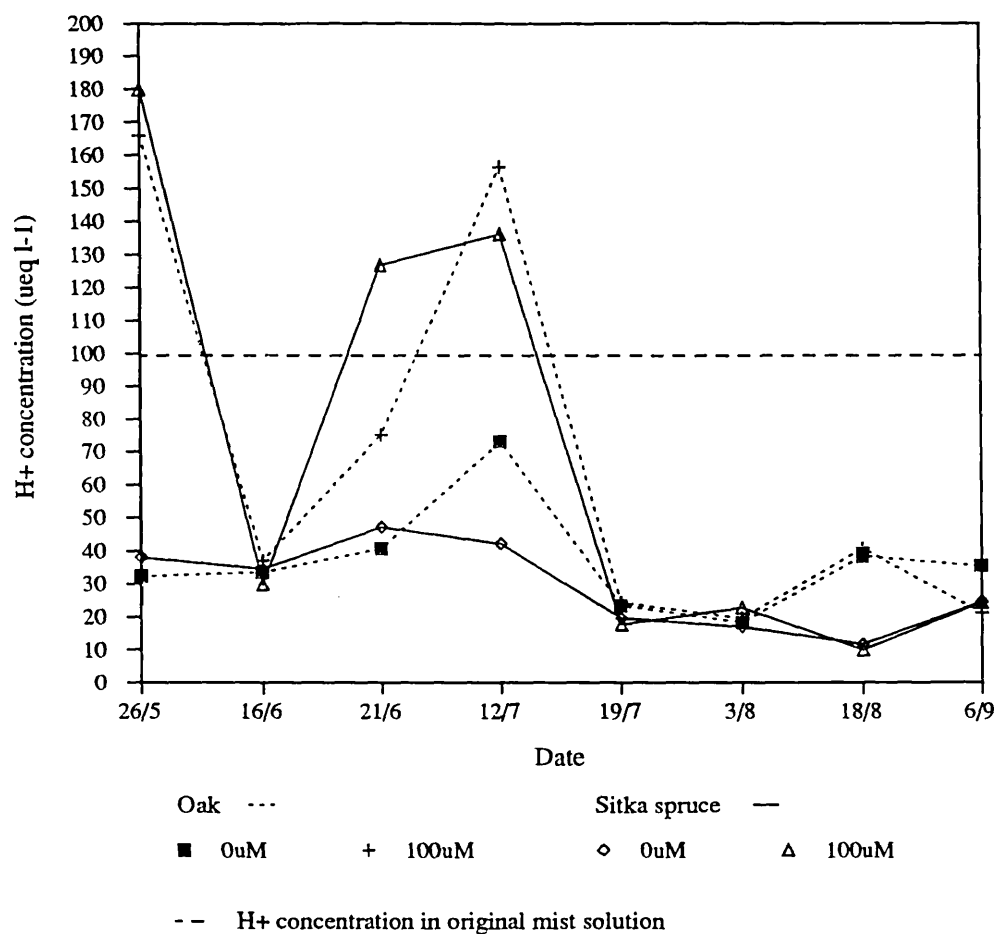
There are a number of reports of changes in the pH of bulk precipitation (= incident precipitation + dry deposition; Parker, 1983), when passing through a tree canopy. The extent to which throughfall pH is increased or decreased relative to incipient precipitation is dependant on a number of factors, namely species, tree age, previous atmospheric deposition, exchange processes at the leaf surface, tree nutrient status and time of year. In general, tree species raise the pH of throughfall above that of incipient precipitation, with hardwoods having a greater capacity to increase the pH than softwoods (Mahendrappa, 1983; Parker, 1983; Cronan, 1984; Miller, 1984; Schaefer and Reiners, 1990). In this study, both species demonstrated equal capacity to increase the pH of surface droplets.

Observations of throughfall acidification relative to incipient precipitation appear to be largely limited to older conifers (>60 yrs). Miller (1984) and Miller *et al* (1987) noted that the effect of age of conifer stands was most marked with a 110 yr old Scots pine stand which decreased throughfall pH, but a 45 yr old stand of the same species, only 500m away, increased throughfall pH. However, in areas subjected to acid precipitation, even hardwood throughfall pH can be less than incipient precipitation (Parker, 1983).

It should be remembered that in this study with oak and Sitka spruce the surface droplets cannot properly be equated with throughfall measurements as the latter in passing through a tree canopy may have come into contact with many layers of foliage or alternatively, not achieved any contact with the foliage.

Seedlings from both species treated with H_2O_2 , during the first half of the season, acidified surface droplets to give pH values less than the applied mist, as reported by Parker (1983) and Miller (1984) above. Acidification of surface droplets was not observed in the control (Fig. 4.15). This change in throughfall pH was also noted by Hoffman *et al* (1980a) and Lindberg *et al* (1989) for a chestnut oak (*Quercus prinus*) stand. Following budbreak and canopy refoliation, throughfall pH from this stand was greater than incipient precipitation, indicating H^+ exchange at the leaf surface. But this was not always maintained through the growing season. In some cases, as seen here for oak and Sitka spruce, throughfall pH was less than that in incipient precipitation. It was suggested that the developing canopy had temporarily lost the ability to remove H^+ . This higher throughfall acidity during full canopy development was due to an increase in both strong and weak acids in throughfall.

Fig.4.15. H⁺ concentration in droplets collected from oak and Sitka spruce seedlings exposed to 0 and 100uM H₂O₂.



These results suggest that both oak and Sitka spruce exposed to H_2O_2 may also have temporarily lost the ability to remove H^+ . It may have been that the H^+ exchange sites were impaired. This pattern was seen in this study and that of Lindberg *et al* (1989), at approximately the same time in the growing season i.e. before full canopy development.

In the study of Hoffman *et al* (1980a), the acidification of throughfall above that in incipient precipitation generally coincided with rain events following increased photochemical concentrations. They suggested that as O_3 was known to attack double bonds and affect membrane permeability, it was possible that O_3 may have reacted with free carboxyl groups in the cuticle altering their ability to behave as H^+ exchange sites. In this study with oak and Sitka spruce, there was no evidence of such a correlation of high levels of O_3 prior to days of droplet acidification.

There are a number of possibilities to explain the decrease in surface droplet pH relative to applied mist in combination with the seedlings' inability to remove H^+ . First, is an increase in the leaching of weak organic acids from the seedlings. pH is a measure of a solution's free acidity; strong acids completely dissociate in solution, whereas weak acids only partially dissociate and the degree of dissociation decreases as pH decreases. In throughfall collected from a chestnut oak canopy (Hoffman *et al*, 1980a; Lindberg *et al*, 1989), weak acids contributed about twice as much to total acidity as that for incipient precipitation, and although the canopy had little influence on total acidity, strong acidity decreased. Hoffman *et al* (1980b) identified a number of organic acids in throughfall of a chestnut oak canopy, which were attributed to the foliage itself.

Secondly, it was possible that the increase in droplet H^+ resulted from solubilisation of particles on the leaf surface having a strong acid reaction. In such a case, the oxidation of previously dry deposited SO_2 by H_2O_2 was considered. This reaction (discussed in section 1.2.2) would be limited by both SO_2 and oxidant concentrations, but relatively independent of the pH of the solution. In this study, the oxidant concentration should not have limited such a reaction, as H_2O_2 was continuously applied to the leaf surface, although not at a constant concentration (due to the natural decay of H_2O_2 with time). The case for such a reaction was strengthened by the observation that, for Sitka spruce at least, H^+ was occasionally significantly correlated with droplet SO_4^{2-} concentration. No such correlation was seen for oak and when the combined data from both species were analysed there was only significant positive correlation between H^+ and SO_4^{2-} on one occasion (21/6/89). However, Alcock and Morton (1981) found no evidence that the acidification of throughfall beneath Scots pine (*Pinus sylvestris*) and birch (*Betula pendula*) when compared with incoming rainfall, was due to wash-off of adsorbed SO_4^{2-} .

It may also be necessary to consider the pollution climate during the misting period, in addition to previous deposition history (as discussed above). A water soluble gas could have readily entered into solution in the droplets captured by the leaves/needles. SO_2 , again was primarily considered because it is readily soluble. The concentration of SO_2 that would be required to produce the change in H^+ and SO_4^{2-} concentration seen is not known. Jayne *et al* (1990) reported the effect of H_2O_2 content on the uptake of SO_2 by aqueous droplets. At $\text{pH} < 4$, SO_2 uptake increased when the aqueous solution contained $< 2\text{M}$ H_2O_2 . It was concluded that the reaction rate of H_2O_2 with S(IV) was consistent with rates measured in bulk solutions, indicating that the droplet surface was not significant in the H_2O_2 - S(IV) reaction.

Therefore the potential for a H_2O_2 - SO_2 reaction in droplets exists, but the concentrations of H_2O_2 in this study (up to $100\mu\text{M}$) were much lower than that of Jayne *et al* (1990). In addition the concentration of SO_4^{2-} in collected droplets was greatly in excess of the H^+ concentration. However, it was possible that some of the H^+ produced was neutralised and this may have accounted for the large excess of SO_4^{2-} compared with the H^+ concentration. Cape (1988b) suggested that the oxidation of SO_2 at the leaf surface by O_2 (with for example, leached Mn^{2+} as catalyst) could be especially important if in situ buffering by the leaf and/or cation exchange prevented the increase in acidity which would otherwise inhibit the solution of SO_2 . In this study the oxidation of SO_2 by H_2O_2 would not have been limited by the change in solution acidity as the rate of reaction is pH independent (see section 1.2.2). Unfortunately, no measurements of ambient SO_2 at the field site were possible in 1989, but Silwood Park is a rural site, and mean SO_2 concentrations for the same period in 1988 was 0.3ppb .

SO_4^{2-} is known to be readily leached from plants (Parker, 1983; Cronan, 1984; Miller, 1984) and it has been suggested that leachates may comprise $>50\%$ of throughfall SO_4^{2-} in pristine regions (Parker, 1983). It was possible droplet SO_4^{2-} in this study may have the result of increased leaching. However, a recent report by Cape *et al* (1990) suggested that the leaching of SO_4^{2-} from a Scots pine (*Pinus sylvestris*) canopy contributed less than 10% of the extra S collected below the canopy.

A final possibility for the increase seen in H^+ droplet content was the concentration of ion droplet content by evaporation. This process was also considered in the interpretation of the droplet content of the other ions. Unsworth (1984) suggested that the evaporation of intercepted cloudwater from forest canopies, proceeding simultaneously with occult deposition, could lead to chemical concentrations on leaf surfaces, that were substantially larger than those measured in the cloud drops themselves. 100% humidity was rapidly established in the chambers once misting had begun, although it is not known whether this

was maintained during each event. It was possible that on warm evenings or when the wind speed was high, that the humidity may have been less than 100% and some degree of simultaneous deposition and evaporation may have taken place. The concentration of ions at leaf surfaces by evaporation was also considered by Parker (1983) and Joslin *et al* (1988).

There is a more obvious concentration effect, namely the drying of leaf surfaces by evaporation after deposition ceases. Unsworth (1984) considered this to be a transient effect, whereas the mechanism of simultaneous deposition and evaporation could persist during long periods of cloud cover. However, it is a factor which needs to be considered here, as the droplets were collected after the misting event, over a period of 3.5 hrs.

Neutralisation or canopy uptake of H^+ , has been associated with the uptake of an oppositely charged ion or a compensating efflux of ions i.e. cation exchange (Miller *et al*, 1987). A canopy can remove almost 70% of the total deposition of free H^+ during the growing season (Lindberg *et al*, 1989). There are numerous reports describing this process of H^+ uptake and subsequent loss of foliar cations by exchange processes at the leaf surface (Tukey, 1970; Scherbatskoy and Klein, 1983; Parker, 1983; Cronan, 1984; Miller, 1984; Skiba *et al*, 1986; Miller *et al*, 1987; Waldman and Hoffman, 1988; Fluckiger *et al*, 1988; Joslin *et al* (1988); Lindberg *et al*, 1989; Scherbatskoy and Tyree, 1990; Turner and Tingey, 1990; Schaefer and Reiners, 1990). For hardwood canopies, a second neutralisation mechanism, involving the leaching of weak Bronsted bases, has been suggested (Cronan, 1984; Lindberg *et al*, 1989). If weak organic or bicarbonate salts were released by the canopy, the free H^+ would be consumed by the weak base and converted to a weak acid. This would give the same increase in base cations leached, but a decrease in H^+ concentration (Cronan, 1984). Acid-base titrations have suggested that soluble weak Bronsted bases could account for 30-50% of the neutralisation of incoming strong acids (Cronan, 1984).

H^+ droplet content was not consistently negatively correlated with any other cation (i.e. a decrease in H^+ droplet concentration could have been associated with an increase in base cation concentration) in either species. However, mean Zn^{2+} droplet content in oak was significantly increased in response to H_2O_2 , but this was not related to changes in H^+ concentration and is discussed in more detail in the following section.

The concentrations of ions in collected droplets were in excess of those in the applied mist. The enrichment factors for Na^+ and K^+ were especially high for both species. Although Na^+ is readily leached (Tukey, 1970), throughfall sodium is believed to originate mostly from dry deposition whereas more than 90% of K^+ is derived by leaching (Parker, 1983). Unfortunately, it was not possible to determine the origin of the ions in the droplets collected.

The volume of mist captured by each seedling and therefore, the total ionic input to each seedling was unknown. It should also be remembered that the values obtained here were the result of two consecutive misting events, so that deposition and evaporation of mist droplets after the first misting event, may have contributed to the large concentrations seen. In addition the volume of mist applied may have added to the high concentrations observed, as Cape (1988b) suggested that large ionic concentrations would arise if the rate of wetting of foliage i.e. the amount of water applied, was small.

It was noticeable that Sitka spruce gave higher surface droplet concentrations than oak. In the field, throughfall quality varies according to the intercepting canopy and hardwood stands have been shown to have much greater annual nutrient deposition than pines, despite the shorter growing season (Parker,1983). However, there are examples of conifers e.g. red spruce, showing increased deposition in throughfall compared to adjacent hardwoods (Parker,1983).

In addition to the ions applied in the mist, the seedlings would have received further input through ambient wet and dry deposition. In view of the dry summer of 1989, dry deposition received greater consideration. Dry deposition tends to dominate during the summer, when the fully developed canopy provides significant surface area for interaction with suspended particles and vapours (Lindberg *et al*,1989). There was no quantification of the dry deposition to oak and Sitka spruce in this study, but there are a number of reports providing estimates of the contribution of dry deposition to throughfall.

Schaefer and Reiners (1990) considered dry deposition to canopies to be the primary source of inorganic ions in throughfall and that the dose of any dry deposited compound to a canopy was the product of its air concentration, deposition velocity and duration of the dry inter-rain period. In this study, the time interval between misting events varied and there appeared to be no relationship between this interval and the concentration of ions observed. Dry deposited materials may only influence throughfall during the early part of a precipitation event (Schaefer and Reiners,1990), which would explain observations of concentrations in initial throughfall collections occasionally being much higher than subsequent ones, presumably because of the washing off of earlier dry deposits (Skeffington and Roberts,1985). However, in this study it would be expected that dry deposition would contribute to the concentrations obtained at each collection date because of exposure to ambient air between misting events. In addition, conifers appear to reflect better the proximity to dry deposition sources (Parker,1983).

4.5.5 Seedling nutrient status.

There was no significant effect of H_2O_2 on the cation content of oak at the EOS harvest. In Sitka spruce, only Zn^{2+} content of 1988 needles at the EOS harvest and 1989 and 1990 needles at the final harvest were significantly affected by exposure to H_2O_2 . 1989 Sitka spruce needles treated with 100uM H_2O_2 had 70% more Zn^{2+} than control needles at the EOS harvest, but at the final harvest, this trend was reversed, with 1989 and 1990 needles exposed to this same H_2O_2 concentration, having 57% and 68% less Zn respectively, when compared to the controls. It was also observed that the levels of Mn^{2+} and Zn^{2+} in oak leaves were generally greater than in Sitka spruce needles.

It has been suggested that a distinct decrease of leaf mineral content as a result of input of acidity may only be detected if nutrient supply is restricted (Leonardi and Fluckiger,1989). In this study of oak and Sitka spruce it was unlikely that the seedlings would have suffered from such a restriction or nutrient deficiency during treatment, unless leaching losses were excessive. Mist application throughout the season would have provided low nutrient inputs to both soil and foliage as the mist contained Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , NO_3^{2-} and SO_4^{2-} . Further inputs could have been achieved through rainfall and dry deposition between misting events. The seedlings were watered with tap water as required, which provided additional cation input. Chemical analysis of the tap water showed it to be rich in Ca^{2+} (116ppm). The Sitka spruce maintained over the winter of 1989/1990 having been repotted in November 1989, would have received further fertilisation.

A comparison of the cation content of Sitka spruce needles seen here with published deficiency levels generally showed no shortage of the minerals assayed (Table 4.38) except for the Zn^{2+} content of 1989 and 1990 needles from all H_2O_2 treated seedlings (discussed later). However, no deficiency values for oak have been found but the concentrations obtained here are within the range of published values for oak and the deficiency values for beech (Table 4.38).

The only significant effect of H_2O_2 treatment was on the Zn^{2+} content of Sitka spruce needles. Zn^{2+} is a plant micronutrient and thus only required in small quantities. Zn^{2+} has intermediate mobility in plants and is a component of many enzymes (Hewitt and Smith,1974; Collins,1981). There is evidence that some retranslocation may take place within plants. Zn^{2+} accumulation in developing ear of maize, between tasselling and maturity, was greater than total uptake by 50%, so that the excess Zn^{2+} came from the surrounding stem and leaf tissue (Collins,1981).

Table 4.38. Deficiency values for foliar cation content in trees.

Species	Ca mg g ⁻¹	Mg mg g ⁻¹	K mg g ⁻¹	Mn ug g ⁻¹	Zn ug g ⁻¹	Comments
Norway spruce (<i>Picea abies</i>)	1-2	0.7- 0.8	4- 4.5	20- 80	< 13	Zoetl <i>et al</i> , 1989.
Oak (<i>Quercus petraea</i>)	7	2	12	na	na	Leaves collected from Cumbria (Allen,1989).
Beech (<i>Fagus sylvatica</i>)	3	1.5	10	na	na	Fluckiger <i>et al</i> ,1984

na = data not available.

There are a number of possible reasons for the accumulation of Zn^{2+} seen at the EOS harvest for Sitka spruce. It has been reported that an increase in soil acidity may increase the mobility of Zn^{2+} in the soil solution, thus making it more available for uptake by plants (McDonald *et al*, 1986; Westman and Temple, 1989). In this experiment however, soil acidity decreased from 5.3 at the initial harvest, to 6.97 - 7.01 (range for all four treatments) at the EOS harvest.

It is also possible that increased concentrations of Zn^{2+} may have been supplied to the seedlings by contamination from the mist delivery system. All metal surfaces in the misting system had been covered with PTFE tape to minimise any contamination and the seedlings were also positioned on the platform away from the stainless steel struts. Analysis of the mist from the control collectors however suggested no such contamination, with no significant difference in mist composition between the start and end of the misting episode. Jacobson *et al* (1989) attributed changes in foliar levels of aluminium (Al) and iron (Fe) in red spruce (*Picea rubens*) to deposition of soil particles and/or contamination with metals from the misting system.

The final consideration is that of atmospheric deposition between misting events. Lindberg *et al* (1989) suggested that atmospheric deposition did not dominate the estimated total flux to the forest floor of Zn^{2+} , but was still significant, contributing approximately 40% of the Zn^{2+} flux. A combustion source was suggested for Zn^{2+} . The annual atmospheric deposition value for Zn^{2+} in the UK (Cawse, 1981) at Chilton, Oxfordshire was $6.1 \mu\text{g cm}^{-2} \text{ yr}^{-1}$. The deposition at a similar site, like Silwood Park is expected to be of the same order.

Some contribution from atmospheric deposition was therefore likely. Interactions between moisture on vegetation and previously dry deposited material could result in dissolved metal concentrations at the leaf surface that were considerably higher than those measured in rain alone. Estimated metal concentrations on leaves due to particle dissolution in rain and partial moisture evaporation gave approximately $120 \mu\text{g l}^{-1} \text{ Zn}^{2+}$ (Lindberg *et al*, 1989). In this experiment with Sitka spruce, the mean concentration of Zn^{2+} in droplets was $0.36 \mu\text{g l}^{-1}$ (0.11 ueq l^{-1}). However, this represented the concentration before evaporation occurred, so that the concentration left on the needles cannot be estimated.

Surface deposited metals can be absorbed by vegetation (Parker, 1983; Scherbatskoy and Tyree, 1990). Long durations of wetting and intermittent wetting and drying are known to increase rates of solute penetration (Jacobson *et al*, 89). Percy and Baker (1988) suggested that interspecies differences in epicuticular wax layer and cuticular properties are of less importance than the properties of each ion in influencing foliar uptake. A negative linear

relationship between the degree of cation penetration and the hydrated radii of the cations was observed by MacFarlane and Berry (1974) for isolated cuticles and suggested that some charge hindrance to permeability was also involved.

There was no significant effect of H_2O_2 treatment on the Zn^{2+} content of droplets collected from Sitka spruce needles. Therefore the possibility that an increase in the leaching of Zn^{2+} may have stimulated an increased uptake of Zn^{2+} from the soil was unlikely. For oak, there was no significant effect on the Zn^{2+} content of foliage at the EOS harvest, but the mean Zn^{2+} content of droplets significantly increased with increasing H_2O_2 .

At the final harvest, there was a significant decrease in the Zn^{2+} content of 1989 and 1990 Sitka spruce foliage that had been treated with H_2O_2 . The mechanism bringing about this reversal is uncertain. It was possible that there was retranslocation of Zn^{2+} from older needles (especially 1989 needles) into new 1990 needles.

4.5.6 Wax content of Sitka spruce.

No significant effect of H_2O_2 treatment was seen on the total wax content of 1988 or 1989 needles, although for both year classes, the 50uM and 100uM treated seedlings had less wax (expressed as a percentage of needle dry weight) than the 0uM and 20uM seedlings.

Cape (1988a) advised caution in the interpretation of wax content and stressed that the amount of wax should be expressed in the correct terms. In a study of the change in wax content with age of Scots pine (*Pinus sylvestris*) needles, there was no significant decrease in total wax content when expressed on a needle area basis. However, when the same data was expressed on a needle dry weight basis, there was a significant decrease with time. This decrease was due to an increase in the ratio of dry weight to surface area with time.

Barnes and Brown (1990) saw no significant effect of ozone and/or acid mist on the wax extracted from Norway spruce (*Picea abies*) seedlings, whether expressed on a needle area or dry weight basis. However, there was an increase in the projected area of needles relative to their dry weight (specific needle area, SNA), so that polluted trees produced larger needles. They again suggested that apparent changes in the amount of wax expressed on a needle dry weight basis could reflect treatment-induced changes in needle area relative to their dry weight, rather than the density of the wax covering. However, in this experiment with Sitka spruce, there was no significant response of specific needle area to H_2O_2 .

An estimate of the projected area of the wax needles (needles from which wax was extracted) was obtained by regression analysis of needle dry weight on needle area for each treatment (the values were for those needles used for area determination). The regression analysis gave

a significant positive correlation between needle dry weight and area. Using the regression equations obtained, estimates of the amount of wax on a needle area basis were calculated (Table 4.39).

Table 4.39. Estimates of the amount of wax on a needle area basis for Sitka spruce.

Year	Wax content (mg cm ⁻²)			
	0	20	50	100
1989 needles	0.231	0.225	0.222	0.163
1988 needles	0.278	0.304	0.216	0.231

The decrease in wax content at the higher H₂O₂ concentrations showed the same trend as that seen when wax amount was expressed on a needle dry weight basis.

There have been many reports of the effects of pollutant exposure on the wax content of plants. Baker and Hunt (1986) found that wax was removed from leaf surfaces after the application of simulated rain. The amount of wax lost varied with species and the duration of the rain event. Ozone increased the degradation of wax on the surface of current and previous year Norway spruce needles and in the majority of clones, had a significantly greater effect on previous year rather than current year needles (Barnes *et al*, 1988). In this study, there was no difference in the decrease in wax content (on a needle dry weight basis) between the two year classes, with both 50uM and 100uM H₂O₂ treated seedlings, having 15-30% less wax than the 0uM and 20uM treatments. The rate of erosion of needle surfaces is related to the average lifetime of the needles and it is highly probable that the wax is not replaced, as wax production ceases when needles become fully expanded (Turunen and Huttenen, 1990).

However, the results of Cape *et al* (1989) suggested a stimulation of wax production in Norway spruce and Scots pine by ozone, whereas Barnes *et al* (1990) saw no significant difference in wax amount of Norway spruce after ozone fumigation. Wax amount in Sitka spruce was unaffected by the application of simulated acid rain at pH2.6 (Percy, 1987; Percy and Baker, 1990).

There are no other reports of the effects of H₂O₂ on wax amount. However, Masuch *et al* (1989) observed cracks in the wax plugs of stomata and fusion of fine wax rods in current year *Picea abies* needles after exposure to 25uM H₂O₂.

4.5.7 Water loss in Sitka spruce.

The mean rate of water loss from excised Sitka spruce shoots was not significantly affected by exposure to H_2O_2 , but the water lost from 1989 shoots (all treatments) was significantly greater than from 1988 shoots on all three days of the experiment. The initial high rate of water loss shown on the drying curves was thought to be a combination of both stomatal and cuticular transpiration, the then steady fall due to cuticular transpiration alone or combined with incomplete stomatal closure (uncontrolled water loss).

The results provided no evidence that exposure to H_2O_2 had led to an increase in cuticular transpiration or had damaged stomatal function. However, these experiments were performed with excised shoots which held many needles, so that differences between needles on the same shoot were also taken into account. A more sensitive method would have been to measure the water loss of individual needles.

Masuch *et al* (1989) observed a decrease in the evaporation rate (expressed as a percentage of original fresh weight) in the current year needles of Norway spruce exposed to 25 μM H_2O_2 . This is in contrast to the results obtained here with Sitka spruce, where although no clear effect of H_2O_2 treatment was seen, it appeared that treatment with H_2O_2 led to an increase in water loss compared to the control.

Exposure to other pollutants has been shown to affect water loss in different species.

Neighbour *et al* (1988) saw an increase in the rate of water loss in excised leaves of silver birch (*Betula pendula*) and downy birch (*B. pubescens*) after exposure to SO_2 and NO_2 alone or in combination. One Norway spruce clone exhibited a significant increase in cuticular water loss from previous year's needles after O_3 fumigation (Barnes and Davison, 1988).

Current year needles excised from one Norway spruce clone dried more rapidly following exposure to O_3 and/or acid mist than needles from control trees, indicating impaired stomatal function (Barnes *et al*, 1990).

Chapter 5

GENERAL DISCUSSION

5 GENERAL DISCUSSION.

The research presented here has demonstrated that H_2O_2 applied to a range of plant species grown under controlled environment and field conditions can affect growth and physiology. The herbaceous species investigation was used as an indicator of the possible effects of H_2O_2 and the tree species examined were important upland species in the U.K.

The H_2O_2 concentrations used (up to 100 μM) correspond to levels that have been measured at high elevation sites in mist and cloudwater (Kelly *et al*, 1985; Dollard *et al*, 1988; Olszyna *et al*, 1989; Gunz and Hoffman, 1990; Sakugawa *et al*, 1990) and the H^+ - H_2O_2 combinations used also fall within reported levels (Daum *et al*, 1983; Kelly *et al*, 1985). However, the lack of long term measurements of H_2O_2 in the field does not allow an assessment of the validity of the H_2O_2 dose (concentration $\times$ time) received by the plants in this study. It was realised that high H_2O_2 concentrations were most commonly observed during the day and that measurements show a diurnal trend. High concentrations have on occasions been recorded at night and it has been suggested that these were the result of either chemical production of H_2O_2 or the mixing down of H_2O_2 from above by nocturnal inversion following a breakdown in the stability of the atmosphere (Davies and Dollard, 1989; Dollard *et al*, 1989b). However, in view of the misting system used in the tree seedling investigation, it was decided to apply mist overnight to prevent scorch damage to the plants. Other experiments involving the application of wet deposited H_2O_2 have also adopted this approach either misting in the early morning or overnight (Slanina *et al*, 1985; Mallant *et al*, 1986, 1988; Masuch *et al*, 1986, 1989; Hanson and McLaughlin, 1989). In addition, it should be taken into consideration that the concentrations used in this study were the concentrations at the start of each exposure and that over the time scale of an exposure period, the mean H_2O_2 concentration would have been less than these nominal concentrations by 10-20% due to the natural decay of H_2O_2 .

Atmospheric pollution has been implicated as a likely contributing factor to observed changes to forests in Europe and North America, but uncertainties continue to surround the mechanisms and specific causes for these changes. McLaughlin (1985) suggested a framework for considering the role of a variety of stresses contributing to forest decline and he categorised the stresses into three classes: predisposing, inciting and contributing (Table 5.01). Predisposing stresses were considered to weaken the tree in the long term and make it more susceptible to shorter-term inciting factors. The latter were suggested to represent sudden physiological shocks produced by episodic stresses like drought which could alter the tree's physiological condition making it more susceptible to secondary biotic stresses. Once the tree began to decline, contributing factors, which were generally biotic stresses, could accelerate the decline.

Table 5.01. Categories of factors influencing declines of forest trees (after McLaughlin, 1985).

	Predisposing	Inciting	Contributing
Functional role	Chronic weakening	Triggering episodes	Accelerators
	Climate	Insect defoliation	Bark beetles
	Soil moisture	Frost	Canker fungi
Stressing agents	Genotype of host	Drought	Viruses
	Soil nutrients	Salt	Root decay fungi
	Air pollutants	Air pollutants	Competition
	Competition	Mechanical injury	

In this scenario, air pollutants can act as both predisposing and inciting factors. However, the difficulty in separating the effects of any one or a combination of air pollutants from the effects of the other stresses has led to the common view that there is no single simple cause of novel forest decline, but rather that it results from a complex interaction between more than one pollutant and other environmental stresses (Ashmore *et al*,1985).

The role of H₂O₂ in this scenario is uncertain in view of the limited experimental evidence and the lack of any field observations. Previous work involving H₂O₂ has been restricted to the exposure of seedlings under controlled environment conditions (Hanson and McLaughlin,1989) or in glass chambers situated outside but shielded from direct sunlight (Slanina *et al*,1985; Mallant *et al*,1986,1988; Masuch *et al*,1986). Seedlings in the work of Masuch *et al* (1989) were grown outside and subjected to ambient air, temperature and light, but were shielded from ambient wet deposition. In these experiments the trees were also adequately supplied with water and nutrients.

The results from these experiments and from the present study can therefore only provide indications of possible effects in the field where microclimate and soil nutrient status are frequently not optimal for growth. Slanina *et al* (1985), Mallant *et al* (1986,1988) and Masuch *et al* (1986,1989) suggested that both Norway spruce (*Picea abies* and beech (*Fagus sylvatica*) exposed to H₂O₂ at concentrations as low as 21µM, caused a number of cellular perturbations, which were suggested to indicate a reduced capacity for water transport. The

translation of this effect to the field would obviously be dependent on the water supply to the tree - if the water supply was not limiting, then it would be unlikely that any adverse effect of H_2O_2 would be seen. However, if these results were interpreted according to the scenario of McLaughlin (1985) given in Table 5.01, then H_2O_2 could be considered to be acting as a predisposing factor. Similarly, the stimulation of growth of red spruce (*Picea rubens*) seen after exposure to 235uM H_2O_2 (Hanson and McLaughlin,1989), were obtained under controlled growing conditions and may not have been achieved in the field.

In this study exposure to 50uM H_2O_2 had an adverse effect on the growth of pea and broad bean with some evidence of a linear growth response to H_2O_2 . Such obvious negative responses to H_2O_2 were not seen in oak and Sitka spruce, although needle dry weight in 100uM Sitka spruce seedlings was significantly reduced compared with the 0uM (at the 1989 EOS harvest) and the 50uM (at the 1990 final harvest) treatment. Again, extrapolation of these results to a field situation would be uncertain and Bell (1986) highlighted the dangers and difficulty of extrapolation from short-term exposure in predicting effects over the lifetime of the tree. However, the results may provide an indication that exposure to 100uM H_2O_2 could affect dry matter accumulation in Sitka spruce. This decrease in needle dry weight may have represented a reduction in the photosynthetic capacity of these seedlings and would be especially important for a tree if this decrease was compounded over a number of years.

In tree species, the effect of pollutant exposure need not be expressed immediately, so that changes in growth patterns induced by pollutants in one summer could have implications for tree performance in the second or subsequent summers (Freer-Smith,1984; Ashmore *et al*,1988; Kozlowski *et al*,1991). Loehle (1988) concluded that attempts to correlate pollutant dose with tree response was complicated by the lagged nature of tree growth responses. The exposure period to H_2O_2 in all experiments undertaken has been for one growing season or less and only in this present study have the seedlings been maintained for the following season. Therefore there is insufficient evidence available to determine the effects of H_2O_2 on tree growth over more than one growing season.

A further drawback in assessing the implications of H_2O_2 exposure on tree growth is that all experiments have been performed with seedlings, whereas decline symptoms in the field are observed on mature trees. In comparing seedlings and mature trees, Ashmore *et al* (1990) noted that although the carbon cycles contained the same components, the relationship and balance between the components would differ and so the response of each cycle to external stress would differ quantitatively. For example, it was considered that the translocation of similar metabolites or storage compounds to other organs would proceed by the same

mechanisms, but the extent of translocation between branches or between age classes of different needles would differ and the distance between sites of carbon acquisition and those at which it is stored or utilised would also differ. It was concluded that there were likely to be substantial differences between the responses of seedlings and mature trees to pollutant stress.

The results from the work investigating H_2O_2 on growth (Hanson and McLaughlin, 1989, and this present study) have not provided sufficient evidence of any consistent growth effects. However, changes in tree growth represent the integration of any reactions that have taken place at lower levels of organisation e.g. cellular and biochemical changes leading to disruption of the physiological function of the tree prior to alterations in growth and there was some evidence from this study of such disruption as a result of H_2O_2 exposure.

There was evidence that 100 μM H_2O_2 increased leaf conductance in oak. It was uncertain whether this was the result of stomatal dysfunction or of increased cuticular transpiration. The rate of drying of excised Sitka spruce shoots was unaffected by H_2O_2 exposure, so that the rate of closure of the stomata and subsequent rate of cuticular water loss in H_2O_2 treated seedlings were not significantly greater than the control. However, the consistent increase in leaf conductance in oak exposed to 100 μM H_2O_2 may have predisposed these seedlings to secondary stresses, especially drought - an inciting factor in the scenario of McLaughlin (1985) discussed earlier (Table 5.01). It is of special note that the development of water stress during a dry summer could coincide with periods of expected high H_2O_2 concentrations in view of its photochemical origins and seasonal variation.

Epicuticular wax acts as a barrier to micro-organisms, pollutants and water loss. Air pollution-induced wax erosion generally resembles natural weathering (Turunen and Huttunen, 1990), but there are also reports of a stimulation of wax production in response to acidity at pH 3.5 (Cape, 1988) and O_3 (Cape *et al*, 1989). Alteration of the wax structure seen as fusion of wax rodlets was observed on *Pinus strobus* needles exposed to various pollutants in the field (Riding and Percy, 1977). The rate of change of the chemistry of the surface waxes of Scots pine (*Pinus sylvestris*) needles in the field was found by Cape (1988) to be greater in polluted air (SO_2 and NO_x) than in non-polluted air. Percy and Baker (1990) recently provided evidence of a direct interaction between pollutants and wax biosynthesis or crystallisation processes, with a statistically significant relationship between acidity and wax composition for one clone of Sitka spruce. However in this study neither wax content of Sitka spruce needles or the structure of pea epicuticular wax was significantly affected by H_2O_2 exposure.

Epicuticular wax affects the wettability of foliage which is important in the retention of wet deposition (Turunen and Huttunen,1990). Percy (1987) saw an increase in the retention of droplets on plants after treatment with simulated acid rain at a pH not causing visible injury. Increased droplet retention may have particular importance in the case of H_2O_2 in view of its high reactivity - its decomposition at the leaf surface to give free radicals could react with fatty acids in wax. This lipid peroxidation reaction would be similar to that proposed by Barnes *et al* (1990) for the acceleration of the natural change in the amount of wax on Norway spruce (*Picea abies*) needles, where decomposition of O_3 in aqueous solution would give rise to H_2O_2 and various free radicals.

In this present study the reaction of H_2O_2 at the leaf surface was apparently complex. H_2O_2 significantly increased the leaching of K^+ and Ca^{2+} from detached pea leaves, with the chemical composition of the incubating solution determining the ion preferentially leached. There was a gradual destruction in the H_2O_2 content of droplets of the surface of a bean leaf with time which was not observed on the control, teflon-coated surface. This destruction of H_2O_2 was associated with an initial increase in H^+ droplet content (over the first 60 min) followed by H^+ neutralisation and a continuous increase in droplet conductivity. For droplets collected from the leaf surfaces of the tree seedlings acidification of droplets above that in the original mist solution was only observed in the H_2O_2 treatments. The control seedlings always neutralised the deposited mist droplets.

H^+ production on the leaf/needle surface of the tree seedlings was not consistent throughout the season, but may have important implications. There could have been direct effects of such localised H^+ production on leaf ionic balance. Neutralisation of the H^+ produced would be undertaken and would represent a loss of foliar cations either by exchange or through leaching (Tukey,1970; Scherbatskoy and Klein,1983; Parker,1983; Cronan,1984; Miller,1984; Skiba *et al*,1986; Miller *et al*,1987; Waldman and Hoffman,1988; Fluckiger *et al*,1988; Joslin *et al*,1988; Lindberg *et al*,1989; Scherbatskoy and Tyree,1990; Turner and Tingey,1990; Schaefer and Reiners,1990). Substantial cation loss would require replenishment from the soil where they are exchanged for H^+ , leading to acidification of the rhizosphere (Abrahamsen,1984). The preferential leaching of any one ion could lead to nutritional imbalances in the soil and/or foliage.

Throughfall consisting of droplets acidified at the leaf/needle surface could be a source of H^+ into the soil environment. Acidification of the soil solution would lead to a destruction of the soil buffering system, an increase the mobility of toxic cations like aluminium and eventually root death (Abrahamsen,1984; Morrison,1984).

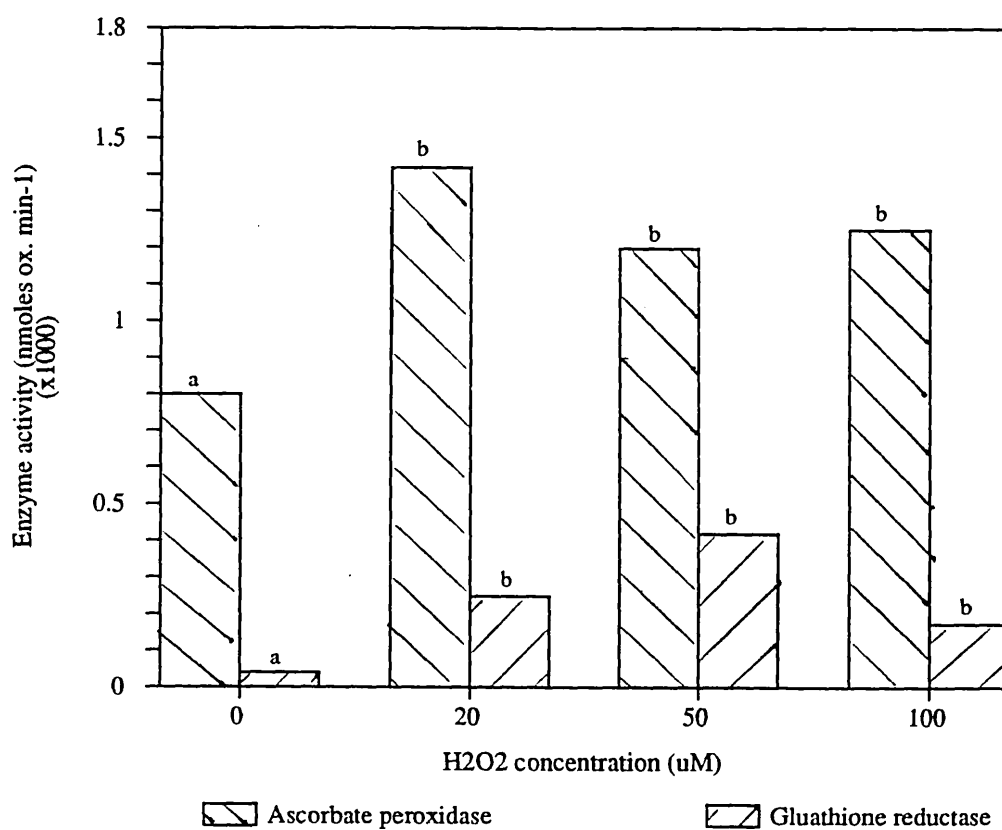
However, the outcome of this scenario would be dependent on soil and foliar nutrient status. In this study there was no evidence of H_2O_2 affecting foliar cation content and the impact of an increase in leaching for these seedlings was likely to have been minimal, as they were adequately supplied with nutrients throughout the experiment. In European and North American forests, some soils have been reported to be deficient in one or more nutrients e.g. magnesium (Roberts *et al*, 1988). The reaction of H_2O_2 at the leaf surface of a mature forest tree over a long time-scale could perturb nutrient relations. This would be in agreement with recent advances in forest decline research which suggest that the symptoms may not be the result of direct effects on the foliage, but rather the result of nutritional imbalances (Oren *et al*, 1988; Zoetl *et al*, 1989).

Acidification of the leaf surface by H_2O_2 appeared to be associated with SO_2 oxidation as H^+ and SO_4^{2-} droplet content were occasionally correlated for Sitka spruce. The role of H_2O_2 in the oxidation of SO_2 in the atmosphere has been well documented and was discussed in section 1.2.2. The reaction is independent of pH and as such is not limited by the decreasing solubility of SO_2 with increasing H^+ concentration. The nature of the reaction is uncertain. The reaction may have taken place in falling droplets, or resulted from SO_2 entering droplets already deposited in the leaf surface. Alternatively, the reaction may be an indication of previous dry deposition.

An alternative path for the removal of H_2O_2 in droplets on a leaf surface would be the detoxification of H_2O_2 and any resultant free radicals by a plant's anti-oxidant system. There are reports of extracellular peroxidases (Heath and Castillo, 1988) and Castillo *et al* (1984) observed a three fold increase in extracellular peroxidase in *Sedum album* leaves exposed to 400ppb O_3 compared with the control.

Analysis of Sitka spruce needles from this study at the time of the final harvest and seven months after the final harvest, revealed a significant increase in foliar levels of ascorbate peroxidase and glutathione reductase in H_2O_2 treated seedlings when compared to the control (Mehlhorn *et al*, in press; Fig.5.01.). These effects were significant even at 20uM H_2O_2 and it should be emphasised that these results were found seven months after the last exposure and indicated the potential for long term perturbations of metabolism by H_2O_2 . This represented a significant biochemical perturbation as a result of H_2O_2 treatment and the effect was only seen in 1989 (year of treatment) and not 1990 needles. This can be viewed as an increased ability to detoxify H_2O_2 and may account for the lack of effect of H_2O_2 seen in this experiment. It appeared as if this species possessed a protective mechanism against the potential impact of H_2O_2 .

Fig.5.01. Effect of H₂O₂ on anti-oxidant levels in 1989 Sitka spruce needles at the 1990 final harvest.



For each enzyme, the means with different letters are significantly different at $P < 0.05$.

From Mehlhorn et al (in press).

However, such a dramatic increase in glutathione reductase requires a corresponding supply of NADPH for the hydrogen peroxide decomposing system to be operational, so that catabolic activity would be expected to increase (Mehlhorn *et al*,1987). There are recent reports showing that ascorbate peroxidase is a good predictor of reductions in growth and yield, so that the results of Mehlhorn *et al* (in press) suggest that H_2O_2 may be harmful to vegetation, especially to plants that retain their foliage for more than one growing season. It was concluded that ambient levels of H_2O_2 impose an oxidant stress on vegetation.

In a final assessment of the possible role of H_2O_2 in forest decline, it should be taken into consideration that H_2O_2 is not present alone in the atmosphere. Rather by the nature of its formation it is often found concurrent with O_3 concentrations. Possible combined exposure to H_2O_2 and O_3 would be an area for future research, together with the interaction of H_2O_2 with secondary stresses, especially drought. The reaction of O_3 with natural (and/or anthropogenic) hydrocarbons represent a further source of H_2O_2 and there are reports of H_2O_2 concentrations above forests being greater than outside the forested area (Jacob *et al*,1987).

Ambient concentrations of H_2O_2 have increased to approximately 50% above pre-industrial values (Sigg and Nefel,1991) and model calculations have predicted a 33% increase between 1985 and 2035 as a result of global climate change (Thompson *et al*,1989). From the results of this study it is not suggested that H_2O_2 is a major force in forest decline. However, in view of the potential reactions at the leaf surface in acidifying droplets and in imposing an oxidant stress on vegetation, it may be a contributing factor in forest decline.

APPENDIX 1.

Fertiliser mix for herbaceous species compost.

KNO ₃	15g
K ₂ SO ₄	15g
Superphosphate	120g
Frit	37g
CaCO ₃	235g
MgCO ₃	295g

Per 100l of peat and sand.

APPENDIX 2.

ANOVA tables.

i) Analysis of growth results from Expt.B.

	df
H2O2	2
Linear	1
Residual	(n-3)

ii) Analysis of growth results from tree experiment.

	df
H2O2	3
Linear	1
Quadratic	1
Block	1
H2O2-Block	3
Residual	(n-8)

REFERENCES.

- Abrahamsen,G. 1984. Effects of acidic deposition on forest soil and vegetation. *Phil.Trans.R.Soc.Lond.*,B 305:111-123.
- Adams,C.M. and Hutchinson,T.C. 1984. A comparison of the ability of leaf surfaces of three species to neutralise acidic rain drops. *New Phytol.*,97:463-478.
- Adams,C.M. and Hutchinson,T.C. 1987. Comparative abilities of leaf surface to neutralise acidic raindrops. II.the influence of leaf wettability, leaf age and rain duration on changes in droplet pH and chemistry on leaf surfaces. *New Phytol.*,106:437-456.
- Alcock,M.R. and Morton,A.J. 1981. The sulphur content and pH of rainfall and throughfalls under pine and birch. *J.App.Ecol.*, 18:835-839.
- Allen,S.E. 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publishers, Oxford.
- Ames,D.L. 1983. A method of determining hydrogen peroxide in cloud and rainwater. CERL report no.,TPRD/L/2552/N83.
- Arnold,A.C. 1993. Comparative droplet size spectrum for three different-angled flat fan nozzles. *Crop protection*,2:193-204.
- Ashenden,T.W. and Bell,S.A. 1988. Growth responses of birch and Sitka spruce to acidified rain. *Environ.Pollut.*,51:153-162.
- Ashmore,M.R., Bell,J.N.B. and Rutter,A.J. 1985. The role of ozone in forest damage in West Germany. *Ambio*, 14(2):81-87.
- Ashmore,M.R., Garretty,C., McHugh,F.M. and Mepsted,R. 1988. Combined effects of ozone and acid mist on tree seedlings. In *Air Pollution and Ecosystems*. P.Mathy (ed.). D.Reidel, Dortrecht. pp 659-664.
- 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*. J.B.Bucher and I.Bucher-Wallin (eds.). EAFV, Birmensdorf, Switzerland. pp 384-386.
- Ashmore,M.R., Bell,J.N.B. and Brown,I.J. 1990. Air Pollution and Forest Ecosystems in the European Community. CEC Air Pollution Research Report 29, Brussels.
- Baker,E.A. and Hunt,G.M. 1986. Erosion of waxes from leaf surfaces by simulated rain. *New Phytol.*,102:161-173.

- Barnes,J.D. and Davison,A.W. 1988. The influence of ozone on the winter hardiness of Norway spruce [*Picea abies* (L.) Karst.]. *New Phytol.*,108:159-166.
- Barnes,J.D., Davison,A.W. and Booth,T.A. 1988. Premature degradation of epicuticular wax on *Picea abies* needles induced by ozone. In *Scientific Basis of Forest Decline Symptomatology*. J.N.Cape and P.Mathy (eds.). E.Guyot,Brussels. pp 245-251.
- Barnes,J.D. and Brown,K.A. 1990. The influence of ozone and acid mist on the amount and wettability of the surface waxes in [*Picea abies* (L.) Karst.]. *New Phytol.*,114:531-535.
- Barnes,J.D., Eamus,D. and Brown,K.A. 1990. The influence of ozone, acid mist and soil nutrient status on Norway spruce [*Picea abies* (L.) Karst.]. I.Plant-water relations. *New Phytol.*,114:713-720.
- Becker,K.H., Brockmann,K.J. and Bechara,J. 1990. Production of hydrogen peroxide in forest air by reaction of ozone with terpenes. *Nature*,346:256-258.
- Bell,J.N.B. 1986. Effects of acid deposition on crops and forests. *Experientia*, 46:363-371.
- Bittisnich,D.J., Entwisle,L.O. and Neales,T.F. 1987. Acid-induced stomatal opening in *Vicia faba* L. and the role of guard cell wall elasticity. *Plant Physiol.*,85:554-557.
- Blank,L.W. 1985. A new type of forest decline in Germany. *Nature*,314:311-314.
- Blank,L.W., Roberts,T.M. and Skeffington,R.A. 1988. New perspectives on forest decline. *Nature*,336:27-30.
- Brown,K.A. and Roberts,T.M. 1988. Effects of ozone on foliar leaching in (*Picea abies* L. Karst): confounding factors due to NO_x production during ozone generation. *Environ.Pollut.*,55:55-73.
- Brown,V.C. Dept.Biology, Imperial College, Silwood Park, Ascot, Berks SL5 7PY.
- Bufalini,J.J., Gay Jr.,B.W. and Brubaker,K.L. 1972. Hydrogen peroxide formation from formaldehyde photooxidation and its presence in urban atmospheres. *Environ.Sci.Tech-nol.*,6(9):816-821.
- Calvert,J.G., Lazrus,A., Kok,G.L., Heikes,B.G., Walega,J.G., Lind,J. and Cantrell,C.A. 1985. Chemical mechanisms of acid generation in the troposphere. *Nature*,317:27-35.
- Cape,J.N. 1986. Effects of air pollution on the chemistry of surface waxes of Scots pine. *Water,Air,Soil Pollut.*,31:393-399.
- Cape,J.N. 1988a. Air pollutant effects on conifer needle surfaces. In *Scientific Basis of Forest Decline Symptomatology*. J.N.Cape and P.Mathy (eds.). E.Guyot,Brussels. pp 149-159.

- Cape,J.N. 1988b. Chemical interactions between cloud droplets and trees. In *Acid Deposition at High Elevation Sites*. M.H.Unsworth and D.Fowler (eds.). NATO ASI Series C, vol.252. Kluwer Academic Publishers, Dordrecht. pp 639-649.
- Cape,J.N., Paterson,I.S. and Wolfenden. 1989. Regional variation in surface properties of Norway spruce and Scots pine needles in relation to forest decline. *Environ.Pollut.*,58:325-342.
- Cape,J.N., Sheppard,L.J., Fowler,D., Harrison,A.F. and Parkinson,J.A. 1990. Contribution of canopy leaching to sulphate deposition in a Scots pine forest. In *Acidic Deposition: its nature and impacts*. Proceedings of International Conference, Glasgow, 16-21 Sept.,1990.
- Capom,S.J.M. and Hutchinson,T.C. 1987. The influence of temperature, water and nutrient conditions during growth on the response of *Brassica oleracea* L. to a single, short treatment with simulated acid rain. *New Phytol.*,106:251-259.
- Castillo,F.J., Penel,C. and Greppin,H. 1984. Peroxidase release induced by ozone in *Sedum album* leaves: involvement of Ca^{2+} . *Plant.Physiol.*,74:846-851.
- Cawse,P.A. 1981. A survey of trace elements in the U.K.:results for 1979. AERE report no.R9886.
- Chameides,W.L. and Davis,D.D. 1982. The free radical chemistry of cloud droplets and its impact upon the composition of rain. *J.Geophys.Res.* ,87(C7):4863-4877.
- Chandler,A.S., Choularton,T.W., Dollard,G.J., Gay,M.J., Hill,T.A., Jones,A., Jones,B.M.R., Morse,A.P., Penkett,S.A. and Tyler,B.J. 1988a. A field study of the cloud chemistry and cloud microphysics at Great Dun Fell. *Atmos.Environ.*,22(4):683-694.
- Chandler,A.S., Choularton,T.W., Dollard,G.J., Eggleton,A.E.J., Gay,M.J., Hill,T.A., Jones,B.M.R., Tyler,B.J., Bandy,B.J. and Penkett,S.A. 1988b. Measurements of H_2O_2 and SO_2 in clouds and estimates of their reaction rate. *Nature*,336:562-565.
- Clapham,A.R., Tutin,T.G. and Warburg,E.F. 1962. Flora of the British Isles. Cambridge University Press.
- Clark,P.A., Gervat,G.P., Hill,T.A., Marsh,A.R.W., Chandler,A.S., Choularton,T.W. and Gay,M.J. 1990. A field study of the oxidation of SO_2 in cloud. *J.Geophys.Res.*, 95(D9):13,985-13,995.
- Collins,J.C. 1981. Zinc. In *Effect of Heavy Metal Pollution on Plants*. Vol.1. Effects of Trace Metals on Plant Function. N.W.Lepp (ed.). Applied Science Publishers,U.K. pp145-170.

- Craker, L.E. and Bernstein, D. 1984. Buffering of acid rain by leaf tissue of selected crop plants. *Environ. Pollut. (A)*, 36:375-381.
- Cronan, C.S. 1984. Biogeochemical responses of forest canopies to acid precipitation. In *Direct and Indirect Effects of Acid Deposition on Vegetation*. R.A. Linthurst (ed.). Ann Arbor Sci. pp65-78.
- Das, T.N., Moorthy, P.N. and Rao, K.N. 1983. Chemiluminescent measurement of atmospheric hydrogen peroxide in the Bombay area. *Atmos. Environ.*, 17(1):79-82.
- Daum, P.H., Schwartz, S.E. and Newman, L. 1983. Studies of the gas and aqueous phase composition of stratiform clouds. In *Precipitation Scavenging, Dry Deposition and Resuspension*, vol. 1. H.R. Pruppacher, R.G. Semonin, W.G.N. Slinn (eds.). Elsevier Science Publishing Co., New York. pp31-46.
- Daum, P.H. 1988. Processes determining cloudwater composition: inferences from field measurements. In *Acid Deposition at High Elevation Sites*. M.H. Unsworth and D. Fowler (eds.). NATO ASI Series C, vol. 252. Kluwer Academic Publishers, Dordrecht. pp139-153.
- Davies, T.J. and Dollard, G.J. 1989. Measurements of gaseous hydrogen peroxide in southern England. AERE R13095.
- Dittrich, P., Mayer, M. and Meusel, M. 1979. Proton stimulated opening of stomata in relation to chloride uptake by guard cells. *Planta*, 144:305-309.
- Dollard, G.J., Unsworth, M.H. and Harvey, M.J. 1983. Pollutant transfer in upland regions by occult deposition. *Nature*, 302:241-243.
- Dollard, G.J., Jones, B.M.R., Chandler, A.S. and Gay, M.J. 1988. Measurements of ambient SO₂ and H₂O₂ at Great Dun Fell and evidence of their reaction in cloud. In *Acid Deposition at High Elevation Sites*. M.H. Unsworth and D. Fowler (eds.). NATO ASI Series C, vol. 252. Kluwer Academic Publishers, Dordrecht. pp 215-229.
- Dollard, G.J., Sandalls, F.J. and Derwent, R.G. 1989a. Measurements of gaseous hydrogen peroxide in southern England during a photochemical episode. *Environ. Pollut.*, 115-124.
- Dollard, G.J., Jones, B.M.R. and Davies, T.J. 1989b. Measurements of gaseous hydrogen peroxide and PAN in rural southern England. AERE R13621.
- Dollard, G.J., Jones, B.M.R. and Davies, T.J. 1989c. Temporal trends in H₂O₂ and PAN in the atmosphere. U.K. DOE interim report.
- Dollard, G.J., Jones, B.M.R. and Davies, T.J. 1991. Measurements of gaseous hydrogen peroxide and PAN in rural southern England. *Atmos. Environ.*, 25A(9):2039-2053.

- Ennis,C.A., Lazrus,A.L., Zimmerman,P.R. and Monson,R.K. 1990. Flux determinations and physiological response in the exposure of red spruce to gaseous hydrogen peroxide, ozone and sulphur dioxide. *Tellus*,42B:183-199.
- Evans,L.S., Curry,T.M. and Lewin,K.F. 1981. Responses of leaves of *Phaseolus vulgaris* L. to simulated acidic rain. *New Phytol.*, 88:403-420.
- Evans,L.S., Lewin,K.F., Cunningham,E.A. and Patti,M.J. 1982. Effects of simulated acid rain on field grown crops. *New Phytol.*,91:429-441.
- Fairfax,J.A.W. and Lepp,N.W. 1975. Effect of simulated acid rain on cation loss from leaves. *Nature*, 255:324-325.
- Farquhar,G.D. and Sharkey,T.D. 1982. Stomatal conductance and photosynthesis, *Ann.Rev.Plant.Physiol.*,33:317-345.
- Fluckiger,W., Fluckiger-Keller,H. and Braun,S. 1984. Untersuchungen uber Waldschaden in der Nordwestschweiz. *Schweiz.Z.Forwest.*,135:389-444.
- Fluckiger,W., Leonardi,S. and Braun,S. 1988. Air pollutant effects on foliar leaching. In *Scientific Basis of Forest Decline Symptomatology*. J.N.Cape and P.Mathy (eds.). E.Guyot, Brussels. pp 160-169.
- Forsline,P.L., Dee,R.J. and Melious,R.E. 1983. Growth changes of apple seedlings in response to simulated acid rain. *J.Amer.Soc.Hort.Sci.*, 108(2):202-207.
- Fowler,D., Cape,J.N., Leith,I.D., Choularton,T.W., Gay,M.J. and Jones,A. 1988. Wet deposition and altitude, the role of orographic cloud. In *Acid Deposition at High Elevation Sites*. M.H.Unsworth and D.Fowler (eds.). NATO ASI Series C, vol.,252. Kluwer Academic Publishers, Dordrecht. pp231-257.
- Freer-Smith,P.H. 1984. The responses of six broadleaved trees during long-term exposure to SO₂ and NO_x. *New Phytol.*,97:49-61.
- Gab,S., Hellpointner,E, Turner,W.V. and Korte,F. 1985. Hydroxymethyl hydroperoxide and bis(hydroxymethyl)peroxide from gas-phase ozonolysis of naturally occurring alkenes. *Nature*,316:535-536.
- Gallagher,M.W., Downer,R.M., Choularton,T.W., Gay,M.J., Stromberg,I., Mill,C.S., Radojevic,M., Tyler,B.J., Bandy,B.J., Penkett,S.A., Davies,T.J., Dollard,G.J. and Jones,B.M.R. 1990. Case studies of the oxidation of sulphur dioxide in a hill cap cloud using ground and aircraft based measurements. *J.Geophys.Res.*, 95(D11):18517-18537.
- Garretty,C. Dept.Biology, Imperial College, Silwood Park, Ascot,Berks SL5 7PY.

- Gascoyne, M. and Patrick, C.K. 1981. Variation in rainwater chemistry and its relation to synoptic conditions in north-west England. *Intern.J.Environ.Stud.*, 17:209-214.
- Gervat, G.P., Clark, P.A., Marsh, A.R.W., Teasdale, I., Chandler, A.S., Choularton, T.W., Gay, M.J., Hill, M.K. and Hill, T.A. 1988a. Field evidence for the oxidation of SO₂ by H₂O₂ in cap clouds. *Nature*, 333:241-243.
- Gervat, G.P., Clark, P.A., Marsh, A.R.W., Chandler, A.S., Choularton, T.W. and Gay, M.J. 1988b. Controlled chemical kinetic experiments in cloud: a review of the CERL/UMIST Great Dun Fell project. In *Acid Deposition at High Elevation Sites*. M.H. Unsworth and D. Fowler (eds.). NATO ASI Series C, vol., 252. Kluwer Academic Publishers, Dordrecht. pp283-297.
- Gunz, D.W. and Hoffman, M.R. 1990. Atmospheric chemistry of peroxides: a review. *Atmos.Environ.*, 24A(7):1601-1633.
- Haines, B., Stefani, M. and Hendrix, F. 1980. Acid rain: threshold of leaf damage in eight plant species from a southern Appalachian forest succession. *Water, Air, Soil Pollut.*, 14:403-407.
- Hanson, P.J. and McLaughlin, S.B. 1989. Growth, photosynthesis and chlorophyll concentrations of red spruce seedlings treated with mist containing hydrogen peroxide. *J.Environ.Qual.*, 18(4):499-503.
- Hart, J.W. 1988. Light and plant growth. Unwin Hyman, London.
- Hausladen, A., Madamanchi, N.R., Fellows, S., Alscher, R.G. and Amundson, R.G. 1990. Seasonal changes in antioxidants in red spruce as affected by ozone. *New Phytol.*, 115:447-458.
- Heath, R.L. and Castillo, F.J. 1988. Membrane disfunction in response to air pollutants. In *Air Pollution and Plant Metabolism*. S.Schulte-Hostede, N.M.Darrall, L.W.Blank and A.R.Wellburn (eds.). Elsevier, London.
- Heikes, B.G. 1984. Aqueous H₂O₂ production from O₃ in glass impingers. *Atmos.Environ.*, 18(7):1433-1445.
- Heikes, B.G., Lazrus, A.L. and Kok, G.L. 1985. Measurements of H₂O₂ in the lower troposphere. *Proc. 17th International Symposium on Free Radicals* at Snow Mountain Ranch, Granby, Colorado, 18-23 August, 1985.
- Heikes, B.G., Kok, G.L., Walega, J.G. and Lazrus, A.L. 1987. H₂O₂, O₃ and SO₂ measurements in the lower troposphere over the eastern United States during fall. *J.Geophys.Res.*, 92(D1):915-931.

- Hewitt, C.N., Kok, G.L. and Fall, R. 1990a. Hydroperoxides in plants exposed to ozone mediate air pollution damage to alkene emitters. *Nature*, 344:56-58.
- Hewitt, C.N., Lucas, P., Wellburn, A.R. and Fall, R. 1990b. Chemistry of ozone damage to plants. *Chem. Ind.* (6/8):478-481.
- Hewitt, E.J. and Smith, T.A. 1974. Plant Mineral Nutrition. English Universities Press Ltd, London.
- Hewitt, H.G. and Ayres, P.G. 1976. Effect of infection by *Microsphaera alphitoides* (powdery mildew) on carbohydrate levels and translocation in seedlings of *Quercus robur*. *New Phytol.*, 77:379-390.
- Hoffman Jr., W.A., Lindberg, S.E. and Turner, R.R. 1980a. Precipitation acidity: the role of the forest canopy in acid exchange. *J. Environ. Qual.*, 9(1):95-100.
- Hoffman Jr., W.A., Lindberg, S.E. and Turner, R.R. 1980b. Some observations of organic constituents in rain above and below a forest canopy. *Env. Sci. Tech.*, 14(8):999-1002.
- Holland, M.R., Mueller, P.W. and Rutter, A.J. 1989. Effects of SO₂ and O₃ on tree growth. A report on measurements of tree growth in the Liphook forest fumigation experiment, taken between March 1985 and October 1988. Dept. Biology, Imperial College of Science, Technology and Medicine. ICTP/LIP/1.
- Hutchinson, T.C., Adams, C.M. and Gaber, B.A. 1986. Neutralisation of acidic raindrops on leaves of agricultural crop and boreal forest species. *Water, Air, Soil Pollut.*, 31:475-481.
- Hutchinson, T.C. and Adams, C.M. 1987. Comparative abilities of leaf surface to neutralise acidic raindrops. I. The influence of calcium nutrition and charcoal-filtered air. *New Phytol.*, 106:169-183.
- Ibusuki, I. 1983. Influence of trace metal ions on the determination of hydrogen peroxide in rainwater by using chemiluminescent techniques. *Atmos. Environ.*, 17(2):393-396.
- Irving, P.M. 1983. Acidic precipitation effects on crops: a review and analysis of research. *J. Environ. Qual.*, 12(4):442-454.
- Jacob, D.J., Waldman, J.M., Munger, J.W. and Hoffman, M.R. 1984. A field investigation of physical and chemical mechanisms affecting pollutant concentrations in fog droplets. *Tellus*, 36B:272-285.
- Jacob, P., Neftel, A. and Klockow, D. 1987. Die peroxyoxalat-chemilumineszenz und ihre anwendung zur bestimmung von wasserstoffperoxid in der außenluft und ihren niederschlägen. *VDI (Ber.)*, 608:377-399.

- Jacobson, J.S. 1984. Effects of acidic aerosol, fog, mist and rain on crops and trees. *Phil.Trans.R.Soc.Lond.*, B 305:69-78.
- Jacobson, J.S., Lassoie, J.F., Osmeloski, J. and Yamada, K. 1989. Changes in foliar elements in red spruce seedlings after exposure to sulphuric and nitric acid mist. *Water, Air, Soil Pollut.*, 48:141-159.
- Jayne, J.T., Gardner, J.A., Davidovits, P., Worsnop, D.R., Zahniser, M.S. and Kolb, C.E. 1990. The effect of hydrogen peroxide content on the uptake of sulphur dioxide (g) by aqueous droplets. *J.Geophys.Res.(Atmos.)*, 95(D12):20559-20563.
- Johnson, A.H. and Siccama, T.G. 1983. Acid deposition and forest decline. *Environ.Sci.Tech.*, 17(7):294A-304A.
- Joslin, J.D., McDuffie, C. and Brewer, P.F. 1988. Acidic cloudwater and cation loss from red spruce foliage. *Water, Air, Soil Pollut.*, 39:355-363.
- Juniper, B.E. 1960. Growth, development and effect of the environment on the ultra-structure of plant surfaces. *J.Linn.Soc.(Bot.)*, 56:413-419.
- Kelly, T.J., Daum, P.H. and Schwartz, S.E. 1985. Measurements of peroxides in cloudwater and rain. *J.Geophys.Res.*, 90(D5):7861-7871.
- Kender, W.J., Taschenberg, E.F. and Schaulis, N.J. 1983. Benomyl protection of grapevines from air pollution injury. *Hort.Sci.*, 8(5):396-398.
- Kleinman, L.I. 1984. Oxidant requirements for the acidification of precipitation. *Atmos.Environ.*, 18(7):1453-1457.
- Klockow, D. and Jacob, P. 1986. The peroxyoxalate chemiluminescence and its application to the determination of hydrogen peroxide in precipitation. In *Chemistry of Multiphase Atmospheric Systems*. W.Jaeschke (ed.). NATO ASI Series Vol.G6. Springer-Verlag, Berlin Heidelberg, pp 117-130.
- Kok, G.L. 1980. Measurements of hydrogen peroxide in rainwater. *Atmos.Environ.*, 14:653-656.
- Kok, G.L., Darnell, K.R., Winer, A.M., Pitts, J.N. and Gay, B.W. 1978a. Ambient air measurements of hydrogen peroxide in the California south coast air basin. *Environ.Sci.Technol.*, 12(9):1077-1080.
- Kok, G.L., Holler, T.P., Lopez, M.B., Nachtrieb, H.A. and Yuan, M. 1978b. Chemiluminescent method for determination of hydrogen peroxide in the ambient atmosphere. *Environ.Sci.Tech.*, 12(9):1072-1076.

- Kozlowski,T.T., Kramer,P.J. and Pallardy,S.G. 1991. The Physiological Ecology of Woody Plants. Academic Press Inc.,U.S.A.
- 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,Soil Pollut.*, 31:647-668.
- Larsen,B.R. 1986. *In vivo* buffering and concentration of simulated acid rain drops on leaves of selected crops. *Water, Air, Soil Pollut.*, 31:401-407.
- Larson,R.A. 1988. The antioxidant of higher plants. *Biochem.*,27(4):969-978.
- Lazrus,A.L., Kok,G.L., Gitlin,S.N. and Lind,J.A. 1985. Automated fluorometric method for hydrogen peroxide determination in atmospheric precipitation. *Anal.Chem.*,57:917-922.
- Lee,J.J. and Weber,D.E. 1979. The effect of simulated acid rain on seedling emergence and growth of eleven woody species. *Forest Sci.*,25(3):393-398.
- Lee,J.J., Neely,G.E., Perrigan,S.C. and Grothaus,L.C. 1981. Effect of simulated sulphuric acid rain on yield, growth and foliar injury of several crops. *Env.Exp.Bot.*,21(2):171-185.
- Lee,Y.-N., Shen,J., Klotz,P.J., Schwartz,S.E. and Newman,L. 1986. Kinetics of hydrogen peroxide-sulphur(IV) reaction in rainwater collected at a northeastern U.S. site. *J.Geo-phys.Res.*, 91(D12):13,264-13,274.
- Leonardi,S. and Fluckiger,W. 1989. Effects of cation leaching on mineral cycling and transpiration: investigations with beech seedlings, *Fagus sylvatica* L. *New Phytol.*,111:173-179.
- Lindberg,S.E., Silsbee,D., Schaefer,D.A., Owens,J.G. and Petty,W. 1988. A comparison of atmospheric exposure conditions at high and low elevation forests in the southern Appalachian mountain range. In *Acid Deposition at High Elevation Sites*. M.H.Unsworth and D.Fowler (eds.). NATO ASI Series C, vol.,252. Kluwer Academic Publishers, Dordrecht. pp 321-345.
- Lindberg,S.E., Harriss,R.C., Hoffman Jr.,W.A., Lovett,G.M. and Turner,R.R. 1989. Atmospheric chemistry, deposition and canopy interactions. In *Analysis of Biogeochemical Cycling in Walker Branch Watershed*. D.W.Johnson and R.I.van Hook (eds.). Springer-Verlag,NY. pp96-163.
- Ling,K.A. and Ashmore,M.R. 1987. Acid Rain and Trees. NCC Report No.19. Nature Conservancy Council, Peterborough, U.K.
- Loehle,C. 1988. Forest decline: endogenous dynamics, tree defences and the elimination of spurious correlation. *Vegetatio*,77:65-78.

- MacDonald,N.W., Hart Jr.,J.B. and Nguyen,P.V. 1986. Simulated acid rain effects on jack pine seedling establishment and nutrition. *Soil Sci.Soc.Am.J.*, 50(1):219-225.
- MacFarlane,J.C. and Berry,W.L. 1974. Cation penetration through isolated leaf cuticles. *Plant Physiol.*,53:723-727.
- Mahendrappa,M.K. 1983. Chemical characteristics of precipitation and hydrogen ion input in throughfall and stemflow under some eastern Canadian forest stands. *Can.J.For.Res.*,13:948-955.
- Mallant,R.K.A.M., Slanina,J., Masuch,G. and Kettrup,A. 1986. Experiments on H₂O₂-containing fog exposures of young trees. In *Aerosols*. S.D.Lee, T.Schneider, L.D.Grant and P.J.Verkerk (eds.). Lewis Publishers, Chelsea, Michigan, USA. pp 901-909.
- Mallant,R.K.A.M., Slanina,J., Masuch,G. and Kettrup,A. 1988. Effects of H₂O₂-containing acidic fog on young trees. In *Air Pollution and Ecosystems*. P.Mathy (ed.). D.Reidel, Dortrecht.
- Martin,L.R. and Damschen,D.E. 1981. Aqueous oxidation of sulphur dioxide by hydrogen peroxide at low pH. *Atmos.Environ.*, 15(9):1615-1621.
- Masuch,G., Kettrup,A., Mallant,R.K.A.M. and Slanina,J. 1986. Effects of H₂O₂-containing acidic fog on young trees. *Intern.J.Environ.Anal.Chem.*, 27:183-213.
- Masuch,G., Kicinski,H.G., Dulme,W. and Kettrup,A. 1989. Hydrogen peroxide dissolved in acidic fog as air pollutant. *Intern.J.Environ.Anal.Chem.*, 37:161-185.
- Matkin,O.H. and Chandler,P.A. 1957. The U-C type soil mixes. In *The UC system for producing healthy container-grown plants*. K.F.Baker (ed.). University of California Agricultural Experimental Station, Extension Service Manual No.23.pp68-85.
- McElroy,W.J. 1986. Sources of hydrogen peroxide in cloudwater. *Atmos.Environ.*, 20(3):427-438.
- McLaughlin,S.B. 1985. Effects of air pollution on forests: a critical review. *J.Air.Poll.Contr.Ass.*, 35(5):512-534.
- Mecklenburg,R.A., Tukey Jr.,H.B. and Morgan,J.V. 1966. A mechanism for the leaching of calcium from foliage. *Plant Physiol.*, 41:610-613.
- Mehlhorn,H., Cottam,D.A., Lucas,P.W. and Wellburn,A.R. 1987. Induction of ascorbate peroxidase and glutathione reductase activities by interactions with air pollutants. *Free Radical Res.Comm.*, 3:193-197.

- Mehlhorn, H., Jones, C.A. and Ashmore, M.R. 199-. The effect of hydrogen peroxidase on antioxidant levels in Sitka spruce. *In press*.
- Miller, H.G. 1984. Effects of acidic deposition on forest soil and vegetation. *Phil.Trans.R.Soc.Lond.*, B305:369-382.
- 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*. M.H.Martin and M.H.Unsworth (eds.). Blackwell Scientific Publishers. pp171-180.
- Moller, D. 1988. Production of free radicals by an ozone-alkene reaction - a possible factor in the new-type forest decline. *Atmos.Environ.*, 22(11):2607-2611.
- Moller, D. 1989. The possible role of H₂O₂ in new-type forest decline. *Atmos.Environ.*, 23(7):1625-1627.
- Mooney, H.A. and Winner, W.E. 1988. Carbon gain, allocation and growth as affected by atmospheric pollutants. In *Air Pollution and Plant Metabolism*. S.Schulte-Hostede, N.M.Dar-rall, L.W.Blank and A.R.Wellburn (eds.). Elsevier Scientific Publishers Ltd. pp272-287.
- Morrison, I.K. 1984. Acid rain: a review of literature on acid deposition effects in forest ecosystems. *Forestry Abs.*, 45(8):483-506.
- Mrose, H. 1966. Measurements of pH and chemical analyses of rain, snow and fogwater. *Tellus*, 18(2):266-270.
- Musselman, R.C. and Sterrett, J.L. 1988. Sensitivity of plants to acidic fog. *J.Envi-ron.Qual.*, 17(2):329-333.
- Neighbour, E.A., Cottam, D.A. and Mansfield, T.A. 1988. Effects of sulphur dioxide and nitrogen dioxide on the control of water loss by birch (*Betula* spp.). *New Phytol.*, 108:149-157.
- Olszyna, K.J., Meagher, J.F. and Bailey, E.M. 1988. Gas phase, cloud and rainwater measu-remnts of hydrogen peroxide at a high elevation site. *Atmos.Environ.*, 22(5):1699-1706.
- Oren, R., Schulze, E.-D., Werk, K.S. and Meyer, J. 1988. Performance of two *Picea abies* (L.) Karst. stands at different stages of decline. VII. Nutrient relations and growth. *Oecologia*, 77:163-173.
- Papple, D.J. and Onrod, D.P. 1977. Comparative efficiency of ozone injury suppression by benomyl and carboxin on turfgrasses. *J.Amer.Soc.Hort.Sci.*, 102(6):792-796.

- Parker, G.G. 1983. Throughfall and stemflow in the forest nutrient cycle. *Adv.Ecol.Res.*, 13:57-133.
- Penkett, S.A., Jones, B.M.R., Brice, K.A. and Eggleton, A.E.J. 1979. The importance of atmospheric ozone and hydrogen peroxide in oxidising sulphur dioxide in cloud and rainwater. *Atmos.Environ.*, 13:123-137.
- Penkett, S.A. 1986b. Hydrogen peroxide in cloudwater. *Nature*, 319:624.
- Percy, K.E. 1986. The effects of simulated acid rain on germinative capacity, growth and morphology of forest tree seedlings. *New.Phytol.*, 104:473-494.
- Percy, K.E. 1987. Effects of simulated acid rain on leaf cuticular characteristics and surface properties. PhD Thesis. University of Bristol.
- 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 Phytol.*, 107:577-589.
- Percy, K.E. and Baker, E.A. 1988. Effects of simulated acid rain on leaf wettability, rain retention and uptake of some inorganic ions. *New Phytol.*, 108:75-82.
- Percy, K.E. and Baker, E.A. 1990. Effects of simulated acid rain on epicuticular wax production, morphology, chemical composition and on cuticular membrane thickness in two clones of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. *New Phytol.*, 116:79-87.
- Radojevic, M. 1983. H_2O_2 in the atmosphere and its role in the aqueous oxidation of SO_2 . *Environ.Tech.Lett.*, 4:499-508.
- Reich, P.B., Schoettle, A.W., and Amundson, R.G. 1986. Effects of O_3 and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings. *Environ.Pollut.(Ser.A)*, 40:1-15.
- Reich, P.B., Schoettle, A.W., Stroo, H.F., Troiano, J. and Amundson, R.G. 1987. Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. I. Net photosynthesis and growth. *Can.J.Bot.*, 65:977-987.
- Review Group on Acid Rain (RGAR). Acid Deposition in the U.K. 1981-1985. Warren Spring Laboratory, Stevenage, U.K. 1987.
- Riding, R.T. and Percy, K.E. 1985. effects of SO_2 and other air pollutants on the morphology of epicuticular waxes on needles of *Pinus strobus* and *Pinus banksiana*. *New Phytol.*, 99:555-565.

- Roberts,T.M., Skeffington,R.A. and Blank,L.W. 1989. Causes of Type 1 spruce decline in Europe. *Forestry*,62:179-222.
- Sakugawa,H., Kaplan,I.R., Tsai,W., Cohen,Y. 1990. Atmospheric hydrogen peroxide. *Environ.Sci.Technol.*, 24(10):1452-1462.
- Schaefer,D.A. and Reiners,W.A. 1990. Throughfall chemistry and canopy processing mechanisms. In *Acidic Precipitation. Volume 3: Sources, Deposition and Canopy Interactions*. S.E.Lindberg, A.L.Page and S.A.Norton (eds.). Springer-Verlag, New York. pp 241-284.
- Scherbatskoy,T. and Klein,R.M. 1983. Response of spruce and birch foliage to leaching by acidic mists. *J.Environ.Qual.*,12(2):189-195.
- Scherbatskoy,T. and Tyree,M.T. 1990. Kinetics of exchange of ions between artificial precipitation and maple leaf surfaces. *New Phytol.*,114:703-712.
- Sigg,A. and Neftel,A. 1991. Evidence for a 50% increase in H₂O₂ over the past 200 years from a Greenland ice core. *Nature*,351:557-559.
- Skeffington,R.A. and Roberts,T.M. 1985. The effects of ozone and acid mist on Scots pine seedlings. *Oecologia (Berl.)*,65:201-206.
- 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 *Picea sitchensis* and heather *Calluna vulgaris*. *Environ.Pollut.(Ser.B)*, 11:255-270.
- Slanina,J., Mallant,R.K.A.M., Masuch,G. and Kettrup,A. 1985. The role of clouds, fogs and dew in tree die-back. In *Physico-Chemical Behaviour of Atmospheric Pollutants*. B.Versino and H.Ott (eds.). D.Reidel,U.S.A.
- Sokal,R.R. and Rohlf,F.J. 1981. Biometry. W.H.Freeman and Co., NY.
- Tanaka,,K., Suda,Y., Kondo.,N. and Sugahara,K. 1985. O₃ tolerance and the ascorbate dependent H₂O₂ decomposing system in chloroplasts. *Plant, Cell Physiol.*,26(7):1425-1431.
- Taylor,G. and Dobson,M.C. 1989. Photosynthetic characteristics, stomatal responses and water relations of *Fagus sylvatica*: impact of air quality at a site in southern Britain. *New Phytol.*,113:265-273.
- Thompson,A.M., Owens,M.A. and Stewart,R.A. 1989. Sensitivity of tropospheric hydrogen peroxide to global chemical and climate change. *Geophys.Res.Lett.*, 16(1):53-56.

- Tukey Jr., H.B. 1970. The leaching of substances from plants. *Ann. Rev. Plant Physiol.*, 71:305-324.
- Turner, D.P. and Tingey, D.T. 1990. Foliar leaching and root uptake of Ca, Mg and K in relation to acid fog effects on Douglas fir. *Water, Air, Soil Pollut.*, 49:205-214.
- Turunen, M. and Huttunen, S. 1990. A review of the response of epicuticular wax of conifer needles to air pollution. *J. Environ. Qual.*, 19:35-45.
- Unsworth, M.H. 1984. Evaporation from forests in cloud enhances the effects of acid deposition. *Nature*, 312:262-264.
- Vong, R.J., Sigmon, J.T. and Mueller, S.F. 1991a. Cloudwater deposition to Appalachian forests. *Environ. Sci. Tech.*, 25(6):1014-1021
- Waldman, J.M., Munger, J.W., Jacob, D.J. and Hoffman, M.R. 1983. Fogwater composition in southern California. In *Precipitation Scavenging, Dry Deposition and Resuspension*, vol. 1. H.R. Pruppacher, R.G. Semonin, W.G.N. Slinn (eds.). Elsevier Science Publishing Co., New York. pp137-146.
- Waldman, J.M., Munger, J.W., Jacob, D.J. and Hoffman, M.R. 1985. Chemical characterisation of stratus cloudwater and its role as a vector for pollutant deposition in a Los Angeles pine forest. *Tellus*, 37B:91-108.
- Waldman, J.M. and Hoffman, M.R. 1988. Nutrient leaching from pine needles impacted by acidic cloudwater. *Water, Air, Soil Pollut.*, 37:193-201.
- Watterson, J.D. 1989. Interception and Retention of Aerosols by Vegetation in Relation to their Surface Cuticular Characteristics. PhD Thesis, Imperial College of Science, Technology and Medicine.
- Westman, W.E. and Temple, P.J. 1989. Acid mist and ozone effects on the leaf chemistry of two western conifer species. *Environ. Pollut.*, 57:9-26.
- Wisniewski, J. 1982. The potential acidity associated with dews, frosts and fogs. *Water, Air, Soil Pollut.*, 17:361-377.
- Wood, T. and Bormann, F.H. 1974. The effects of an artificial acid mist upon the growth of *Betula alleghaniensis* Britt. *Environ. Pollut.*, 7:259-267.
- 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, Soil Pollut.*, 7:479-488.

- Wolfe, M.H., Joslin, J.D. and Brewer, P.F. 1987. Cloudwater interactions with high elevation spruce forests. In *Proc. 4th Annual Gatlinburg Acid Rain Conf.*, Gatlinburg, TN. 26-27 Oct. Tennessee Valley Authority, Knoxville, TN, U.S.A. p15.
- Yoshizumi, K., Aoki, K., Nouchi, I., Okita, T., Kobayashi, T., Kamakura, S. and Tajima, M. 1984. Measurements of the concentration in rainwater and of the Henry's Law constant of hydrogen peroxide. *Atmos. Environ.*, 18(2):395-401.
- Zika, R., Saltzman, E., Chameides, W.L. and Davis, D.D. 1982. H₂O₂ levels in rainwater collected in south Florida and the Bahamas Islands. *J. Geophys. Res.*, 87(C7):5015-5017.
- Zoetl, H.W., Huettl, R.F., Fink, S., Tomlinson, G.H. and Wisniewski, J. 1989. Nutritional disturbances and histological changes in declining forests. *Water, Air, Soil Pollut.*, 48:87-109.