ASSESSMENT REPORT ON
SULPHUR DIOXIDE
FOR DEVELOPING
AMBIENT AIR QUALITY
OBJECTIVES

**EFFECTS ON
VEGETATION**

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SULPHUR DIOXIDE
FOR DEVELOPING
AMBIENT AIR QUALITY OBJECTIVES**

- Effects on Vegetation

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PREFACE

A multi-stakeholder workshop was held in October 2000 to obtain input from stakeholders on priorities for development or review of ambient air quality guidelines (AAQG). Workshop participants identified the sulphur dioxide ambient air quality guideline as one of the existing guidelines that required review. This document presents sulphur dioxide effects on vegetation and is one of two science assessment documents for sulphur dioxide.

The purpose of this document is to summarize selected primary literature on the effects of SO₂ exposure on vegetation. This document is not intended to be a comprehensive review of the published literature. Instead an attempt was made to select work that would provide relevant information for reviewing the existing ambient air quality objective (AAQO) for SO₂ for Alberta. Basic criteria for selecting studies included:

- Ambient and/or experimental SO₂ concentrations were provided
- The use of realistic SO₂ exposure concentrations
- Species native (or similar) to ones grown in Alberta
- Measurements of vegetation effects were quantifiable and repeatable

Part I provides an over view of studies conducted prior to 1990. Although an attempt was made to select studies that used realistic concentrations of SO₂, many of the early studies discussed in Part I used high SO₂ concentrations (many exceeded 1,000 µg m⁻³). However, as the understanding of sulphur dioxide effects on plants advanced, the concentrations of SO₂ used in research were generally reduced to more realistic levels, many that might be observed in the environment. Part II presents literature from 1990 to 2002, in which the majority of the studies used more realistic concentrations of SO₂.

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LIST OF ABBREVIATIONS

AAQO	Ambient air quality objective(s)
AAQG	Ambient air quality guideline(s)
ABA	Absciscic acid
ATP	Adenosine triphosphate
avg	Average
CF	Charcoal filtered
CO ₂	Carbon dioxide
Cu	Copper
cv	Cultivar
d	Day(s)
DW	Dry weight
FBPase	Fructose-1,6-bisphosphatase
FeSOD	Iron superoxide dismutase
FC	Fusicoccin
g	Gram
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate-pyruvate transaminase
>	Greater than
GSH	Reduced glutathione
h	Hour(s)
HCl	Hydrogen chloride or hydrochloric acid
HSO ₃ ⁻	Hydrogen sulphite ion (bisulphite)
IAP	Index of atmospheric purity
K	Potassium
km	Kilometre
kPa	Kilopascals
<	Less than
L	Litre
m	Meter
min	Minute(s)
µg m ⁻³	Micrograms per cubic meter
mon	Month(s)
N	Nitrogen
Na ₂ S ₂ O ₅	Sodium hydrogensulfite
NAAQO	National Ambient Air Quality Objectives

NADP-GPD	NADP-dependent glyceraldehyde-phosphate dehydrogenase
NF	Non-filtered
Ni	Nickel
NO ₂	Nitrogen dioxide
O ₃	Ozone
P	Phosphorous
PES	Energy used for photosynthesis
PGK	Phosphoglycerate kinase
PMCs	Pollen mother cells
PPB	Parts per billion
PPM	Parts per million
PRK	Phosphoribulokinase
RH	Relative humidity
RuDPC	Ribulose-diphosphate carboxylase
S	Sulphur
SO ₂	Sulphur dioxide
SO ₃ ²⁻	Sulphite ion
SO ₄ ²⁻	Sulphate anion
SOD	Superoxide dismutase
VAM	Vesicular arbuscular mycorrhizae
wk	Week(s)
y	Year(s)

1.0 INTRODUCTION

Sulphur (S) is one of the essential elements plants require for growth and reproduction. Plant roots can absorb sulphate anions (SO_4^{2-}) from the soil; alternatively, leaves can absorb sulphur dioxide (SO_2) from the atmosphere. When soil S is inadequate, atmospheric SO_2 can be used as an alternate S source. However, uptake of excess S from the soil or the atmosphere, or a combination of the two can result in injury to vegetation.

When exposed to air pollutants the amount of pollutant that enters the plant has the greatest effect on the degree of injury. In addition, many biotic and abiotic factors play a role in the degree of damage when plants are exposed to SO_2 . Biotic factors include growth stage, genetic make-up, plant nutrient status, insects, and disease. Abiotic factors include light, moisture availability, temperature, relative humidity, and the presence of other air pollutants.

The major route of atmospheric gases, including SO_2 , into plants is through the stomata (apertures in the leaf epidermis, which can open and close to control gas exchange). When exposed to SO_2 , some plants close the stomates thus reducing SO_2 uptake, but not all plants close their stomates upon exposure (Black, 1985). Water stress can also result in reduced stomatal opening, which would result in reduced SO_2 uptake. In order to enter through open stomata, SO_2 must first diffuse across a layer of “unstirred” air surrounding the leaf called the boundary layer. Increased air movement can decrease the diffusive resistance of the boundary layer (Salisbury and Ross, 1992) resulting in increased SO_2 uptake.

After absorption, SO_2 dissolves in the aqueous phase of the cell wall to form bisulphite (HSO_3^-) or sulphite (SO_3^{2-}), which then undergoes enzymatic conversion to SO_4^{2-} . The SO_4^{2-} is then transported into leaf cells where it is incorporated into organic molecules, including amino acids such as cysteine and methionine, which are then incorporated into proteins (Rennenberg and Herschbach, 1996). When excess SO_2 enters the plant the resulting injury may:

- become visible as foliar injury (e.g. necrotic or dead tissue, chlorosis)
- result in disruption of metabolic or physiological processes, which lead to growth and yield reductions
- result in accumulation of excess S in plant tissues.

Sulphur dioxide injury to vegetation is characterized as being a result of either acute or chronic exposure and is generally thought of as follows (Legge *et al.*, 1998):

- Acute exposures are a few minutes to a few hours at concentrations that result in visible injury (chlorosis or necrosis of tissue) within a few hours or days.
- Chronic exposures are long-term (weeks to years), to low concentrations that result in metabolic injury and may or may not result in visible injury.

The concentrations of SO₂ in the literature were reported using various units, for example: parts per billion (ppb), parts per million (ppm), or micrograms per meter cubed (µg m⁻³). To facilitate comparison between studies all concentrations were converted to µg m⁻³, assuming standard temperature (25°C) and pressure (101.3 kPa).

The report has been divided into sections based on the effects of sulphur dioxide on different aspects of plant physiology (e.g. sulphur content, visible injury, growth); however, many of the studies examined more than one of these possible effects. For ease of reading, each study is included in only one section of the report.

PART I

2.0 LITERATURE PRIOR TO 1990

Physiological changes in plants in response to exposure to SO₂ can involve from a few cells to the whole plant. These changes can include: reduced photosynthetic activity, increased respiration, alteration of enzyme production, changed growth (increased or decreased), and/or altered uptake and accumulation of nutrients (including S). The extent of change is a result of the concentration of SO₂ and exposure duration, the relative sensitivity of the plant to SO₂, and what mechanisms the plant can employ to detoxify SO₂, or the ability of the plant to repair damage that has occurred due to exposure. The following studies describe the effects that can occur as a result of exposure to SO₂.

2.1 Effects on Growth

Cowling and Lockyer (1976) studied the effects of SO₂ exposure on the growth and sulphur nutrition of perennial ryegrass (*Lolium perenne* L.). They compared the growth of plants exposed for 87 d, in chambers, to a daily average of 50 µg m⁻³ SO₂ or filtered air, with or without adequate soil sulphur. Plants grown without additional soil S exhibited S deficiency symptoms and growth was reduced. Exposure to SO₂ increased the root and shoot dry weight of plants grown in sulphate deficient soil, but did not restore growth to levels observed with adequate soil S. However, SO₂ exposure did not impact the shoot growth of plants grown in sulphate-sufficient soil. Shoot sulphur declined in all treatments throughout the exposure period, but exposure to SO₂ lessened the decline. It was also observed that exposure to SO₂ resulted in an increase of shoot sulphate-S as a proportion of the total S. It was indicated that although SO₂ was used as a S supply, in these experiments only the soil applied sulphate provided adequate sulphur (Table 1).

In a further study on how nutrient supply influences the effect of SO₂ on perennial ryegrass, Cowling and Lockyer (1978) exposed plants for 85 d, in chambers, to a daily average of 55 µg m⁻³ SO₂ or filtered air. Plants were grown with or without added soil sulphur and two rates of added nitrogen (N: low and high). Plants grown with high N and no added soil S developed S deficiency symptoms. The S deficiency symptoms were reduced in the high N treatment in the presence of SO₂ while shoot dry weight more than doubled and root dry weight almost doubled. When high N plants were supplied with soil sulphate, the addition of SO₂ to the atmosphere did not alter root or shoot dry weight. The number of tillers increased with increased N, with added soil sulphate, and when exposed to SO₂. There was little difference in shoot or root dry weights and number of tillers in the low N treatments in the presence or absence of SO₂. Total shoot sulphur and sulphate-S generally increased with the addition of soil sulphate and exposure to SO₂; although, high N treatments had lower levels of both which was attributed to dilution, as the high N plants were larger (Table 1).

The effects of SO₂ exposure on red pine (*Pinus resinosa* Alt) seedlings in the cotyledon stage were examined to determine possible effects on seedling establishment (Constantinidou *et al.*, 1976). Seedlings were harvested 11 weeks after exposure to 1,310, 2,619, 7,857 or 10,476 µg m⁻³ SO₂ in Plexiglas exposure cubes for 15, 30, 60 or 120 minutes. Exposure of cotyledons resulted in reduced chlorophyll content which decreased as exposure concentration or time increased. Cotyledon dry weight also decreased as exposure concentration and time increased, with significant differences from controls in the highest two concentrations for the 60 and 120 minute exposures. Analysis indicated that exposure time was more important than concentration for chlorophyll and dry weight of cotyledons. Primary needles exhibited the same pattern of response as the cotyledons (chlorophyll content and dry weight decreased as exposure time or concentration increased). In addition, emergence of primary needles appeared to be inhibited but the effect was not significant. Visible injury was apparent within 1 day of fumigation in 15% (7,857 µg m⁻³ SO₂) and 25% (10,476 µg m⁻³ SO₂) of seedlings when exposed for 60 or 120 minutes (Table 1).

Ashenden and Mansfield (1977) recognized that air movement could influence SO₂ uptake into plants so they investigated the influence of wind speed on the effects of SO₂ on perennial ryegrass (*Lolium perenne* L.). Plants were exposed in wind tunnels to 288 µg m⁻³ SO₂ at two wind speeds, 10 and 25 m min⁻¹. Each experiment was conducted twice, and day and night temperatures varied slightly between the experiments, which altered plant growth but did not change the pattern of response. When exposed to SO₂ with a wind speed of 25 m min⁻¹ there were significant reductions in leaf area, root/shoot ratio, and dry weight of: green leaf material, dead leaves + stubble, total shoot, and roots. In contrast, dry weights of plants exposed with a wind speed of 10 m min⁻¹ were not significantly reduced but there was a significant increase in the number of fully expanded green leaves. The authors concluded that the greater sensitivity to SO₂ exhibited at the higher wind speed was probably a result of reduced boundary layer resistance of the leaf (Table 1).

Roberts (1975) compared the growth of seedlings of SO₂-sensitive white birch (*Betula papyrifera* Marsh) and SO₂-tolerant pin oak (*Quercus palustris*). Seedlings were grown in pots (sunk into the ground and mulched), in the field, where they were exposed for three months to SO₂ levels of <10 (ambient) or 70.8 µg m⁻³ (annual averages). Plants were harvested biweekly from mid-June until the end of August. Dry weight of white birch leaves, stems, and roots showed no significant differences until the mid-August harvest, at which time there was a significant increase in leaf and stem weights of plants in high SO₂ compared to low SO₂. Final height of the birch seedlings was also significantly greater in the high SO₂ plots. The author thought that the increase in growth of birch was due to the additional S supply. In contrast, pin oak height was significantly less and dry weight was reduced in the high SO₂ compared to the low SO₂ treatment. It was suggested that the reduction in growth of pin oak was probably due to stomatal closure (a common resistance mechanism observed in SO₂ resistant species) in response to high ambient SO₂ exposure, which resulted in reduced photosynthesis (Table 1).

2.2 Sulphur Dioxide Uptake and Plant Sulphur Content

When exposed to SO₂ there can be wide variation in the absorption of and accumulation of sulphur compounds between plant species. The following studies describe some of the differences that have been observed.

In a study on the capacity of alfalfa (*Medicago sativa* L.) to neutralize and metabolize absorbed SO₂, Thomas *et al.* (1944) found that exposures of 628 to 338 h, to 492 or 762 µg m⁻³ SO₂ respectively, did not change the pH of leaf extracts but did reduce their buffering capacity. The plants were able to neutralize the absorbed acid, which is one mechanism of SO₂ resistance. It was also found that the absorbed SO₂ was transformed principally to sulphate and small amounts of organic S compounds. In addition, alfalfa and sugar beets (*Beta vulgaris*) were exposed to 487 µg m⁻³ SO₂ for 5.2 h d⁻¹, for 60 days, while being grown under various nutrient regimes. When the nutrient solution was deficient in sulphate, fumigated leaves had higher levels of labile organic sulphur than the non-fumigated leaves. When sulphate was at sufficient levels in the nutrient solution there was little difference in labile S between treatments (Table 1).

The differences in SO₂ absorption of four cultivars of cucumber (*Cucurbitaceae*) were studied after exposure to SO₂ (1,048 to 23,571 µg m⁻³ for 360 to 1,430 minutes) (Bressan *et al.*, 1977). Sulphur dioxide concentrations used in this study were much higher than would be expected to occur at a polluted site but the authors were able to show that the most resistant cultivars absorbed the least SO₂. Differential absorption between the cultivars could not be attributed to stomatal behaviour, as it was not examined in this study. It was also observed that young leaves were more resistant than old leaves but there was no difference in SO₂ absorption between the two. It was concluded that a biochemical mechanism conferred greater resistance to the younger leaves.

Miller and Xerikos (1979) treated 'sensitive' and 'tolerant' cultivars of soybean (*Glycine max* L. Merr.) in 0.08 M sodium sulphite (Na₂SO₃) solutions (equivalent to 10 mg ml⁻¹, or 26,190 µg m⁻³ SO₂) for 30 minutes to determine if the residence time of sulphite in plant cells relates to varietal or species resistance. The 'resistant' cultivars had consistently less sulphite at the end of the treatment period than 'sensitive' cultivars despite taking up more of the treatment solution. It was suggested that the reduction of sulphite levels was a result of conversion to sulphate and that 'resistant' cultivars metabolized sulphite more efficiently than 'sensitive' cultivars. A further test indicated that after a 30-minute treatment period, leaves of 'resistant' cultivars contained significantly less of the absorbed sulphite, 4.9%, compared to 11.5% in 'sensitive' cultivars.

2.3 Visible Injury

Symptoms of SO₂ injury can be described as either acute or chronic. Acute injury results from short-term exposures to high concentrations that result in cellular death of all or part of the plant (Legg *et al.*, 1998). Chronic injury is the response to long-term exposures to sub-lethal concentrations that result in alterations in cellular metabolism and may or may not exhibit visible injury (Legg *et al.*, 1998). The following studies investigated the effects of both acute and chronic exposures to SO₂ on the appearance of visible injury symptoms.

Katz and McCallum (1939) conducted a series of field plot experiments to determine the relative sensitivity of a number of conifer species to SO₂. The experiments used a number of SO₂ concentrations up to 13,933 µg m⁻³ with exposure times of a few hours to 69 d. The appearance of injury symptoms was influenced by a number of factors and varied by species and SO₂ exposure (see Table 1). No visible injury was observed on any of the exposed trees at 576 µg m⁻³ for up to a 69 d exposure (Oct 8 to Dec 18). Injury was delayed when exposures were conducted in late fall (Nov 23 to Dec 2). The observed delay in onset of injury was attributed to lowered plant activity as the temperature decreased. Relative humidity (RH) was shown to be one of the factors affecting injury as injury to western larch (*Larix occidentalis*) appeared in 8 h at 786 µg m⁻³ (May 29) at 67% RH but injury did not appear in a 76.5 h exposure up to 1,100 µg m⁻³ (May 29) at 45% RH. The conifers were ranked for their susceptibility to SO₂ using the degree of injury sustained along with the length of time for initial injury symptoms to appear. The species were ranked (in order of susceptibility) as follows: larch, Douglas fir, Engelmann spruce, white pine, yellow pine, cedar, lodgepole pine, silver fir and white fir. In addition, total needle sulphur content was determined in four of the experiments and results indicated that up to the time of incipient injury the rate of SO₂ absorption was influenced more by the concentration of SO₂ than the time of exposure (Table 1).

The results of a five year study by Linzon (1966) indicated that the deterioration of eastern white pine (*Pinus strobes* L.), in permanent plots to the northeast of Sudbury, correlated with the increase in atmospheric SO₂ as the distance to the source (smelter) decreased. When the distance to the smelter exceeded 25 miles and ambient SO₂ levels were consistently below 655 µg m⁻³ the health of the pines had not deteriorated. Linzon concluded that SO₂ higher than 655 µg m⁻³ for several hours could damage foliage of eastern white pine.

O'Connor *et al.* (1974) tested 131 native Australian plant species to determine their sensitivity to acute SO₂ exposure. Plants were exposed to 800 µg m⁻³ for 27 h, 2,620 µg m⁻³ for 3 and 6 h, 5,240 µg m⁻³ for 4 h, or 7,860 µg m⁻³ for 0.5, 1, 2, 4 and 6 h. The measure of sensitivity used was the percent of necrotic leaf tissue that had developed 3 or 4 days after fumigation. It was observed that most species showed increased sensitivity with increased concentrations of SO₂ or with increased exposure times. Also, "injury occurred most rapidly and extensively to fully-developed actively-photosynthesising leaves" while young expanding leaves were more resistant. It was also noted that sensitivity of species within genera could be highly variable in their response to acute exposure to SO₂.

Taylor *et al.* (1986) investigated physiological differences of two ecotypes of geranium (*Geranium carolinianum*) that differed in their sensitivity to SO₂. Although SO₂ uptake was equivalent between sensitive and resistant ecotypes, leaf necrosis (as % of the total leaf area) for the sensitive ecotype averaged 31.1% while the resistant ecotype averaged only 4.6% after 5 h exposure to 2,095 µg m⁻³ SO₂. Photosynthesis was measured every hour for 5 hours during exposure to 786 to 1,571 µg m⁻³ SO₂. Inhibition of photosynthesis in the resistant ecotype was dependent on concentration but independent of time. From 3 to 5 h exposure the percentage inhibition increased from an average of 11.5% at 786 µg m⁻³ to 29.9% at 1,571 µg m⁻³ SO₂. In contrast, photosynthesis in the sensitive ecotype was inhibited at all concentrations of SO₂, 786 µg m⁻³ and higher and the percentage inhibition increased significantly with exposure time. Measurement of efflux of hydrogen sulphide (H₂S) (one of the mechanisms plants use for removal of excess S) indicated the rate of efflux increased with SO₂ concentration but did not differ significantly between the ecotypes. Long-term exposure to 1,179 µg m⁻³ SO₂ for 6 h d⁻¹, 4 d wk⁻¹, for 4 weeks resulted in a 28% increase (resistant ecotype) and 26% decrease (sensitive ecotype) in photosynthesis from controls. In addition, photoassimilate retention in the foliage was reduced 6% in the resistant ecotype and increased 7% in the sensitive ecotype. Average shoot or root dry weight of the sensitive ecotype was 10 to 25% lower than the resistant ecotype at equivalent SO₂ concentrations and exposures. The results suggested the genetic differences between the ecotypes resulted in physiological expression of different biochemical threshold levels of response to SO₂, or homeostatic processes governing repair and compensation for injury (Table 1).

2.4 Other Metabolic Processes

Exposure of plants to SO₂ can result in disruption of normal metabolic activity. Enzymatic activity can be increased or decreased or photosynthesis and respiration can be altered. The degree of metabolic change is dependent on the concentration of SO₂ and the length of exposure. It is possible that metabolic disturbance can occur without visible signs of injury. Following are studies that indicate the broad range of metabolic response that may be observed when plants are exposed to SO₂.

Horsman and Wellburn (1977) used broad-leaved dock (*Rumex obtusifolius*) collected from areas of high ambient (annual mean 150 µg m⁻³) and low ambient (30 µg m⁻³) SO₂ to determine effects of SO₂ exposure on enzyme activities. Plants were fumigated with 524 µg m⁻³ SO₂ for 11 days in a wind-tunnel fumigation system. No visible injury was observed on any of the plants, and leaf fresh weights were not affected. Ribulose-diphosphate carboxylase (RuDPC) in low ambient area leaves was reduced to 65% (young) and 68% (old) of controls. Fumigated high ambient plants exhibited little change in RuDPC in either young (97%) or old leaves (109%). Peroxidase levels in the low ambient plants increased following exposure (140% young leaves, 135% old leaves). The high ambient area plants had significantly higher levels of peroxidase (>140%) than low ambient plants under control conditions and experienced relatively small changes in peroxidase following SO₂ exposure (114% young leaves, 105% old leaves). Glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) levels increased in fumigated young leaves (138% and 127% low ambient; 119% and 123% high

ambient), from both areas, while old leaves were not affected. It was concluded that the differences in enzyme activities between low and high ambient plants could be a result of development of sulphite tolerance in the high ambient plants and could be the reason for maintenance of RuDPC activity. The high activity of peroxidase in high ambient plants could reflect adaptation to the SO₂ levels of their natural environment (Table 1).

Harvey and Legge (1979) studied the effects of SO₂ on adenosine triphosphate (ATP) metabolism in lodgepole-jack pine (*Pinus contorta x banksiana*) hybrid foliage. Hybrid seedlings were grown under controlled chamber conditions then exposed in Siemens cuvettes to 200 to 800 µg m⁻³ SO₂ for 0.5 h. In addition, needles were collected from trees, growing around a natural gas processing plant near Whitecourt, Alberta, during SO₂ fumigations. Sulphur dioxide levels at the field site were normally 0 to 656 µg m⁻³ with the maximum level of SO₂ measured during this experiment being 542 µg m⁻³. It was found that ATP in needles of field samples decreased linearly as SO₂ increased. Branches collected from the field site and used for controlled fumigations in the laboratory showed a similar response. In contrast, the previously unexposed foliage grown in growth chambers did not normally show a reduction in ATP during fumigation. It was concluded that the difference in response could be explained by adaptation of the field grown trees to low-level SO₂ fumigation (Table 1).

Tanaka and Sugahara (1980) used poplar (*Populus euramericana*) and spinach (*Spinacia oleracea* L.) cv. New Asia to show that increased resistance to SO₂ can be correlated with high levels of superoxide dismutase (SOD) activity in leaves. Plants were exposed in fumigation chambers to the SO₂ concentrations and times indicated below. They observed that young poplar leaves had up to five times more SOD activity than old ones when exposed to 5,238 µg m⁻³ SO₂ for 22 hours. The higher SOD activities resulted in reduced chlorophyll destruction and lower malondialdehyde formation (a product of lipid peroxidation). In order to confirm the role of SOD in reducing SO₂ toxicity, diethyldithiocarbamate (DDTC, a SOD inhibitor) was applied to spinach leaves. This resulted in 65% reduction in SOD activities after 2 h exposure. Exposure of spinach leaves that had been treated with DDTC to 1,310 µg m⁻³ SO₂ for 22 h resulted in enhanced chlorophyll destruction over untreated leaves. In addition, it was shown that pre-treatment of poplar leaves with 262 µg m⁻³ SO₂ for 20 days increased SOD activities to 4.4 times the control. The pre-treatment resulted in a decrease in chlorophyll destruction when exposed to 5,238 µg m⁻³ SO₂ for up to 24 h (Table 1).

Shimazaki *et al.* (1980) presented further evidence that SOD plays a role in SO₂ resistance. Photosynthetic pigments and lipids of spinach (*Spinacia oleracea* L.) cv. New Asia were studied in a series of experiments to determine the role of active oxygen species in SO₂ toxicity. Whole plants were exposed in fumigation chambers to 5,238 µg m⁻³ SO₂ for the indicated times. Visible injury appeared around leaf veins 2-3 h after start of fumigation, in the light, and injured areas were completely white in 10 – 15 h; whereas, no injury occurred when leaves were exposed for up to 10 h in the dark. After 2 h exposure to SO₂, chlorophyll *a* and carotenoids were destroyed in the light but not in the dark. Anaerobic conditions reduced pigment destruction. When SOD was added to leaf reaction mixtures after 3 h fumigation, the destruction of chlorophyll *a* was reduced. After 2 h SO₂ fumigation the formation of malondialdehyde (a product of lipid peroxidation) was inhibited by the addition of scavengers of superoxide radicals (O₂⁻) (e.g. hydroquinone, tiron, or ascorbate). These results indicated that the destruction of

chlorophyll *a* required both light and oxygen. It was concluded that lipid peroxidation in fumigated leaves resulted from the action of singlet oxygen produced from O_2^- (Table 1).

Peiser *et al.* (1982) used ethane formation to test if lipid peroxidation in chloroplasts is induced by free radicals from sulphite oxidation. Isolated spinach chloroplasts (*Spinacia oleracea* L.) were incubated with solutions containing sulphate or sulphite and lipid peroxidation was measured by ethane production. In comparison to sulphate, it was found that sulphite produced approximately a 10-fold increase in ethane production in intact chloroplasts. They also observed that light was important for sulphite induced ethane production as little ethane was produced in the dark with or without sulphite. Ethane production was not inhibited in the presence of a scavenger of singlet oxygen but was inhibited by SOD, once again suggesting the involvement of O_2^- in lipid peroxidation. While this study cannot be compared directly with gaseous exposures, it does provide support for the role of SOD in protecting against oxidative stress resulting from sulphite oxidation.

Olszyk and Tingey (1984a) studied the effects of light on the phytotoxicity of SO_2 using garden pea (*Pisum sativum* L.) cv Alsweet and a tomato mutant (*Lycopersicon esculentum flacca* Mill.). To ensure similar internal SO_2 exposure in dark and light exposures, the peas were sprayed with fusicoccin (FC), which induces opening of stomata in both light and dark. The stomata of the tomato mutant do not close in the dark due to a deficiency in abscisic acid (ABA). Pea plants were exposed, in fumigation chambers, to 1,310 to 2,619 $\mu g\ m^{-3}$ SO_2 and tomato plants to 1,571 to 2,095 $\mu g\ m^{-3}$ SO_2 for 2 hours, in the dark and in the light. Exposure in the light resulted in up to 80% (pea) or 64% (tomato) less leaf injury compared to exposure in the dark and the reduction in injury was most prominent in the oldest leaves. It was concluded that light-activated metabolism may have detoxified SO_2 itself, or repaired the SO_2 injury. It was also noted that these results are only applicable to acute exposures as plants may respond differently to chronic exposures (Table 1).

Further experiments were performed using garden pea (*Pisum sativum* L.) cv Alsweet and a tomato mutant (*Lycopersicon esculentum flacca* Mill.) in an attempt to determine the possible role of cellular transport processes in SO_2 toxicity (Olszyk and Tingey, 1984b). Plants were sprayed with fusicoccin (FC) to ensure stomata were open during fumigation. Peas were exposed to 1,833, 2,357 and 2,619 $\mu g\ m^{-3}$ SO_2 and tomato to 1,571 $\mu g\ m^{-3}$ SO_2 for 2 hours, in the light. When tomato was exposed to SO_2 , FC treated plants had an average 21% necrotic leaf area; whereas, plants not treated with FC had significantly less injury, only 3% of the leaf area. Exposure to SO_2 of FC treated peas resulted in 2 to 8 times more leaf injury than untreated plants. The results of these experiments led the researchers to conclude that in addition to ensuring the stomata were open, FC stimulated the movement of SO_2 or one of its metabolites across the plasma membrane, which resulted in the enhancement of SO_2 injury (Table 1).

2.5 Effects on Pollen

As many studies had shown that exposure to SO₂ damaged foliage, reduced growth, and caused metabolic and enzymatic changes, many researchers believed it was important to evaluate the effects of SO₂ on reproduction. This section describes studies that examined the effects of SO₂ on pollen germination, pollen tube growth, and pollen chromosomal abnormalities after SO₂ exposure.

Ma *et al.* (1973) used spiderwort (*Tradescantia paludosa* Anders. Sax clone-3) to determine if exposure to SO₂ would increase the number of chromosomal aberrations in exposed pollen. Pollen was exposed in sealed Plexiglas containers to 131 to 262 µg m⁻³ SO₂ from 5 to 10 minutes after sowing to the metaphase stage of mitosis (18 – 20 h). It was found that SO₂ enhanced the chromatid aberration rate under their experimental conditions. The average increase in aberrations was 46.4 breaks per 100 cells during summer experiments and 11.7 breaks per 100 cells in fall and winter experiments. The difference in aberration rates from summer to winter was attributed to higher light intensities in summer. It was suggested that SO₂ might interfere with the repair of damage such as enhanced sister chromatid exchange or lesions in chromosomes, which can be caused by photochemical reactions (Table 1).

Further experiments by Ma and Khan (1976) were conducted to determine if there was a dosage effect on pollen tube development and mitotic activity of the generative nucleus of spiderwort (*Tradescantia paludosa* Anders. Sax clone-3). Results indicated that mitotic activity of the generative nucleus was inhibited by exposure to 196 to 130,950 µg m⁻³ SO₂ for 18.5 h in sealed Plexiglas chambers. Mitotic activity decreased linearly as the SO₂ concentration increased. Pollen tube growth was also measured after exposure to 26,190 to 5,238,000 µg m⁻³ SO₂ for 18.5 h. It was found that there was no reduction in growth from 196 to 26,190 µg m⁻³ SO₂, but from 130,950 to 2,619,000 µg m⁻³ SO₂ pollen tube length was reduced from an average of approximately 880 to 140 µm. It was suggested that the response was saturated at 2,619,000 µg m⁻³ SO₂ as there were no further growth reductions at the highest SO₂ concentration. It was concluded that the results of this and the previous study (Ma *et al.*, 1973) indicated that SO₂ damaged sites that are related to DNA metabolism (Table 1).

In vitro studies were used to determine if SO₂ exposure inhibited pollen germination and pollen tube growth of cottonwood (*Populus deltoides* Bartr.), Red pine (*Pinus resinosa* Ait.), Austrian pine (*Pinus nigra* Arnold), and blue spruce (*Picea pungens* Engelm.) (Karnosky and Stairs, 1974). Wet or dry pollen was exposed to 786 to 26,190 µg m⁻³ SO₂ for 4 h. Pollen of cottonwood dehydrated and did not germinate after dry exposure; as a result, only wet exposure results were reported. Significant reductions in cottonwood pollen germination and pollen tube elongation were observed at or above 1,960 µg m⁻³ and 786 µg m⁻³ SO₂, respectively. When exposed dry, concentrations up to 26,190 µg m⁻³ SO₂ had no effect on germination and tube elongation of the conifer pollen. In contrast, when wet, exposure to 3,667 µg m⁻³ SO₂ resulted in greatly reduced germination and tube elongation (to a level < 20% of controls). It was suggested that the inhibition of pollen germination and tube elongation in cottonwood could have resulted from acidification of the germination media resulting from SO₂ absorption. For conifer pollen it

appeared that the form of absorbed SO₂ in the media had affected germination and elongation rather than the degree of acidification (Table 1).

Varshney and Varshney (1981) exposed chick pea, (*Cicer arietinum* L.); nasturtium (*Nasturtium indicum* L.); petunia, (*Petunia alba* Juss.); and spiderwort, (*Tradescantia axillaris* L.) pollen to a range of SO₂ concentrations when dry (1,310 to 13,100 µg m⁻³), or wet (26.2 to 2,620 µg m⁻³) for 1 to 5 h, in sealed chambers. Germination of pollen under moist exposure conditions was almost completely inhibited at 262 µg m⁻³ SO₂ for the four species (1 to 3% of controls); whereas, dry exposure was not affected up to 7,860 µg m⁻³ SO₂. It was suggested that the greater toxicity of SO₂ to moist pollen was due to its conversion to sulphite (SO₃²⁻) or hydrogen sulphite (HSO₃⁻) ions or a dissolved or unreacted form of SO₂. Moist exposure to SO₂ also greatly reduced pollen tube growth. There were reductions in length of 5.5 to 28.5% after 1 h exposure to 26.2 µg m⁻³ SO₂, and 91.3 to 96.6% after 1 h exposure to 262 µg m⁻³ SO₂. Pollen fumigation under dry conditions resulted in approximately 35% reduction in pollen tube growth for all species after 5 h exposure to 13,100 µg m⁻³ SO₂. It was suggested that the reduction in growth was due to SO₂ effects on the generative nucleus (Table 1).

A number of in vitro studies demonstrated that SO₂ has a detrimental effect on pollen germination and growth. In order to determine the specific stage of sexual reproduction that was affected by SO₂, DuBay and Murdy (1983) conducted whole plant studies with geranium (*Geranium carolinianum* L.). Plants were exposed to 1,571 µg m⁻³ SO₂ for 8 h at 70, 80, or 90% relative humidity (RH). In the 90% RH treatment, seed set, mean number of pollen germinated on the stigma and percent germination were significantly lower than the control by 34, 42, and 34%, respectively. At 70 or 80% RH there was slight visible injury to the leaves but there were no effects of SO₂ on any of the measured parameters. It was concluded that at the highest RH, SO₂ dissolved into the moist surface of the stigma and pollen grains, and affected the ability of the pollen to germinate (Table 1).

Many of the experiments on the effects of SO₂ on pollen were performed with extremely high concentrations that are unlikely to be observed in the environment. Keller and Beda (1984) decided to evaluate the effects of lower SO₂ concentrations on pollen of mugo pine (*Pinus mugo* Turra), scotch pine (*Pinus sylvestris* L.), Austrian pine (*Pinus nigra* Arnold), and silver fir (*Abies alba* Mill.). Pollen was exposed, in chambers, to 65, 195 or 589 µg m⁻³ SO₂ for 16 h (pines) and/or 24 h (pines and fir) then germinated on agar medium in "moist chambers". Germination of pollen decreased by more than 25% (pines, 16 h exposure) and 30% (fir, 24 h exposure) at 195 µg m⁻³ SO₂ and increasing the length of exposure of pine pollen from 16 to 24 h did not alter the magnitude of the response. Complete inhibition of germination was observed at the highest SO₂ level for all four species. It was concluded that tree reproduction could be affected in the field (Table 1).

Linskens *et al.* (1985) exposed petunia (*Petunia hybrida* Vilm.) Clone W166H pollen in vitro to 52,380 µg m⁻³ SO₂, generated by mixing hydrochloric acid (HCl) and sodium hydrogen sulphite (Na₂S₂O₅). Pollen germination and tube growth were measured after 2 and 3 hours incubation, respectively. There were significant reductions from the controls in both pollen germination (45%) and tube length (32%). The reductions were attributed to reductions in pH (acidification) of the medium (Table 1).

Experiments in which Monterey pine (*Pinus radiata*) pollen was exposed to 262 to 26,190 $\mu\text{g m}^{-3}$ SO_2 for 24 h resulted in noticeably reduced pollen germination and approximately 50% reduction in pollen tube growth at 2,619 $\mu\text{g m}^{-3}$ SO_2 (O'Connor *et al.*, 1987). Chromatography assays were also performed to determine the effects of SO_2 on the metabolites of early germination. It was found that percent activity was reduced by 24% (aspartate), 13% (glutamine), 10% (alanine), and 26% (γ -amino butyrate) at 262 $\mu\text{g m}^{-3}$ SO_2 . In addition, the number of identifiable metabolites was decreased. It was concluded that SO_2 inhibited the enzymes that catalyzed the formation of the metabolites (Table 1).

Faba bean (*Vicia faba* V. Giza 2) plants were exposed to SO_2 concentrations of 13,095 to 654,750 $\mu\text{g m}^{-3}$ for 5 h or 13,095 $\mu\text{g m}^{-3}$ for 5 h d^{-1} for 1, 2 or 4 days to determine if there were cytogenetic or viability effects on pollen (Amer *et al.*, 1989). The number of non-viable pollen grains increased significantly as the concentration of SO_2 increased. The authors used chromosome fragmentation as the criterion for mutagenic potential. Statistical analysis of the results indicated the percentage of abnormal pollen mother cells (PMC's) increased significantly after exposure to 13,095 $\mu\text{g m}^{-3}$ SO_2 for 5 h and the percentage of abnormal cells increased as the concentration of SO_2 increased or as the period of fumigation increased (Table 1).

2.6 Multiple Pollutant (Interaction) Experiments

Exposure to a single pollutant results in damage and/or growth losses that can be predicted based on previous experiments. However, when exposed to more than one pollutant, the response of plants can be difficult to interpret as the pollutants may 'act against each other' resulting in less damage than would be predicted, or 'act together' resulting in greater damage than predicted, or have no interaction at all. For clarity in reading the following, interactions of multiple pollutants will be defined as:

- When growth is less than that predicted, **synergistic** interactions are implied.
- When growth is greater than predicted, **antagonistic** interactions are implied.

Houston (1974) used greenhouse fumigation chambers to expose tolerant and sensitive clones of eastern white pine (*Pinus strobus* L.) to SO_2 and ozone (O_3) singly, and in combination, to determine the minimum concentrations that would result in foliage injury. Plants were exposed to 65 to 1,179 $\mu\text{g m}^{-3}$ SO_2 , 98 to 1,117 $\mu\text{g m}^{-3}$ O_3 , or 65 $\mu\text{g m}^{-3}$ SO_2 plus 98 $\mu\text{g m}^{-3}$ O_3 , for 6 hours. Sensitive clones showed reduced needle elongation and visible foliar injury at all concentrations of SO_2 . Tolerant clones did not exhibit foliar injury until exposed to 131 $\mu\text{g m}^{-3}$ SO_2 and injury increased in severity as the concentration of SO_2 increased. Ozone and SO_2 in combination resulted in synergistic effects on growth and foliar injury for sensitive clones. However, tolerant clones had no foliar injury but needle growth was depressed. It was concluded that tolerant clones might have suffered physiological injury without visible symptoms (Table 1).

In an attempt to determine threshold concentrations for injury of trembling aspen (*Populus tremuloides* Michx.) clones to O₃ and SO₂, Karnosky (1976) exposed plants for 3 h to 524 to 1,702 µg m⁻³ SO₂, 98 to 392 µg m⁻³ O₃, and performed two interaction experiments with concentrations of 524 or 916 µg m⁻³ SO₂ plus 98 µg m⁻³ O₃. The response of the 5 clones varied, 3 of the 5 clones showed injury to SO₂ at 916 µg m⁻³ with injury generally increasing as SO₂ concentration increased. All five clones exhibited injury symptoms at 1,702 µg m⁻³ SO₂. In the interaction experiments exposure to the combination of pollutants resulted in more of the clones and a greater percentage of the foliage being injured than when exposed to a single pollutant.

Experiments were conducted in which O₃ sensitive and non-sensitive loblolly pine (*Pinus taeda*) seedlings were exposed in fumigation chambers to O₃ (98 µg m⁻³), SO₂ (367 µg m⁻³) or NO₂ (188 µg m⁻³) singly, and in combination 6 h per day for 28 days. Experiments were to determine if ambient concentrations of the pollutants would cause foliar injury or affect growth (Kress *et al.*, 1982). The results indicated that the pollutants generally caused greater foliar injury and growth suppression when exposed in combination than when exposed singly. The authors also noted that when exposed to O₃ + SO₂ or O₃ + SO₂ + NO₂ the non-sensitive trees exhibited >10% height reductions and the sensitive trees exhibited significant height reductions (>18%). The height reductions were not always correlated with foliar injury. Less than 4% of the foliage of the sensitive family exhibited injury symptoms and the non-sensitive trees exhibited less injury than the sensitive trees in all treatments (Table 1).

Long-term exposure of Kentucky bluegrass (*Poa pratensis* L.) cv. Monopoly to 177 µg m⁻³ SO₂ and 127 µg m⁻³ NO₂ singly, and in combination was examined to determine how the response would change over an extended period (Whitmore and Mansfield, 1983). Seedlings were exposed from 0900 h Monday to 1700 h Friday each week, from emergence on October 26 to May 7. Six harvests were made at intervals between these dates. A significant interaction of SO₂ + NO₂ on dry weight was observed by the 2nd harvest (February) while individual effects of the two pollutants did not become apparent until the 4th harvest (April). At the final harvest, dry weight was reduced to about 55% of controls in the SO₂ treatment and to about 26% in the SO₂ + NO₂ treatment. A significant increase in the shoot:root ratio was observed in the SO₂ treatment. A similar experiment was conducted the following year, with the same pollutant treatments and measurements continuing until late summer. The effects on dry weight and shoot:root ratio were generally the same as the previous experiment up until the May harvest; however, as this was the last harvest in which it was feasible to separate the roots subsequent data was only for shoots. Shoot growth recovered over the growing season and by the final harvest the SO₂ and SO₂ + NO₂ treatments showed a slight stimulation of growth. In contrast, both the SO₂ and SO₂ + NO₂ treatments, delayed the start of stem elongation and severely reduced the number of flowering heads at the final harvest. The authors concluded that the observed reductions in growth could be attributed to lower net assimilation rates while the increased shoot:root ratio was a compensatory mechanism to counteract reduced photosynthetic activity. It was also indicated that the increased growth observed over the summer in the second experiment was associated with reduced flowering and that reduced flowering may have been a result of delayed development (smaller plants during culm development in the spring) in the SO₂ and SO₂ + NO₂ treatments (Table 1).

A chamberless field exposure system was used to determine if there would be yield effects from exposure of soybean to SO₂ (26, 144 and 288 µg m⁻³) and O₃ (82, 122 and 157 µg m⁻³) singly, and in all combinations for 5.25 h d⁻¹ for 16 of 23 days (Reich and Amundson, 1984). Fumigations were not conducted on days in which wind would greatly alter pollutant concentrations from designed levels. When exposed singly, O₃ significantly decreased all yield parameters measured; whereas, SO₂ only significantly reduced mass per seed by 4 to 7%. However, a non-significant seed yield reduction of 7% per plant and 12% per hectare occurred in the high SO₂ treatment (288 µg m⁻³). No significant interactions were observed between SO₂ and O₃ and there were no visible signs of leaf injury in any treatments (Table 1).

Heggestad *et al.* (1986) investigated the impact of increasing SO₂ concentrations (13 to 1,226 µg m⁻³) in the presence (non-filtered; NF), and absence (charcoal-filtered; CF) of ambient ozone. Tomato (*Lycopersicon esculentum*) cv Jet star plants were exposed, in open-top chambers, for 5 h d⁻¹ (daylight), 5 d wk⁻¹ for 57 days, July to September. Leaf injury increased, as the season progressed with significantly greater injury in the NF air indicating other pollutants, probably O₃ were a more important cause of injury than SO₂ alone. Increased SO₂ had no significant effects on plant height; however, plant and fruit weights decreased linearly with increasing SO₂. The ambient O₃ treatment and all non-filtered SO₂ treatments reduced the weight of ripe fruit. Statistical analysis indicated there were no interactive effects of exposure to the SO₂ and O₃. Increased SO₂ resulted in a linear increase in foliar S in both NF and CF treatments but did not change fruit S levels. There was no consistent change in the other foliar elements tested (Table 1).

2.7 Lichens

The study of physiological responses of lichens to pollutants is complex since each member (the fungus and the algae) play a specific role in the symbiosis. The algal portion of lichens occupies only 5-10% by weight (Farrar, 1973) but through photosynthesis fixes carbon and passes the carbon to the fungal portion in the form of simple carbohydrates. As lichens do not have roots, they depend on the atmosphere for nutrients and have developed nutrient concentrating mechanisms that are required for survival (Nash, 1996). These mechanisms have led to the use of lichens as monitors of atmospheric deposition. This section summarizes a few of the studies on lichen diversity and physiological responses to SO₂.

2.7.1 Lichen Diversity in Response to Sulphur Dioxide

The lichen flora in a region reflects the average, cumulative effects of air pollution over time; therefore, a change in the diversity could indicate a change the quality of the atmosphere. Many studies have been done to determine the effects of increasing or decreasing SO₂ on lichen abundance and diversity.

Gilbert (1968) examined lichens and bryophytes along a belt transect from the city centre of Newcastle upon Tyne to 32 km in the direction of the prevailing winds. Sulphur dioxide measurements from several standard volumetric gauges on or near the transect indicated there was a steep gradient from 200 to 40 $\mu\text{g m}^{-3}$ (annual average) from the city centre to 19 km out. *Evernia prunastri*, a sensitive epiphytic lichen first appeared about 13 km from the city and exhibited an increase in luxuriance, biomass and percentage cover as the distance increased up to about 27 km. Over 90% of the bryophytes and over 95% of the lichens studied exhibited a similar pattern. In contrast, the resistant epiphytic lichen, *Lecanora conizaeoides*, reached its maximum cover on ash trees at the point where sensitive lichens were eliminated and maintained this cover into the suburbs then declined toward the city centre where it only survived in slightly sheltered sites. It was also found that sheltered environments (e.g. sheltered valleys, dense woodland) or high pH of the substrate could alleviate the toxic effects of SO_2 . In addition, total sulphur in tissues of bryophytes and lichens declined as distance from the city centre increased. It was concluded that assemblages of, or individual bryophytes or lichens could be used as indicators of SO_2 pollution as long as the habitats surveyed were standardized to ensure that the environmental conditions of the habitat were not alleviating the effects of SO_2 (Table 1).

The results of a survey of lichen diversity and abundance in the Montreal area exhibited the same pattern as Gilbert's 1968 survey (LeBlanc and De Sloover, 1970). The number of species, frequency in each zone and coverage of species present generally increased as the distance from the most industrialized zones increased. The data was used to generate a single number for each site, the index of atmospheric purity (IAP), as the authors believed that the epiphytic vegetation at each site was directly related to the quality of the ambient air. However, measurements of ambient sulphur dioxide were not performed so the reduction in number of species, frequency of appearance at the different sites, and coverage or abundance at each site could not be related to SO_2 concentrations.

In a similar study in the Sudbury area, LeBlanc *et al.* (1972) surveyed 31 sites for epiphytic bryophytes and lichens and then calculated the IAP. The greater the IAP the more sensitive the epiphytes were to atmospheric pollution. The IAP's were used to separate the studied area into five zones then compared to a map that delineated 12 years of SO_2 monitoring data (12-year average concentration) into five pollution zones. It was found that the two maps were comparable; as the levels of pollution increased the IAP decreased (i.e. the frequency and diversity of epiphytes decreased). For example, zone one had an average SO_2 concentration of 79 $\mu\text{g m}^{-3}$ with only four species of epiphytic lichens present; whereas, in unpolluted zone five there were 40 species of epiphytic lichens (Table 1).

Another study of lichen abundance and species diversity was conducted around two gas plants that had been operating for 3 years in the Rocky Mountain House area of Alberta. The results indicated that the lichen vegetation within 1.5 to 3 km of the gas plants was already severely damaged by SO_2 emissions (Skorepa and Vitt, 1976); however, atmospheric SO_2 concentrations were not documented.

Rose and Hawksworth (1981) surveyed the abundance of lichens at 29 sites north and west of Greater London to determine if lichen populations had changed with the 50% reduction in atmospheric SO_2 since 1960. Comparison of the estimated time of recolonization with mean

annual SO₂ concentrations indicated that there was a direct response of recolonization with falling SO₂ levels. Mean annual SO₂ concentrations in Inner London declined from 200-250 µg m⁻³ to less than 130 µg m⁻³ between 1960 and 1980. The authors also indicated that growth rates of the species studied indicated that the recolonization occurred within the previous 3 to 7 years.

In a subsequent study, Hawksworth and McManus (1989) surveyed the same 29 sites plus 22 additional sites to determine how the lichen communities had responded to further reductions in mean winter SO₂ levels. Sulphur dioxide levels had decreased from a winter average of about 130 µg m⁻³ in 1980 to 29-55 µg m⁻³ for 1985 to 1987. This survey indicated that many species had recolonized throughout the study area. Of the species observed, 25 had not been recorded within 16 km of the centre of London in the twentieth century and of these, eight had not been observed for the last 200 years.

2.7.2 Physiological Response of Lichens

Kuziel (1974) used lichen and maize (*Zea mays* L.) to study the effects of SO₂ on chlorophyll content and catalase activity. Several species of epiphytic lichens were collected in the field, brought into the lab, then exposed alongside seedlings of maize (*Zea mays* L.) to 10 mg m⁻³ SO₂ for six days (in fumigation chambers). Both were then measured for chlorophyll content and catalase activity. It was found that exposed lichens had up to 50% (*Parmelia furfuracea*) less chlorophyll than controls and up to 85% (*Ramilina fraxinea*) lower catalase activity than controls. The smallest differences were observed in maize; 6% reduction in chlorophyll and 9% reduction in catalase activity (Table 1).

Holopainen and Karenlampi (1984) studied visible injury and ultrastructural changes that occurred when the lichens *Bryoria capillaris* and *Hypogymnia physodes* L. were exposed to a range of SO₂ concentrations (140-2,860 µg m⁻³). The lichens were exposed in fumigation chambers for 5.5 to 8.0 h d⁻¹ for 7 to 21 days. Visible injury, bleaching of the thalli of *H. physodes* was apparent after 4 days (22 h, fumigation at 2,860 µg m⁻³) with bleaching increasing as the length of exposure increased. In contrast, electron micrographs indicated the presence of ultrastructural injury to the algal cells at all SO₂ levels, with the incidence of injury increasing as the concentration and duration of exposure increased. The type of injury observed was separated into five stages, which have been briefly described in Table 1, and related to exposure concentration and duration. While the pattern of injury for both lichens was similar, *B. capillaris* tended to have greater injury especially at the lower concentrations of SO₂. It was indicated that the ultrastructural changes observed in the lowest concentrations and shortest exposures were similar to changes observed in field material from industrial environments (Table 1).

Huebert *et al.* (1985) used *Evernia mesomorpha* Nyl. to determine the effects of low concentrations of SO₂ on net photosynthesis and respiration. The lichens were exposed to 0 to 856 µg m⁻³ SO₂, for 1 to 6 h in a flow-through cuvette and net photosynthesis and respiration were measured immediately after exposure. Net photosynthesis decreased significantly when exposed to 205 µg m⁻³ SO₂ for 1 h and these reductions were greater as SO₂ concentration

increased. Respiration decreased significantly after being exposed to $639 \mu\text{g m}^{-3}$ SO_2 for 4 h or to $856 \mu\text{g m}^{-3}$ SO_2 for 2 hours. In a second experiment, recovery of net photosynthesis was measured after a 1 or 4 h SO_2 exposure followed by a recovery period of 4 or 24 h in clean air. Exposure to 639 or $856 \mu\text{g m}^{-3}$ SO_2 for 1 h required a 4 or 24 hour recovery period, respectively, for net photosynthesis to recover to near the pre-fumigation rate. When exposed for 4 h, net photosynthesis in only the $205 \mu\text{g m}^{-3}$ SO_2 treatment recovered to the pre-fumigation rate. From their results the authors concluded that the most important factor in lichen response to SO_2 was exposure to short-term, high concentrations (Table 1).

Lichens in natural habitats can be exposed to dry deposition of pollutants such as SO_2 during periods of desiccation. In order to determine what effects dry deposition could have, Coxson (1988) studied the effects of rehydration on net photosynthesis and dark respiration of *Cladonia mitis* (Sandst.) Hale & Culb. after a 2 or 3 week exposure of desiccated thalli to 524 or $2,619 \mu\text{g m}^{-3}$ SO_2 . After exposure, the dessicated thalli were rehydrated and net photosynthesis and respiration were measured for up to 800 minutes after rewetting. The pattern of photosynthetic recovery for the lichens exposed to $2,619 \mu\text{g m}^{-3}$ SO_2 was no different than that of the controls for either exposure period. In contrast, the rate of recovery and maximum net photosynthesis was significantly higher in the lichens exposed to the $524 \mu\text{g m}^{-3}$ SO_2 treatment (both exposure times) than in controls. Dark respiration increased significantly only in the $524 \mu\text{g m}^{-3}$ SO_2 treatment (for both exposure times) after 200 minutes rehydration. It was concluded that the physiological response to periods of pollution exposure might not occur at the same time as the exposure (Table 1).

Table 1 Summary of effects of sulphur dioxide on vegetation

Note: When percent increases or decreases are given, these values are comparisons with controls unless indicated otherwise.

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Effects on Growth						
Ashenden and Mansfield (1977)	<i>Lolium perenne</i> L. (perennial ryegrass)	288	4 wk	Wind tunnel fumigation	Wind speed 10 m min⁻¹: # of fully expanded green leaves Dry weights Wind speed 25 m min⁻¹: # of fully expanded green leaves Leaf area Root/shoot ratio Green leaf dry weight Dead leaves + stubble dry weight Total shoot dry weight Root dry weight Visible injury	Wind speed 10 m min⁻¹: Increased significantly greater than (>) 17% No significant differences Wind speed 25 m min⁻¹: No significant change from control Decreased significantly > 13% Decreased significantly > 34% Decreased significantly > 18% Decreased significantly > 22% Decreased significantly > 17% Decreased significantly > 45% Some necrotic leaf lesions (1-2% of the leaf area per plant)
Constantinidou et al. (1976)	<i>Pinus resinosa</i> Alt (red pine seedlings)	1,310 to 10,476	15 to 120 min	Plexiglas cubes	Cotyledon chlorophyll 1° needle chlorophyll 1° needle dry weight # of 1° needles Cotyledon dry weight	Reduced significantly at 1,310 µg m ⁻³ , 120 min and at ≥ 2,619 for ≥ 30 min Reduced significantly at ≥ 7,857 for ≥ 60 min Reduced significantly at 1,310 & 2,619 µg m ⁻³ for 120 min and at 7,857 for ≥ 30 min and 10,476 at all exposure times Emergence appeared to be inhibited Reduced significantly at ≥ 7,857 & 10,476 for ≥ 60 min

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Cowling and Lockyer (1976)	<i>Lolium perenne</i> L. (perennial ryegrass)	50 (daily avg)	87 d	Fumigation chambers	Shoot dry weight Stubble dry weight Root dry weight Shoot sulphur Shoot dry weight Stubble dry weight Root dry weight Shoot sulphur	% increase over no SO ₂ , no added soil SO ₄ ²⁻ : 66% 9% 31% 43% % increase over no SO ₂ , added soil SO ₄ ²⁻ : 4% 8% 17% 24%
Cowling and Lockyer (1978)	<i>Lolium perenne</i> L. (perennial ryegrass)	55 (daily avg)	85 d	Fumigation chambers	Shoot and root dry weight, # of tillers Shoot dry weight Root dry weight # of tillers	Low N: No change High N no soil S: > 120% increase > 80% increase > 20% increase
Roberts, Bruce R. (1975)	<i>Betula papyrifera</i> Marsh. (White birch)	annual avgs <10 or 70.8	3 mon – with biweekly harvests	Potted seedlings in the field	Height - % increase over 3 mon Dry weight - % increase over 3 mon	93% - high SO ₂ 45% - low SO ₂ No significant difference until mid-August harvest 448% - high SO ₂ 229% - low SO ₂
	<i>Quercus palustris</i> Muenchh (Pin oak)				Height - % increase over 3 mon Dry weight - % increase over 3 mon	42% - high SO ₂ 73% - low SO ₂ 111% - high SO ₂ 261% - low SO ₂

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
SO₂ Uptake and Plant S Content						
Thomas <i>et al.</i> (1944)	<i>Medicago sativa</i> L. (alfalfa)	492	628 h	Plot fumigation	Leaf extract buffering capacity	Reduced up to 46% of controls
		762	338 h		Leaf extract pH	No change
	<i>Beta vulgaris</i> (sugar beet) and <i>Medicago sativa</i> L. (alfalfa)	487	5.2 h d ⁻¹ , 60 d		Labile organic sulphur in fumigated leaves	Increased when nutrient supply deficient in sulphate No change when nutrient supply sufficient in sulphate
Visible Injury						
Katz and McCallum (1939)	11 conifer species	576	1,656 h (69 d)	Plot fumigation	Visible injury	None
		1,205	736 h (Sept 30 – Nov 5)			First appearance of visible injury: 530 h – Engelmann spruce
		1,833	483 h (Oct 9 – 30)			48 h – Douglas fir 120 h – White pine 162 h – Western red cedar 354 h – Engelmann spruce
		2,488	287 h (Sept 26 – Oct 8)			28 h – Douglas fir 144 h – lodgepole pine, silver fir, cedar 168 h – Engelmann spruce
		2,409	214 h (Nov 23 – Dec 2)			96 h – Douglas fir 144 h – Yellow pine, lodgepole pine
		2,095	5 h	Fumigation chamber	Leaf necrosis	Resistant ecotype – 4.6% of leaf area Sensitive ecotype – 31.1 % of leaf area
Taylor <i>et al.</i> (1986)	<i>Geranium carolinianum</i> L. (geranium)	786 to 1,571	5 h		Photosynthesis	Resistant – inhibited avg 11.5% (786 µg m ⁻³) and 29.9% (1,571 µg m ⁻³), 3-5 h exposure Sensitive – inhibited 30% at 1,571 µg m ⁻³
		1,179	6 h d ⁻¹ , 4 d wk ⁻¹ , 4 wk		Photosynthesis – change from controls	Resistant – increased 28% from control Sensitive – decreased 26% from control
					Photoassimilate retention in foliage	Resistant – decreased 6% from control Sensitive – increased 7% from control
		0 to 1,179			Shoot and root DW	Sensitive 10 to 25% lower than resistant ecotype

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Other Metabolic Processes						
Harvey and Legge (1979)	<i>Pinus contorta</i> x <i>banksiana</i> (lodgepole - jack pine)	200 to 800	0.5 h	Siemens cuvette	ATP level	No effect on chamber grown seedlings Decreased in branches from field site
		Ambient to 542		Field fumigation	ATP level	Decreased as SO ₂ level increased
Horsman and Wellburn (1977)	<i>Rumex obtusifolius</i> (Broad leaved dock)	524	11 d	Wind tunnel fumigation	Low-ambient area:	
					RuDPC	Activity in leaves (% of control) Young - 65, old - 68
					GPT	Young - 138, old - 98
					GOT	Young - 127, old - 104
					Peroxidase	Young - 140, old - 135
					High-ambient area:	
					RuDPC	Activity in leaves (% of control) Young - 97, old - 109
					GPT	Young - 119, old - 104
					GOT	Young - 123, old - 107
					Peroxidase	Young - 114, old - 105
Olszyk and Tingey (1984a)	<i>Pisum sativum</i> L. cv Alsweet (pea)	1,310 - 2,619	2 h - dark	Fumigation chamber	Leaf injury	55 - 72% necrotic tissue
			2 h - light		Leaf injury	10 - 22% necrotic tissue
			2 h - dark		Leaf injury	20 - 50% necrotic tissue
	<i>Lycopersicon esculentum</i> flacca Mill (tomato)	1,571 - 2,095	2 h - light		Leaf injury	5 - 15% necrotic tissue
			2 h - light	Fumigation chamber	Leaf injury	2 to 8 times higher in FC treated plants than untreated plants
			2 h - light		Leaf injury	7 times higher in FC treated plants than untreated plants
Shimazaki <i>et al.</i> (1980)	<i>Spinacia oleracea</i> L. cv. New Asia (spinach)	5,238	8 h - light	Growth cabinet fumigation	Visible injury	Appeared 2-3 h after start of fumigation
					Chlorophyll <i>a</i>	Decreased from 2 h after start of fumigation

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Tanaka and Sugahara (1980)	<i>Populus euramericana</i> (poplar)	262	10 h - dark	Fumigation chamber	Chlorophyll <i>b</i>	No decrease
					Visible injury	None
					Chlorophyll <i>a</i>	No decrease
	<i>Spinacia oleracea</i> L. cv. New Asia (spinach)	1,310	22h	Fumigation chamber	SOD activity	Increased up to 4.4 times in leaves that were youngest at the start of fumigation
					SOD activity after treatment with a SOD inhibitor (DDTC)	5 times more in young leaves than old leaves 65% inhibition after 2 h, reduced to 77% inhibition at end of fumigation
Effects on Pollen						
Amer <i>et al.</i> (1989)	<i>Vicia faba</i> (Faba bean)	13,095 to 654,750	5 h	Fumigation chamber	Abnormal PMC's	Significant increase (3.5 to over 50% as concentration increased)
					Nonviable pollen	Significant increase as concentration increased
					Abnormal PMC's	Significant increase (4.6 to over 30% as exposure time increased)
					Nonviable pollen	Significant increase as exposure time increased
DuBay and Murdy (1983)	<i>Geranium carolinianum</i> L. (geranium)	1,571	8 h	Fumigation chamber	At 90% RH: Seed set	Changes from the control: Significantly reduced by 34%
					Mean number of pollen germinated on stigma	Significantly reduced by 42%
					Pollen germination	Significantly reduced by 34%
					Pollen tube growth in the style	No effects
					At 70 or 80% RH	No effects on measured parameters
Karnosky and Stairs (1974)	<i>Populus deltoides</i> Bartr. (cottonwood)	0 to 3,667	4 h	Fumigation chamber	Pollen tube elongation	Significantly reduced $\geq 786 \mu\text{g m}^{-3}$
					Pollen germination	Significantly reduced $\geq 1,964 \mu\text{g m}^{-3}$
	<i>Pinus resinosa</i> Ait. (Red pine) <i>Pinus nigra</i> Arnold	0 to 26,190	Dry pollen	Pollen germination and tube elongation	No effects	

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	(Austrian pine) <i>Picea pungens</i> Engelm. (Blue spruce)					
			Wet pollen			
Keller and Beda (1984)	<i>Pinus mugo</i> Turra (Mugo pine), <i>Pinus sylvestris</i> L. (Scotch pine), <i>Pinus nigra</i> Arnold (Austrian pine)	65, 195 or 589	16 or 24 h	Fumigation chamber	Pollen germination and tube elongation	Significantly reduced to less than 20% of controls $\geq 3,667 \mu\text{g m}^{-3}$
	<i>Abies alba</i> Mill. (Silver fir)		24 h		Pollen germination	> 25% reduction at $195 \mu\text{g m}^{-3}$ at 16 h exposure Complete inhibition at $589 \mu\text{g m}^{-3}$
Linskens <i>et al.</i> (1985)	<i>Petunia hybrida</i> Vilm. (petunia)	52,380	2 h	Petri dish	Pollen germination	> 30% reduction at $195 \mu\text{g m}^{-3}$ with complete inhibition at $589 \mu\text{g m}^{-3}$
			3 h		Pollen tube growth	45% reduction, significant 32% reduction, significant
Ma <i>et al.</i> (1973)	<i>Tradescantia paludosa</i> Anders (Spiderwort)	131 to 262	18 to 20 h	Sealed Plexiglas chamber	Average number of chromatid aberrations	Increased by 46.4 breaks per 100 cells in summer and by 11.7 breaks per 100 cells in fall and winter
Ma and Khan (1976)	<i>Tradescantia paludosa</i> Anders (Spiderwort)	26,190 to 2,619,000	18.5 h	Sealed Plexiglas chamber	Pollen tube growth	No effect at $26,190 \mu\text{g m}^{-3}$ > 80% reduction in length at $\geq 130,950 \mu\text{g m}^{-3}$
		196 to 130,950	18.5 h		Mitotic activity of generative nucleus	Decreased linearly with increased SO ₂
O'Connor <i>et al.</i> (1987)	<i>Pinus radiata</i> (Monterey pine)	262 to 26,190	24 h	Fumigation chamber	Pollen germination	Reduced more than 50% at $26,190 \mu\text{g m}^{-3}$
					Pollen tube length	50% reduction at $2,619 \mu\text{g m}^{-3}$
					Activity of aspartate, glutamine alanine, and γ -amino butyrate	Reduced 24, 13, 10, and 26%, respectively at 262 $\mu\text{g m}^{-3}$
Varshney and Varshney (1981)	<i>Cicer arietinum</i> L. (Chick pea), <i>Nasturtium indicum</i> L. (<i>nasturtium</i>), <i>Petunia alba</i> Juss. (petunia),	1,310 to 13,100	1 to 5 h Dry pollen	Sealed chamber	Pollen germination	No effect up to $7,860 \mu\text{g m}^{-3}$, > 10% reduction at $13,100 \mu\text{g m}^{-3}$

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	<i>Tradescantia axillaries</i> L. (Spiderwort)	26.2 to 2,620	Wet pollen		Pollen tube growth	Approximate 35% reduction after 5 h exposure to $13,100 \mu\text{g m}^{-3}$
					Pollen germination	Almost complete inhibition at $262 \mu\text{g m}^{-3}$
					Pollen tube growth	91.3 to 96.4% reduction after 1 h at $262 \mu\text{g m}^{-3}$
Multiple Pollutant (Interaction) Experiments						
Heggestad <i>et al.</i> (1986)	<i>Lycopersicon esculentum</i> cv. Jet star (tomato)	13 to 1,220	5 h d^{-1} , 5 d wk^{-1} , 57 d	Open-top chambers		Charcoal filtered (CF)
					Visible injury	Increased as season progressed
					Plant and fruit weight	Decreased significantly
					Ripe fruit weight	Decreased significantly
					Foliar sulphur content	Increased linearly with increased SO ₂
					Fruit sulphur content	No effect
		29 to 1,226 (110 O ₃)				Non-filtered (NF)
					Visible injury	Increased significantly over CF treatment
					Plant and fruit weight	Decreased linearly with increased SO ₂
					Ripe fruit weight	Decreased linearly with increased SO ₂
					Foliar sulphur content	Increased linearly with increased SO ₂
					Fruit sulphur content	No effect
Houston (1974)	<i>Pinus strobes</i> L. (Eastern white pine)	65 to 1,179	6 h	Fumigation chamber	Needle elongation	Sensitive clones 48% reduction compared to controls at $65 \mu\text{g m}^{-3}$
					Visible injury	Sensitive clones - all SO ₂ treatments Tolerant clones - $\geq 131 \mu\text{g m}^{-3}$ SO ₂
		65 SO ₂ + 98 O ₃			Needle elongation	Sensitive clones 52% reduction compared to controls
					Visible injury	Sensitive clones only
Kress <i>et al.</i> (1982)	<i>Pinus taeda</i> (Loblolly pine)	367	6 h d^{-1} , 28 d	Fumigation chamber	Foliar injury	< 1% of the needles in any treatment

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		Plus 93 O ₃ 188 NO ₂	singly or in combination		Foliar injury	Sensitive trees < 4% of the needles in any treatment Non-sensitive - less injury than sensitive trees - numbers not provided
					Height	>18% reduction (sensitive), >10% reduction (non-sensitive) compared to controls in O ₃ + SO ₂ and O ₃ + SO ₂ + NO ₂ treatments
Reich and Amundson (1984)	<i>Glycine max</i> (soybean)	26, 144, 288	5.25 h d ⁻¹ for 16 d	Field exposure	Yield	Reduction of 7% plant ⁻¹ and 12% hectare ⁻¹ at 288 $\mu\text{g m}^{-3}$
					Mass per seed	Significant 4 - 7% reduction
					Visible injury	None
		Plus: 82, 122, 157 O ₃				No significant interactions
Whitmore & Mansfield (1983)	<i>Poa pratensis</i> L. (Kentucky bluegrass)	177	7 months	Fumigation chamber	Plant dry weight	55% of controls
			11 months		Shoot:root ratio	Significant increase
					Shoot dry weight	Significant increase
					Flowering	> 50% reduction
		Plus 127 NO ₂	7 months		Plant dry weight	26% of controls
					Shoot:root ratio	Significant increase
			11 months		Shoot dry weight	Significant increase
					Flowering	> 50% reduction
Lichens						
Coxson (1988)	Mat-forming lichen (<i>Cladonia mitis</i> (Sandst.) Hale & Culb.)	524	2 or 3 wks	Temperature-controlled cuvette	Net photosynthesis	Significant increase in rate of and maximal recovery over controls
		2,619			Dark respiration	Significant increase over controls 200 min after rewetting
					Net photosynthesis	No effect compared to controls
					Dark respiration	No effect compared to controls

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Gilbert (1968)	Epiphytic lichens (e.g. <i>Lecanora conizaeoides</i> , <i>Evernia prunastri</i>)	40 to 200	Survey of natural populations	Transect from city centre out 32 km	Luxuriance, biomass and percentage cover	Increased outward from city centre
Holopainen and Karenlampi (1984)	Epiphytic lichens - (<i>Bryoria capillaries</i> <i>Hypogymnia physodes</i> L.)			Fumigation chamber	Description of stages of ultrastructural injury: Stage 0 - normal algal cell Stage 1 - mitochondria swollen & deformed Stage 2 - swelling of chloroplasts, degeneration of nucleus, mitochondrial cristae and matrix Stage 3 - Mitochondria and nucleus badly degenerated, thylakoids appear stretched Stage 4 - All cell organelles badly injured but their outlines were still visible Stage 5 - no organelles visible, cell contents collapsed	
		140	8 h d ⁻¹ - 21 d		Stage of injury after indicated exposure:	14-21 d - stage 1
		290	5.5 h d ⁻¹ - 9 d			4 d - stages 0 to 1 9 d - stages 1 to 3
		860	5.5 h d ⁻¹ - 6 d			3 d - stages 1 to 2 6 d - stages 2 to 3
		2,860	5.5 h d ⁻¹ - 7 d			1 d - stages 1 to 2 4-7 d - stages 4 to 5
					Visible injury	Bleaching of thalli visible after 4 d exposure
Huebert <i>et al.</i> (1985)	<i>Evernia mesomorpha</i> Nyl.	0 to 856	1 to 6 h	Flow-through cuvette	Net photosynthesis	Significant reduction at 205 $\mu\text{g m}^{-3}$ for 1 h or more - reductions increased as concentration increased
					Respiration	Significant reduction at 639 $\mu\text{g m}^{-3}$ or higher, for 4 h or more
		0 to 1342	1 h		Recovery of net photosynthesis	639 $\mu\text{g m}^{-3}$ - 4 h after exposure recovery almost complete 856 $\mu\text{g m}^{-3}$ - 24 h after exposure recovery almost complete
						Higher concentration, recovery incomplete
		0 to 856	4 h		Recovery of net	205 $\mu\text{g m}^{-3}$ - only treatment to recover to

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Kuziel (1974)	Epiphytic lichens	10,000	6 days	Fumigation chamber	photosynthesis	pre-treatment levels 4 h after exposure
					Chlorophyll	50% reduction (<i>Parmelia furfuracea</i>) compared to controls
					Catalase activity	85% reduction (<i>Ramilina fraxinea</i>) compared to controls
					Chlorophyll	6% reduction compared to controls
					Catalase activity	9% reduction compared to controls
LeBlanc <i>et al.</i> (1972)	Epiphytic lichens	79 52 to 79 26 to 52 < 13	12-y avg. ambient	Natural ecosystem	Number of species	Reduced to 4 from unpolluted area
					Number of species	Reduced to 14 from unpolluted area
					Number of species	Reduced to 21 from unpolluted area
					Number of species	40 (considered an unpolluted area)
					Number of species	40 (considered an unpolluted area)

PART II

3.0 LITERATURE 1990 TO 2002

This section summarizes selected primary literature published from 1990 to 2002 on the effects of SO₂ exposure on vegetation. Table 2 summarizes the results of the studies.

3.1 Effects on Growth

Qifu and Murray (1991) used potato (*Solanum tuberosum*) to study the interactive effects of soil water stress and SO₂. Plants were exposed to 288 or 786 µg m⁻³ SO₂, for 105 days in closed top field chambers for 4 h per day under well-watered or water stressed conditions. Visible symptoms appeared after 9 weeks exposure to 288 µg m⁻³ and after six weeks exposure to 786 µg m⁻³. At harvest, the leaf S content of well-watered plants had increased by more than 100% and 125% in the 288 and 786 µg m⁻³ SO₂ treatments, respectively. When water stressed, the lower SO₂ treatment had little effect on S content; whereas, the 786 µg m⁻³ treatment resulted in a 100% increase in leaf sulphur. Leaf chlorophyll of 35-day-old leaves from well-watered plants decreased significantly, by approximately 30% at 288 µg m⁻³ SO₂ and by 40% at 786 µg m⁻³ SO₂. In contrast, water stress resulted in a maximum chlorophyll loss of 11% in the 786 µg m⁻³ SO₂ treatment. Exposure of well-watered potato plants to 786 µg m⁻³ SO₂ resulted in significant decreases in leaf (25%) and tuber (35%) dry weight compared to controls. In contrast, dry weight reductions of water stressed plants did not usually occur when plants were exposed to SO₂. It was suggested that the reduced response of water stress plants when exposed to SO₂ might be a result of increased stomatal resistance in response to mild water stress that may have reduced SO₂ uptake.

Coleman *et al.* (1990) presented results on the variability of biomass production for wild radish (*Raphanus sativus* x *raphanistrum*) and cultivated radish (*Raphanus sativus*) cv. Cherry Belle when plants were exposed to SO₂. Plants were exposed in fumigation chambers during a 10 h light period to 262, 629 or 1,048 µg m⁻³ SO₂ for 24, 30 or 35 days. They found that variability associated with biomass production increased as SO₂ exposure increased while biomass itself was only significantly reduced in one experiment using Cherry Belle. It was indicated that genetic differences between the individual plants (differential sensitivity to SO₂) might be the reason for the increased variability as the SO₂ concentration increased.

Top-covered chambers were used to investigate visible injury, growth, and stomatal resistance of soybean (*Glycine max* L.) cv. Buchanan when exposed to salt stress followed by SO₂ or SO₂ followed by salt stress (Qifu and Murray, 1994). Plants were exposed to 380 or 786 µg m⁻³ SO₂ for 5 h per d, for 3 weeks followed by exposure to low (320 g/200 L) or high (540 g/200 L) salt stress for an additional 3 weeks. The reverse conditions were also looked at. They found that exposure to either concentration of SO₂ alone resulted in a significant increase in chlorophyll content of leaves and the shoot:root ratio, and significant decreases in leaf area, root and shoot dry weight, and fresh weight of root nodules. Subsequent exposure to salt stress resulted in reduced chlorophyll in all treatments. Exposure to high salinity after SO₂ resulted in visible injury within 5 days, death of all plants from the high SO₂ treatment within 12 days, and death of

half of the low SO₂ treatment plants by the end of 3 weeks. In contrast, when high SO₂ exposure was preceded by low salinity stress, the adverse effects of high SO₂ were mitigated for all growth variables. It was concluded that prior treatment to SO₂ physiologically weakened plants mainly through the increased shoot:root ratio (root surface inadequate to supply water needs of shoots).

Kropff (1990) performed field exposures with an open-air fumigation system and used the data in a crop growth model, which was used to interpret and explain the observed yield loss by quantifying the contribution of different physiological effects. Fumigation of broad bean (*Vicia faba* L.) with 74 µg m⁻³ SO₂ for the growing season, resulted in a 9% decrease in total dry matter accumulation and an 10% decrease in pod yield at the final harvest. These losses were accompanied by visible injury (brown/red spots), which progressed from the oldest leaves upward and also resulted in some leaf abscission. When exposed to 165 µg m⁻³ SO₂, dry matter was reduced by 17% and yield was reduced by 23%. The author indicated the loss of dry matter production was primarily a result of loss of green leaf area in exposed plants.

In an open-top chamber study examining sulphur accumulation, and alteration of growth and yield in barley (*Hordeum vulgare* L.) cv. Schooner, Murray and Wilson (1990) found that exposure to 110 µg m⁻³ SO₂ for 4 h day⁻¹ for 79 days produced an increase in mean shoot height and weight of approximately 10%. This increase in growth was attributed to a fertilizer effect of SO₂. At 317 µg m⁻³ SO₂ or higher, significant linear decreases in barley growth (height, weight, and number of tillers), and yield (head number and weight) were observed. These decreases were proportional to the concentration of the SO₂ exposure. In addition, the shoot sulphur content increased linearly from 0.14% in the control plants to 0.77% at 1,354 µg m⁻³ SO₂.

Colls *et al.* (1992) used an open-air fumigation system to expose winter barley (*Hordeum vulgare* L.) cv. Igri to a single dose (defined as concentration x time) of SO₂, to determine if concentration peaks or long-term averages had the greatest effects on the plants. The treatments were based upon achieving an equivalent dose of 534 µg m⁻³ days. The treatments were: continuous exposure to 89 µg m⁻³ for 6 days, 178 µg m⁻³ for 3 days followed by ambient air for 3 days, or 534 µg m⁻³ for 1 day followed by 5 days of exposure to ambient air. This six-day cycle was repeated 24 times during the growing season. There were no effects on shoot dry weight accumulation or on grain yield in any treatment. The lack of effects due to the concentration peaks was attributed to plants being able to metabolize excess sulphate during the SO₂ free days.

Open-top chambers were used to investigate the response of barrel medic (*Medicago truncatula* Gaerm.) cv. Paraggio when exposed to SO₂ (Murray and Wilson, 1991). Plants were exposed to 107 to 1,349 µg m⁻³ SO₂ for 4 h day⁻¹, 7 d week⁻¹ for 72 days. Growth was affected very little at concentrations up to 314 µg m⁻³ SO₂ (<10% reduction); however, at 668 µg m⁻³ SO₂ there was a 40 to 50% reduction in growth that was accompanied by an approximately 85% increase in the S concentration. At 1,349 µg m⁻³ SO₂, there was little or no growth. One important aspect of the response of barrel medic was the significant reduction in flowering as SO₂ concentration increased. It was indicated this could have implications for pasture maintenance, as self-sown seed is the method by which annual medics regenerate each year.

In a field study of long-term effects of emissions on two sites near a point source of SO₂ (sour gas plant), and a control site, Clapperton and Parkinson (1990) studied the inoculum potential of vesicular arbuscular mycorrhizae (VAM). The study sites were 5 (site 1) and 13 km (site 2) downwind of the plant while the control site (site 3) was 20 km upwind. Daily average SO₂ concentrations, May to September were 80, 27 and <27 µg m⁻³. When timothy (*Phleum pratense*) seedlings were grown for 4 weeks in soil from the 3 sites, it was found that the rate of infection by VAM was significantly lower at the 2 sites closer to the gas plant. The number of spores (inoculum potential) also decreased at sites closer to the plant; 714, 872 and 1,372 per 100 g dry weight soil for sites 1, 2 and 3, respectively. It was indicated that the reduction in inoculum potential was probably a result of lowered productivity of the host (plants at the study sites) and reduced host productivity was probably due to long-term exposure to SO₂.

Ashenden *et al.* (1996) conducted greenhouse studies of SO₂ effects on growth of 41 British herbaceous species to determine if the species differed in their sensitivity to SO₂. Plants were exposed to a constant background concentration of 262 µg m⁻³ SO₂ with peaks applied during daylight. During the first 4 weeks, peaks were 524 µg m⁻³ for 2 h, twice per week. For the next 3 weeks 786 µg m⁻³ was applied for 3 h, 3 times per week. Finally, for the last 3 weeks peaks of 786 µg m⁻³ were applied for 3 h, 5 times per week. These concentrations were chosen to maximize any growth differences between the tested species. Of the 18 statistically significant responses in terms of total dry mass reductions, there was an average decrease of 43%. The mean response of all 41 species was a 25% decrease in total dry mass. Of the seven statistically significant responses of total leaf area, there was an average decrease of 40%. The mean response for all 41 species was a decrease of 10% in total leaf area. For leaf area ratio (total leaf area:total dry mass), there was an average increase of 45% for the 20 statistically significant responses, and an average increase of 23% for all 41 species. An average decrease of 36% in the root:shoot ratio occurred due to SO₂ exposure (for the 13 species with statistically significant responses); whereas, for all 41 species the decrease was 14%. The authors indicated that while there were differences in growth response of the species tested here, the same responses may not be observed under field conditions because SO₂ concentrations in the field are not expected to be as high as those used in this study. Plants growing in natural communities may also respond differently than plants grown in individual containers. In addition, the nutrient supply in this study was non-limiting while in the field nutrients may be limiting and may alter responses to SO₂.

Julkunen-Tiitto *et al.* (1995) studied the effects of SO₂ exposure on growth and secondary metabolism (production of phenolics and soluble sugars) in six clones of willow (*Salix myrsinifolia*). A disruption in secondary metabolism could cause a reduction in plant defence mechanisms to herbivory and microorganisms. Clones were exposed, in fumigation chambers, to 300 µg m⁻³ SO₂ for 7 h day⁻¹, 5 days week⁻¹, for 3 weeks. Salicin and chlorogenic acid content were significantly decreased by 15% to over 70% (depending on clone) while there was no significant effect on salicortin, 2'-O-acetylsalicortin, (+)-catechin and two unknown phenolics. Since SO₂ exposure did not affect salicortin and 2'-O-acetylsalicortin (key molecules in the defence chemistry) it was concluded that willow resistance to herbivory and microorganism attack was not reduced. Glucose, fructose and sucrose contents were not significantly affected. They found that willow exposed to 300 µg m⁻³ SO₂ produced a 14% to 48% greater phytomass

(leaf, stem, and root dry weights) compared to control plants. This increased growth was attributed to SO₂ acting as an additional nutrient source.

Clarke and Murray (1990) studied the effects of long-term SO₂ exposure on growth and development of *Eucalyptus rudis* Endl. The plants were exposed to 132 and 274 µg m⁻³ SO₂ for 8 h day⁻¹ in open-top chambers, for 17 weeks. There was no effect on S content at the lower concentration of SO₂, but the 274 µg m⁻³ treatment significantly increased total leaf S. They found that a 17-week exposure to 132 µg m⁻³ SO₂ increased the height, average leaf area, and average dry weight of leaves. The increased leaf area and dry weight were attributed to increased size of leaves, as there was no effect on total number of leaves. Sulphur dioxide levels of 274 µg m⁻³ had no effect on plant height, average leaf area and average dry weight of leaves, but produced an increased rate of leaf abscission. However, new leaf production replaced the fallen leaves and the total number of leaves did not change.

Agrawal and Verma (1997) investigated whether varying the levels of nitrogen (N), potassium (K), and phosphorous (P) in the growth medium would affect the response of wheat (*Triticum aestivum* L.) cvs. Malviya 206 and Malviya 234 to SO₂. Wheat was grown in the field with 6 nutrient treatments applied after a basal nutrient addition. Treatments were: no additional nutrients, a recommended application rate, and 4 combinations of N, P, and K. Thirty days after sowing plants were exposed, in open-top chambers to 390 ± 20 µg m⁻³ SO₂ for 4 h day⁻¹, 5 d week⁻¹, for 8 weeks. Visible injury symptoms appeared earlier and were the greatest in both cultivars grown with no additional nutrients. Unfertilized plants exposed to SO₂ had the greatest dry weight, height, and yield reductions while plants grown with recommended or 2 times the recommended levels of NPK were able to alleviate SO₂ effects to the greatest extent. Leaf area and total chlorophyll content decreased significantly when plants were exposed to SO₂. Ascorbic acid was significantly reduced in treated plants, which was attributed to consumption of ascorbic acid for removal of free radicals generated when SO₂ entered foliar tissue. Sulphur dioxide treatment also resulted in an increase in sugars that was associated with a decrease in starch, which suggested increased hydrolysis of polysaccharides for use in the repair process and synthesis of other metabolites. Sulphate-S increased significantly in treated plants with the greatest increase observed in plants grown with no additional nutrients. An increase in the root:shoot ratio was also observed in SO₂ treated plants that suggested a modification in the carbon allocation pattern when plants were exposed to SO₂. They concluded that both nutrient deficiency and SO₂ caused the observed reductions of measured parameters but that the addition of NPK in different combinations was able to ameliorate the adverse effects of SO₂.

3.2 Sulphur Dioxide Uptake and Plant Sulphur Content

In some species foliar sulphur and sulphate content may be used as an indicator of SO₂ exposure. Agrawal and Singh (2000) studied six species of tropical trees from a low rainfall area along a pollution gradient (seasonal average of 49 to 233 µg m⁻³ SO₂) around two coal-fired power plants in India. The focus of this study was to determine the effects of the power plant emissions on the nutrient status of trees transplanted from a plantation into an area in which most of the natural forest had been destroyed. Two species of evergreens (mango, *Mangifera indica*; eucalyptus,

Eucalyptus hybrid) and four species of deciduous trees (guava, *Psidium guajava*; cassod tree, *Cassia siamea*; flame-tree, *Delonix regia*; bougainvillea, *Bougainvillea spectabilis*) were studied. Total foliar sulphur content was higher in all six species at the most exposed location compared to the reference location. In deciduous species there was a greater increase in the foliar sulphate-S content after the onset of new leaves during the summer, which the authors indicated was possibly due to translocation of sulphur from woody plant parts.

In an attempt to assess the critical level of SO₂ in the air which would cause adverse effects on vegetation, Manninen and Huttunen (1995) examined total sulphur content in needles of Scots pine (*Pinus sylvestris* L.) growing at ambient SO₂ levels. Monthly mean concentrations ranged from 10 to 100 µg m⁻³ in 1980, 8 to 61 µg m⁻³ in 1985 and 0 to 95 µg m⁻³ in 1989. They found significant correlations between total sulphur content of the two youngest age-classes of needles (7 and 19 months) and the monthly mean and daily mean ambient SO₂ concentrations. There also appeared to be a relationship between total needle sulphur content and the extent of cuticular damage on current year growth and 12-month-old needles (i.e. decreased damage with decreased sulphur content). Sample size was small here so no statistical analysis was performed.

The Liphook Forest Fumigation Project was established to determine the long-term effects of exposing specific tree species to realistic levels of SO₂ and O₃ within field fumigation plots. One of the studies investigated sulphur and nutrient accumulation in needles of Sitka spruce (*Picea sitchensis* Bong. Carr.), Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.) exposed to 34 or 58 µg m⁻³ SO₂ for 43 months (Shaw and McLeod, 1995). Plants were also exposed to 1.3 times the ambient ozone concentration from spring to December of each year. Ozone concentrations were not given but the authors indicated there was little evidence for effects of ozone on the foliar chemistry. In all three species, both SO₂ concentrations resulted in increased sulphur content and ratio of sulphur to cation levels, in exposed needles.

3.3 Visible Injury

Clapperton and Reid (1994) collected genotypes of timothy (*Phleum pratense*) from two field sites, located in the foothills of southern Alberta, 5 and 20 km from a gas plant. Plants were screened for SO₂ sensitivity in experiments conducted in closed fumigation chambers. In the first experiment, plants were exposed to 393 to 524 µg m⁻³ SO₂. The experiment was terminated after 3 weeks when sensitive plants developed chlorotic areas, browning of the leaves and dead tissue. In a second experiment, plants were exposed to 170 µg m⁻³ SO₂ and the experiment was terminated when plants showed the first signs of damage (two weeks). Plants were considered tolerant when they exhibited no signs of visible injury and no significant decreases in shoot or root dry weights compared to control plants. Subsequent field trials that were also done in this study are not described here, because ambient SO₂ concentrations were not provided.

3.4 Photosynthesis

Several studies have been conducted on the effects of SO₂ on metabolic processes in plants that can affect photosynthesis or be altered as a result of changes in photosynthesis. The following studies examined one or more of the processes closely related to photosynthesis, including: stomatal conductance, photochemical efficiency, carbon dioxide assimilation, chlorophyll content, dark respiration, and carbohydrate metabolism.

The effects of SO₂ on photosynthetic carbon metabolism in winter barley (*Hordeum vulgare* L.) cv. Igri were investigated by assaying the activity of key enzymes in carbon fixation and sucrose synthesis in a 2-year field experiment at Littlehampton, Great Britain (Montiel-Canobra *et al.*, 1991). The crop was exposed to three SO₂ concentrations plus an ambient control using an open air fumigation system. In the first year of the study samples were collected from the ambient (18 µg m⁻³) and high (126 µg m⁻³) SO₂ treatments and in the second year samples were collected from the ambient, medium (73 µg m⁻³) and high (100 µg m⁻³) SO₂ treatments. Significant reductions in flag leaf area of 20% (for each year) and flag leaf dry weight of 23 and 21% were observed in the 1st and 2nd seasons, respectively. The data also indicated there was a delay in flag leaf emergence. The authors indicated that the reduction in size and weight of flag leaves could restrict grain filling due to a reduction in photosynthate production of the leaves. Exposure to the highest SO₂ treatment in each growing season (100 and 126 µg m⁻³), resulted in a significant reduction in fructose-1,6-bisphosphatase (FBPase) activity being observed for the post-anthesis period. It was concluded that reductions in FBPase could influence photosynthetic carbon partitioning in leaves, assimilate distribution, and export of fixed carbon to developing tissues. There were no significant treatment effects on chlorophyll content, phosphoribulokinase (PRK), NADP-dependent glyceraldehyde-phosphate dehydrogenase (NADP-GPD) or phosphoglycerate kinase (PGK) in either growing season.

Lorenzini *et al.* (1995) evaluated the gas-exchange response of two-year-old seedlings of oak (*Quercus pubescens* Wild.) and Turkey oak (*Quercus cerris* L.) exposed to 73, 160 and 244 µg m⁻³ SO₂ for 23 weeks, in fumigation chambers. After 11 weeks of exposure *Q. pubescens* exhibited a significant linear decrease in photosynthetic activity, stomatal conductance, transpiration rate, and water use efficiency. In addition, the vapour pressure deficit increased with increasing SO₂ concentration, and the internal/ambient CO₂ concentration ratio was not affected. For *Q. cerris* there was a significant linear decrease in photosynthetic activity, vapour pressure deficit, and water use efficiency, but there was no effect on stomatal conductance or the transpiration rate. The internal/ambient CO₂ concentration ratio increased 15% at 244 µg m⁻³ SO₂. At the conclusion of the 23 week exposure; tree height and leaf area were not affected in either species; whereas, foliar starch and total S content increased linearly with increasing SO₂ concentration for both species (up to four times more than controls with no visible injury symptoms). Leaf dry weight for *Q. cerris* was linearly depressed as SO₂ increased. The authors attributed the differences in response of the two *Quercus* species to intrageneric variability of physiological response to SO₂. It was also suggested that this type of exposure should be conducted with mature trees to determine if seedling and mature tree responses differed.

Veeranjaneyulu *et al.* (1991) used photoacoustic techniques (detection of heat and photosynthetic O₂ pulses from leaves) to study responses of photosynthetic activity (oxygen evolution and energy used in photosynthesis (PES)) to 131 to 5,238 µg m⁻³ SO₂ in the broad-leaf tree, sugar maple (*Acer saccharum*). They found that at 131 µg m⁻³ SO₂, O₂-evolution and PES increased by 65% and 24%, respectively. However, at 5,238 µg m⁻³ SO₂, these two parameters decreased by 50% and 22%, respectively compared to controls. It was concluded that the enhanced O₂ evolution and PES observed at the lowest concentration was a result of detoxification of SO₂, which in turn stimulated photosynthetic electron transport and that the higher SO₂ concentrations inhibited this electron transport reduced O₂ evolution and PES.

In order to determine if airborne pollutants such as SO₂, copper (Cu), and nickel (Ni) affected the photosynthetic efficiency of natural forests, Odasz-Albrigtsen *et al.* (2000) studied 16 sites along a pollutant gradient. Ambient SO₂ measurements for 1989 to 1992 were modelled by the Norwegian Institute of Air Research to cover the entire region (100x100 m grid). Means ranged from 30 µg m⁻³ (7 km from smelters) to non detectable at the control site 386 km away. Concentrations of Ni and Cu, measured at ground level were highest 7 km from the smelters, 8.0 and 5.0 mg m⁻², respectively. Both metals were non detectable at the control site. Chlorophyll fluorescence was measured in the field and photosynthetic efficiency was estimated using these measurements. It was found that photosynthetic efficiency was generally lower closer to the smelters; although, most of the affected plants failed to show any visible signs of stress or damage. While photosynthetic efficiency was reduced in more than 50% of species investigated, only six species exhibited a weak but significant correlation that indicated photosynthetic efficiency decreased with an increase in SO₂ levels. The six species were: birch (*Betula pubescens*), bilberry (*Vaccinium myrtillus*), crowberry (*Empetrum hermaphroditum* Hagerup), Branch lichen (*Hypogymnia physodes* L.), ground lichen (*Cladina* spp.) and snow-level lichen (*Parmelia olivacea*). Pine (*Pinus sylvestris* L.) exhibited no decrease in photosynthetic efficiency, which was attributed to mitigation of pollution by upper branches (measurements were taken in the lower canopy). Photosynthetic efficiency of all lichen species tested in the mixed forest was inversely correlated with Ni and Cu deposition. Although the vegetation was exposed to SO₂ and metals (Cu, Ni) simultaneously, the potential for interactions was not discussed.

To determine the effect of SO₂ on chlorophyll content, Panigrahi *et al.* (1992) exposed rice (*Oryza sativa* L.) at 20, 40, 60, 80 and 100 days old; and mung bean (*Phaseolus aureus* R.) cv. Dhauli at 15, 30, 45 and 50 days old to 655 to 5,240 µg m⁻³ SO₂ for 6 to 48 h. For both species, chlorophyll content decreased significantly with increased SO₂. In addition, chlorophyll content decreased in each SO₂ treatment as the exposure time increased. It was found that chlorophyll content decreased between 20 and 40% in rice and mung bean at SO₂ concentrations of 655 and 1,310 µg m⁻³ respectively. Exposure to the highest concentration of SO₂ (5,240 µg m⁻³) resulted in almost complete destruction of chlorophyll. It was concluded that a decrease in chlorophyll leads to a decrease in growth parameters including biomass, productivity, and yield; however, growth parameters were not measured in this study so these could not be directly related to the observed chlorophyll reductions.

In a study designed to examine changes in leaf gas exchange resulting from SO₂ exposure, Gerini *et al.* (1990) exposed maize (*Zea mays* L.), in fumigation chambers to 113, 186 or 291 µg m⁻³ SO₂ for 4 weeks. A 20% decrease in photosynthetic activity was observed in plants exposed to 113 and 186 µg m⁻³ SO₂. At 291 µg m⁻³ SO₂, photosynthetic activity was decreased by 10% compared to control plants. Stomatal conductance, transpiration rate, and intercellular/ambient CO₂ were enhanced at the lowest SO₂ treatment, but then declined to near control levels at the highest SO₂ treatment. In contrast, water use efficiency and CO₂ assimilation rate declined at the lowest concentration then increased (but not back to control levels). Sulphur dioxide levels used in the study were representative of ambient SO₂ levels observed in the environment. The decrease in photosynthetic activity was attributed to reduced mesophyll assimilation capacity. Stomatal effects were ruled out as stomatal conductance and intercellular CO₂ were enhanced at these levels of SO₂.

Darrall (1991) utilized an open-air fumigation system to examine the effects of SO₂ (ambient, low, medium, and high) on photosynthesis, dark respiration, transpiration, stomatal conductance, and internal CO₂ concentration and discussed observed changes to effects on grain yield in winter barley (*Hordeum vulgare* L.) cv Igri. Experiments were conducted for 3 years and the SO₂ concentrations varied within each year. The average concentrations for the highest SO₂ treatment, for each year were 100, 113, and 126 µg m⁻³. Although SO₂ significantly increased net photosynthesis on some occasions, significant decreases were observed on other occasions. Most of the photosynthetic changes were transient and were attributed to simultaneous changes in stomatal conductance and transpiration. Dark respiration was significantly enhanced at 84 and 113 µg m⁻³ SO₂ (medium and high SO₂ plots, respectively). It was suggested that increases in dark respiration could have resulted from enhanced detoxification and repair processes. No significant effect on grain yield or total plant weight occurred as a result of SO₂ treatment. The author also noted that powdery mildew infested the lower leaves of the plants, which may have affected the results of the experiment.

Ranieri *et al.* (1999) investigated long-term exposure of barley (*Hordeum vulgare* L.) cvs. Panda and Express to 210 µg m⁻³ SO₂, in a greenhouse, to establish if negative impacts of SO₂ could be linked to specific changes in the photosynthetic apparatus. Exposure for 75 days did not result in visible injury to either cultivar, Panda or Express. Results are given as per cent compared to controls. However, the 75 day exposure to 210 µg m⁻³ SO₂ decreased photosynthetic activity by 29 and 49% in cultivars Panda and Express, respectively. Stomatal conductance was reduced by 56% (Panda) and 58% (Express), and whole electron transport chain activity was reduced by 27% (Panda) and 29% (Express). Electron transport activities of photosystem I and II were reduced by 7 and 11%, respectively, in Panda and 18 and 24% respectively, in Express. Significant decreases in leaf pigments were also observed. For example, chlorophyll *a* decreased by 44% (Panda) and 10% (Express), while carotenoids decreased by 46% (Panda) and 10% (Express). Pigment-protein complexes from thylakoid membranes did not show any qualitative or quantitative differences between control and SO₂ exposed plants. The authors concluded that the reduction in photosynthetic activity could be attributed to stomatal closure and a generalized negative impact on the photosynthetic apparatus.

3.5 Other Metabolic Processes

Gupta *et al.* (1991) studied the effects of SO₂ exposure on soybean (*Glycine max*) cv. Elf to determine effects on abscisic acid (ABA) content at the end of the exposure period and after a recovery period of 18 hours. Thirty day old soybean seedlings were exposed in growth chambers to 131, 524 or 1,048 µg m⁻³ SO₂ for 1, 2 or 4 hours. No visible injury was observed at a SO₂ level of 131 µg m⁻³. Although the authors indicated there was slight injury (chlorosis) of top leaves after the 18 hr recovery period in the 524 µg m⁻³ SO₂ treatment but it was not indicated which exposure length resulted in the observed symptoms. Damage (leaf curl and necrotic areas) was also visible in the 1,048 µg m⁻³ SO₂ treatment within 4 h of start of treatment. They found that both exposure concentration and duration significantly increased abscisic acid (ABA) content in leaves. At a SO₂ concentration of 131 µg m⁻³, ABA content increased 28% after 1 h, 87% after 2 h and 141% after 4 h exposure. The 18 hour recovery period resulted in a reduction of ABA levels in all treatments, but ABA levels were still higher than the controls.

Madamanchi and Alscher (1991) examined antioxidants and antioxidant enzymes in an attempt to understand the metabolic differences of two cultivars of pea (*Pisum sativum* L.) known to differ in their sensitivity to SO₂ exposure (cvs. Progress, insensitive and Nugget, sensitive). Plants were exposed in continuously stirred tank reactors to 2,095 µg m⁻³ for 210 min after SO₂ reached the target concentration (this took about 40 min.). Total glutathione (ratio of exposed/control) content increased from 1.11 (at 0 min.) to 2.04 (at 210 min. exposure) in Progress and 1.42 (at 0 min.) to 1.69 (at 210 min. exposure) in Nugget. Reduced glutathione (GSH) increased in Progress from 1.11 to 1.93 and in Nugget from 1.37 to 1.59 for 0 and 210 minutes exposure, respectively. No significant effects were found on ascorbic acid or oxidized glutathione content. Superoxide dismutase activity increased 90% in Progress, but was unaffected in Nugget. Mean glutathione reductase activity increased 35% and 21% in Progress and Nugget, respectively. The authors suggested that the significantly increased glutathione content, glutathione reductase, and superoxide dismutase activities of Progress might be a part of its metabolic resistance to SO₂ exposure.

Rao and Dubey (1990) studied antioxidant production and its role in protecting four tropical tree species (ber, *Zizyphus mauritiana*; jamun, *Syzygium cumini*; neem, *Azadirachta indica*; mango, *Mangifera indica*) from air pollution. Four exposure sites were selected downwind from an industrial source, while the control site was 10 km upwind. Samples were collected once a month for 12 months. The monthly average SO₂ concentration at the 4 sites ranged from 48 to 90 µg m⁻³. The authors considered SO₂ to be the primary pollutant with respect to the plants responses but did not rule out the possible interactive effects of the other pollutants. Sulphate accumulation in the leaves corresponded with the ambient SO₂ level. When exposed to 90 µg m⁻³ SO₂, the sulphate content in the leaves increased by 72%, 69%, 65%, and 92% for ber, jamun, neem, and mango, respectively, in comparison to the control site. Increased sulphate content in the four species ranged from 26% to 48% at the site with an ambient level of SO₂ (48 µg m⁻³). Stomatal conductance decreased by 26 to 28% in the four species at the site with the highest SO₂ level in comparison to the control site. Oxidation of proteins, superoxide dismutase activities and peroxidase activities increased in all four species. The magnitude of the response varied with species and was related to the ambient SO₂ concentration. It was concluded

that increased peroxidase and superoxide dismutase activities could increase SO₂ tolerance under field conditions.

Borland and Lea (1991) investigated the long-term effects of 39, 73, and 100 µg m⁻³ SO₂ (growing season means) on nitrate reductase, nitrite reductase, glutamine synthetase, glutamate dehydrogenase, glutathione reductase activity and total glutathione content in winter barley (*Hordeum vulgare* L.) cv. Igri. Experiments were conducted using an open-air fumigation system at Littlehampton, Great Britain. Nitrate reductase activity in tissues harvested in February, March and April was significantly decreased by 100 µg m⁻³ SO₂. Nitrite reductase activity was relatively constant except for significant increases in April (at 100 µg m⁻³ SO₂) and May (at 39 µg m⁻³ SO₂). There was no effect at any concentration of SO₂ on glutamine synthetase or glutathione reductase. Exposure to SO₂ significantly increased glutamate dehydrogenase activity in samples obtained in December, January and June. Total glutathione varied with the season but there was no accumulation with SO₂ exposure. It was concluded that the concentrations of SO₂ were too low to generate a significant response.

Chauhan (1990) performed a study on the early diagnosis of SO₂-stress and the mechanism of SO₂ damage in crop plants by measuring volatile emissions from treated tissues. Emissions from tomato (*Lycopersicon esculentum* Mill.), mung bean (*Vigna radiata* L.) and maize (*Zea mays* L.) were measured after exposure to 262 µg m⁻³ for 2 h per day or 524 µg m⁻³ for 1 h per day. Tomato and maize were exposed for 60 days and mung bean was exposed for 45 days as it has a shorter life span. Ethylene, ethane, acetaldehyde, and ethanol were measured at 15 day intervals. Ethylene emissions increased substantially in all three species, until visible injury symptoms (chlorosis followed by necrosis) appeared, after which ethylene declined until the end of the exposure. Ethane emissions were detected just prior to the appearance of visible injury symptoms and increased as injury increased. It was suggested that ethane production was a result of lipid peroxidation caused by sulphate oxidation. To verify this, an additional experiment with mung bean was performed to establish if the addition of antioxidants would reduce SO₂ induced damage. Antioxidants substantially reduced ethylene and ethane production supporting the idea that lipid peroxidation was caused by free radicals resulting from sulphite oxidation. Acetaldehyde and ethanol emissions increased as exposure duration increased up to 45 days, but emissions declined after the appearance of visible injury symptoms. As acetaldehyde and ethanol are not normal by-products of aerobic metabolism the author suggested that their production was a result of SO₂ alteration of respiratory metabolism. The rates of emissions of ethane, acetaldehyde, and ethanol were related to the degree of SO₂ resistance displayed by the species in the study (the greater the resistance the greater the rates of emissions).

Bernardi *et al.* (2001) studied levels of soluble leaf proteins and the response of the superoxide dismutase (SOD) complex of bean plants (*Phaseolus vulgaris* L.) cv. Groffy after exposure to SO₂ (79, 157 or 236 µg m⁻³) for 2, 4, or 7 days. No visible injury symptoms were observed in any of the treated plants. Newly synthesized polypeptides were detected in all treatments and there were quantitative differences between the control and treated plants for 6 other protein subunits. The authors indicated that the observed changes in protein synthesis might be linked to a SO₂ resistance mechanism. In addition, SO₂ exposure induced the activation of an additional SOD isoform, which when tested exhibited the characteristics of an iron superoxide dismutase

(FeSOD). It was concluded that the increased activity of the FeSOD represented the initial activation of the antioxidant system in response to radical formation due to oxidation of SO₂.

3.6 Plant Resistance to Other Stresses

An open-air fumigation system was used to assess the development of mycoflora (yeast and filamentous fungi) populations on winter barley (*Hordeum vulgare* L.) cv. Igri in the presence of SO₂ or two fungicides (Magan and McLeod, 1991). Exposures (for the length of each growing season) to 37, 76 or 123 µg m⁻³ (1986) or 37, 73 or 100 µg m⁻³ (1987) caused a consistently lower number of colony forming units on flag leaves while the fungicides had no consistent effect. There was a significant decrease in pink yeast (*Sporobolomyces roseus*) populations in the high SO₂ plots for both years. White yeast (*Cryptococcus* spp.) populations also decreased with SO₂ exposure while populations of *Cladosporium* spp. were not significantly affected. Both fungicide treatments significantly reduced *Cladosporium* spp. populations in 1986. A significantly greater percentage of green flag leaf area was found in the SO₂ treated plots compared to the fungicide treated plots. The authors indicated that yeast and *Cladosporium* species are an important component of cereal mycoflora and they can act as antagonists of foliar pathogens through competition for nutrients on the leaf surface. Thus, a reduction in these populations by SO₂ could reduce plant defences to pathogen attack.

Over a three-year period, Mansfield *et al.* (1991) examined the effects of SO₂ exposure in an open-air fumigation system on the development of fungal diseases of winter barley (*Hordeum vulgare* L.) cv. Igri. Sulphur dioxide concentrations of 24 (ambient), 55, 84 and 113 µg m⁻³ were applied in 1984/85, 18 (ambient), 37, 76 and 126 µg m⁻³ in 1985/86 and 13 (ambient), 64, 73 and 100 µg m⁻³ in 1986/87, during the growing season. There was a general trend of increased powdery mildew infection (significant increases at some sample times) in plots exposed to SO₂, and decreased leaf blotch infection (significant decreases at some sample times). There was no effect of SO₂ treatment in any year on the incidence of sharp eyespot, *Fusarium* foot rot, brown rust, glume blotch, and net blotch on the flag leaves while variable effects were observed for eyespot and black ear moulds. The authors indicated that the variable response of disease to SO₂ could be a result of several interacting factors that have been shown to be affected by SO₂ including; host biochemistry, structure of wax layer, crop growth and growth of the pathogen.

The performance of the grain aphid (*Sitobion avenae* (F.)) on winter wheat (*Triticum aestivum* L.) cv. Rapier and winter barley (*Hordeum vulgare* L.) cv. Igri were assessed at varying levels of SO₂ exposure (in an open-air fumigation system) by Aminu-Kano *et al.* (1991) in a 3 year trial. Winter wheat was grown in the first year and winter barley for the final two years. The number of aphids on plants, plant sugar and plant nitrogen content were examined in treated and untreated plants. Aphid numbers on winter wheat treated with concentrations of SO₂ ranging from 63 to 149 µg m⁻³ through the growing season increased linearly with increasing SO₂ exposure. Total nitrogen levels also increased with increasing SO₂ treatment and soluble nitrogen was significantly greater in all SO₂ treatments compared to the control. There was no effect of SO₂ on plant sugar content in wheat. In the first year of the winter barley exposure, the relationship between exposure (growing season average concentrations of 55 to 113 µg m⁻³ SO₂)

and aphid numbers was very similar to the relationship observed in winter wheat; however, no effect of SO₂ treatment (seasonal means of 37 to 126 µg m⁻³) was noted in the second year of the experiment. It was concluded that field exposures using low levels of SO₂ enhance aphid populations, probably through increased availability of soluble nitrogen in the plants (enhanced food quality for aphids).

3.7 Effects on Pollen

Bosac *et al.* (1993) studied the effects wet and dry exposure, both *in vivo* (on the anthers) and *in vitro* (culture dishes) on germination of pollen from oilseed rape (*Brassica napus*) cvs. Tapidor and Libravo. For *in vivo* treatments inflorescences were exposed in special chambers (excluding the rest of the plant) to 524 µg m⁻³ SO₂ for 6 h. *In vitro* exposures (either wet or dry) were for 3 h. Exposure to 524 µg m⁻³ SO₂ had no effect on germination or pollen tube length, *in vivo* or *in vitro* (dry); however, there was a significant reduction in germination when pollen was exposed to SO₂ while in unbuffered medium droplets. Pollen tube length was also greatly reduced under these conditions but too few pollen grains germinated and grew to calculate reliable means. It was concluded that the reduction in germination in the unbuffered medium was due to acidification of the medium (pH dropped from 6.5 to 5.5) during SO₂ exposure. Sulphur dioxide is highly soluble; therefore, would dissolve and acidify the medium during exposures.

Agrawal *et al.* (1995) utilized a nightshade (*Solanum nigrum*) complex, which exhibits three natural cytotypes [diploid (*S. americanum*), tetraploid (*S. villosum*), and hexaploid (*S. nigrum*)] to determine the effects of SO₂ on pollen chromosomes. Flowering plants were exposed to 524 µg m⁻³ SO₂ for 2 h d⁻¹ for 3, 7 or 11 days. When pollen mother cells (PMC's) were examined it was found that meiotic chromosomal abnormalities were highest in diploid plants (19.67 to 26.0%) and least in hexaploid plants (4.45 to 7.0%). In addition, abnormalities increased with length of exposure for all plants. Pollen sterility followed the same pattern as chromosomal abnormalities, 19.5 to 21.6% in diploid, 13 to 15% in tetraploid and 10 to 13% in hexaploid, with sterility increasing with length of exposure. The authors concluded that the observed abnormalities might have resulted from free radical splitting of phosphodiester linkages of DNA or from bisulphite combining with cytosine or uracil which may result in alteration of DNA or RNA functions.

3.8 Multiple Pollutant (Interaction) Experiments

Frost sensitivity in Scots pine (*Pinus sylvestris* L.) in response to SO₂ exposure, alone or in combination with NH₃, was investigated by Dueck *et al.* (1990). After a 5 month exposure in open-top chambers to 92 µg m⁻³ SO₂, alone or in combination with 53 µg m⁻³ NH₃, branches were frozen at -4, -7, or -10°C. Branches were then thawed, and electrolyte leakage (an indication of cellular damage due to freezing) was measured. No effect of SO₂ on frost hardiness was observed in branches exposed to -4 or -7°C. However, a significant increase in electrolyte leakage from SO₂-treated needles exposed to -10°C was observed compared to needles not

exposed to SO₂. The lack of an effect at -4 and -7°C was attributed to a low degree of frost hardness in these plants. The authors suggested this might have resulted from the mild winter that preceded these experiments. In addition, the combination treatment resulted in a synergistic effect (more than additive) on electrolyte leakage in all freezing treatments. The combination treatment was the only treatment to cause needle injury, which was attributed to damage to the epicuticular wax layer.

Murray *et al.* (1992) examined the growth and yield responses of barley (*Hordeum vulgare* L.) cv. Schooner and subterranean clover (*Trifolium subterraneum*) cv. Trikkala to determine if co-exposure to NO₂ would alter the response to SO₂ alone. Plants were exposed for 108 days to SO₂ (<13 to 1,424 µg m⁻³) and/or NO₂ (<10 to 337 µg m⁻³) in open-top chambers for 4 h day⁻¹. Barley and clover had severe foliar injury at SO₂ concentrations of 686 µg m⁻³ or higher, with no visible injury observed at the lower concentrations. Sulphur dioxide concentrations of 390 µg m⁻³ or less produced no effect on vegetative shoot growth (dry weight) of barley while reductions of up to 70% were observed at 686 µg m⁻³. Most growth parameters decreased linearly as SO₂ concentrations increased. When barley was exposed to SO₂ (144 or 390 µg m⁻³) and NO₂ (337 µg m⁻³), the number and weight of grain increased but protein content and foliar sulphur decreased. Exposure of subterranean clover to 390 µg m⁻³ SO₂ resulted in a 30% increase in the longest branch length but higher concentrations resulted in sizeable reductions in branch length and above ground plant dry weight. When exposed to the SO₂ and NO₂ mixture, above ground plant dry weight and branch length were reduced. Shoot protein and shoot sulphur increased with increasing SO₂ exposure when exposed to SO₂ alone and to the mixture. It was concluded that barley and clover responded differently because of their differential access to nitrogen. Clover is a nitrogen fixer and likely has sufficient amounts of nitrogen compounds; thus, toxic levels of nitrogen derivatives could accumulate when exposed to NO₂.

Adaros *et al.* (1991a) used open-top chambers to examine interactive effects of SO₂, O₃ and NO₂, in two cultivars of spring barley (*Hordeum vulgare* L.) cvs. Arena and Alexis, and two cultivars of spring wheat (*Triticum aestivum* L.) cvs. Star and Turbo. Experiments were conducted over two growing seasons. In the first season (1988) plants were exposed to 56 µg m⁻³ SO₂, 47 µg m⁻³ O₃ and 55 µg m⁻³ NO₂ singly, or in all combinations. During the second growing season (1989) exposures were 63, 121 and 60 µg m⁻³ for SO₂, O₃ and NO₂, respectively. Sulphur dioxide exposures were continuous, while O₃ was for 8 h day⁻¹ followed by NO₂ for 16 h day⁻¹. Significant decreases of 5 and 6% in the 1,000 seed weight were observed for barley cv. Arena in the 1st and 2nd seasons, respectively. Ear length exhibited a significant 3% increase in length; however, there was no significant effect on yield. No other effects of SO₂ were observed for this cultivar. The only significant effect observed in cv. Alexis was an 8% decrease in dry weight per 1,000 seeds in 1989. Significant interactions were observed for most of the parameters measured in the SO₂-NO₂ treatment for cv. Arena in 1988 but there were no significant interactions in 1989. There was little evidence of interactive effects in any of the treatments for cv. Alexis. Both wheat cultivars showed variable effect due to SO₂. In 1988 cv. Turbo had significant decreases in ear dry weight (8%), total plant dry weight (7%), grains/ear (4%), 1,000 seed weight (4%), and in grain yield (9%), while in 1989 the only significant decrease observed was a 5% decrease in the 1,000 seed weight. In contrast, the wheat cv. Star showed little effect of SO₂ in 1988 and a significant increase in yield (9%) in 1989. Plant weight and yield of both wheat cultivars were affected negatively in the SO₂-O₃ treatment in both years. The three-pollutant

combination generally had a negative effect on growth and yield in both years; however, there were no consistent significant effects of any of the pollutant mixtures. The authors indicated they were unable to adequately explain the differences in response for the two growing seasons.

In a similar set of experiments, Adaros *et al.* (1991b) exposed oil seed rape (*Brassica napus* L.) cv. Callypso for three growing seasons to SO₂, O₃ and NO₂ to determine growth and interactive effects. In the first growing season (1987) plants were exposed to SO₂ (46 and 88 µg m⁻³) and O₃ (44 and 85 µg m⁻³) singly, and in combination. In the final two growing seasons (1988 and 1989) exposures were the same as described above in Adaros *et al.* (1991a). In 1987, exposure to 46 µg m⁻³ SO₂ resulted in small increases in some growth parameters but there was no effect on yield. Exposure to 88 µg m⁻³ SO₂ negated these increases. For 1988 and 1989, there was also little effect of SO₂ on plants, only the number of seeds per pod was significantly increased (5%) in 1989. There were few significant interactions observed and they were not consistent over the 3 growing seasons. However, it was indicated that the few interactions observed were mostly antagonistic (eg. the positive growth in the presence of SO₂ was reduced or became negative when O₃ was added) and that interpreting interactive effects of low levels of air pollutants is difficult as plant responses are often highly variable.

In later experiments, Murray *et al.* (1994) studied the growth responses of barrel medic (*Medicago truncatula*) cv. Paraggio and alfalfa (*Medicago sativa*) to intermittent exposures of SO₂ and NO₂ to determine if the addition of NO₂ would alter the growth response to SO₂. Plants were exposed to 144 to 686 µg m⁻³ SO₂ singly, or in combination with 320 µg m⁻³ NO₂ for 4 h day⁻¹, 7 d week⁻¹ for 108 days. Sulphur dioxide significantly reduced stem length and shoot dry weight of barrel medic. In contrast, shoot sulphur content and digestible dry matter increased as SO₂ concentration increased. Addition of NO₂ further decreased stem length, shoot dry weight, and shoot nitrogen content. Significant interactions were observed at the highest SO₂ concentration and the results indicated an antagonistic interaction. For alfalfa, significant reductions were observed for stem length and shoot dry weight while digestible dry matter, shoot sulphur content, and shoot nitrogen content were increased. Sulphur accumulation was significantly reduced when NO₂ was added to the 390 and 686 µg m⁻³ SO₂ treatments. Average leaf dry weight was not decreased at the highest SO₂ level in the presence of NO₂. This along with the reduction in sulphur accumulation suggested a protective effect of NO₂; however, addition of NO₂ generally resulted in greater growth reductions, for both species, than were observed in SO₂ treatments alone.

In another of the series of studies conducted as part of the Liphook Forest Fumigation Project, Holland *et al.* (1995) examined heights and basal diameters in three conifers: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L. Karst.) and Sitka spruce (*Picea sitchensis* Bong. Carr.) in response to long-term (4 years) fumigation with 10, 34 and 58 µg m⁻³ SO₂. Plants were also exposed to 1.3 times the ambient O₃ for a portion of each year but the authors indicated there was no evidence of any O₃ effects. No relationship was found between SO₂ and growth parameters for Scots pine (despite visual foliar injury). Growth of Sitka spruce was poor after 1988 (the second year of fumigation); however, this was attributed to nitrogen deficiency (information from another study at Liphook) and low rainfall during the second half of the experiment. The annual extension growth of leading shoots between October 1988 and 1990, of Sitka spruce was 38 and 93% greater than ambient controls in the 34 and 58 µg m⁻³ SO₂

treatments, respectively. This growth increase was attributed to increased foliar nitrogen, a result of co-deposition of NH_3 with SO_2 (information from another study at Liphook). In contrast, from 1987 to 1989 Norway spruce showed decreases in stem basal diameter growth, which was related to SO_2 treatment

In a study investigating the effects of SO_2 and/or O_3 on foliar injury and stomatal leaf diffusive resistance Tripathi and Tripathi (1992) exposed rice (*Oryza sativa*) and white bean (*Phaseolus vulgaris*) plants in fumigation chambers to $524 \mu\text{g m}^{-3}$ SO_2 , $392 \mu\text{g m}^{-3}$ O_3 , or a combination of the two, for 6 h per day for 10 days. They found that visible injury first appeared on two-week-old seedlings of rice and bean after 2 days of exposure. By the end of the exposure period 20.8% (white bean) and 20.1% (rice) of the leaf area was injured. For white bean visible leaf injury was least in the combined SO_2 and O_3 mixture (16% of total area) while in rice the mixture resulted in the greatest amount of injury (28.6% of total area). They also observed a decrease in leaf diffusive resistance in both species when they were exposed to $524 \mu\text{g m}^{-3}$ SO_2 . Leaf diffusive resistance for both species increased, > 4-fold when plants were exposed a mixture of SO_2 and O_3 compared to SO_2 alone. It was concluded that the observed differences in leaf diffusive resistance and visible injury could not be explained on the basis of stomatal response alone as several morphological and physiological factors influence stomatal function.

In an attempt to correlate observed canopy damage of Norway spruce (*Picea abies* L. Karst.) with ambient SO_2 , NO_2 and O_3 , Slovík *et al.* (1996) used field data and modelling exercises. Measurements of the indicated gases were taken in six spruce stands in Germany and the annual averages were pooled. For the period of the study, the SO_2 concentration ranged from a high of approximately $50 \mu\text{g m}^{-3}$ in 1985 to approximately $15 \mu\text{g m}^{-3}$ in 1992. Nitrogen dioxide remained fairly constant and averaged between 19 and $24 \mu\text{g m}^{-3}$ while the average ambient O_3 increased from 41 to $65 \mu\text{g m}^{-3}$ during the study period. Damage was defined as "more than 25% needle loss" or "chlorosis of at least 60% of the canopy". Results indicated that there were no correlations between ambient NO_2 or O_3 and observed damage; however, from 1984 to 1989 there was a significant correlation between damage to spruce and ambient SO_2 (damage increased as ambient SO_2 increased). Possible interactions between the pollutants were not discussed.

Shaw *et al.* (1993) reported the effects of SO_2 (34 and $58 \mu\text{g m}^{-3}$) exposure on needle necrosis in Scots pine (*Pinus sylvestris* L.) during the fumigation period of the Liphook Forest Fumigation Project. This study was initiated at the end of July 1988 as unusual foliar symptoms were noticed. Regression analysis indicated that the appearance of foliar injury was related to the mean SO_2 concentration during a critical growth period although injury did not become visible until 5 weeks later. This critical period when the majority of the trees were at the end of the bud bursting stage and needle elongation was about to start. At $58 \mu\text{g m}^{-3}$ SO_2 a greater number of trees exhibited foliar injury in 2 of the 3 survey years and foliar injury appeared on the same trees in consecutive years suggesting that the sensitivity was genetic. A subsidiary fumigation chamber experiment was performed to see if the injury symptoms observed in the field could be duplicated. Concentrations of SO_2 (655 , $1,310$ and $2,619 \mu\text{g m}^{-3}$ SO_2) for 4 hours on Scots pine seedlings produced no effects in any treatment. It was suggested that this may have been due to a low replicate number resulting in a few plants at the most sensitive stage of growth, and/ or low humidity during fumigation.

In another of the Liphook Forest Fumigation Project experiments, Peace *et al.* (1995) studied carbohydrate metabolism of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.). In Scots pine they found considerable decreases in soluble carbohydrates and activity of the regulatory enzyme, sucrose phosphate synthase, during the summer months in all treatments (34 and 58 $\mu\text{g m}^{-3}$ SO_2 , with or without 1.3 times ambient O_3). Norway spruce showed a similar pattern of reductions but of a smaller magnitude. It was concluded that the observed reductions were a result of lower photosynthetic rates and CO_2 assimilation (data taken from other research measurements at the Liphook site).

Growth chambers were used to study the physiological effects of long-term exposure (7 weeks) to SO_2 (45 and 112 $\mu\text{g m}^{-3}$), NH_3 (64 $\mu\text{g m}^{-3}$) or a combination of the two (46 and 69 $\mu\text{g m}^{-3}$, respectively) on poplar (*Populus euramericana* L.) cv. Flevo (van Hove *et al.*, 1991). Net CO_2 assimilation and stomatal conductance were reduced by 15% at 112 $\mu\text{g m}^{-3}$ SO_2 and the reduction in CO_2 assimilation was irreversible. It was concluded that the reduction in CO_2 assimilation was not a result of reduced activity of Photosystem II, but they could not speculate on the causative mechanism(s). Scanning electron microscopy indicated there was no injury to the leaf cuticle, stomatal complex or epidermal cells adjacent to the stomatal pores. No significant effects in measured parameters were observed following treatment with SO_2 and NH_3 together.

An investigation in the Liphook Forest Fumigation Project studied the effect of SO_2 and O_3 , alone or in combination, on the levels of astringin and isorhaptin (antifungal components) produced in spruce bark (Pearce and McLeod, 1995). In addition, *in vitro* assays were used to determine resistance to the root- and butt-rot pathogen *Heterobasidion annosum* (Fr.) Bref. Sulphur dioxide exposure (34 or 58 $\mu\text{g m}^{-3}$, for 2 years) singly, or in combination with 1.3 times ambient ozone, did not affect concentrations of astringin and isorhaptin in Sitka spruce (*Picea sitchensis* Bong. Carr.) or Norway spruce (*Picea abies* (L.) Karst.) seedlings. An induced defence mechanism would require the use of energy reserves such as starch; therefore, starch reserves of the stem were assessed in branches from 3-year-old Sitka spruce. No significant differences were found between treatments. Susceptibility to infection by *H. annosum* was not affected by exposure to SO_2 and O_3 in either species.

3.9 Lichens

Bates *et al.* (1996) used a quantitative study of three lichen species (*Hypogymnia physodes* (L.) Nyl., *Lecanora conizaeoides* Nyl. ex Crombie, and *Evernia prunastri* (L.) Ach.) to determine the effects of pollutant treatments on colonization of three conifer species (Scots pine, *Pinus sylvestris* L.; Norway spruce, *Picea abies* (L.) Karst.; and Sitka spruce, *Picea sitchensis* (Bong.) Carr). Trees were exposed in a field fumigation system to 11 (ambient), 32, or 53 $\mu\text{g m}^{-3}$ SO_2 from May 1987 to December 1990, and ambient or 1.3 times ambient ozone from March-December 1988, May-December 1989, and February-December 1990. Observations of lichen abundance were performed 5-7 months after fumigation stopped. *Evernia prunastri* only colonized trees in plots with ambient SO_2 and was most abundant on Norway spruce. The

number of *H. physodes* was significantly reduced as SO₂ concentration increased, with the greatest numbers observed on Norway spruce. In contrast, the colonization of *L. conizaeoides* increased as SO₂ concentration increased, which was attributed to its tolerance or requirement for SO₂. Colonization by the three lichens was not influenced by the O₃ treatment.

Ranta (2001) conducted one of the more recent studies of lichen recolonization after ambient SO₂ levels had decreased from 160 µg m⁻³ in 1973 to 2 µg m⁻³ in 1999. Lichen diversity and percent cover were monitored once every 5 years from 1980 to 2000 at 25 sites in Tampere, Finland. Six reference sites away from the city centre were also monitored. In 1980, the study sites had an average of 0.7 species with 0.06% cover while the reference sites had a mean of 6.83 species with 7.79% cover. Recolonization was rapid after 1985, and by 2000 study sites had an average of 7.6 species with 10.91% cover and reference sites had an average of 13.83 species with 19.6% cover. The authors noted that there was no geographical pattern to the colonization but that recolonization of the city centre was probably due to the rapid decrease in SO₂ and the small size of the city.

Table 2 Summary of effects of SO₂ on vegetation

Note: When percent increases or decreases are given, these values are comparisons with controls unless indicated otherwise.

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Effects on Growth						
Agrawal and Verma (1997)	<i>Triticum aestivum</i> L. cv. M 206 and M 234 (wheat)	390 ± 20	4 h d ⁻¹ , 5 d wk ⁻¹ , 8 wks	Open-top chambers	Visible injury Results below are for SO ₂ + recommended nutrient treatment (lower nutrients resulted in greater injury): Height Total biomass Total chlorophyll Ascorbic acid content Sulphate-S content Yield	Greatest when no nutrients added Reduced: 3.9% (M206), 4.8% (M234) Reduced: 10.0% (M206), 8.6% (M234) Reduced: 20.0% (M206), 18.7% (M234) Reduced: 18.3% (M206), 16.2% (M234) Increased significantly: both cvs. Reduced significantly: 13.3% (M206), 10.3% (M234)
Ashenden <i>et al.</i> (1996)	Native British species: <i>Agrostis capillaris</i> <i>Anthoxanthum odoratum</i> <i>Arrhenatherum elatius</i> <i>Avena sativa</i> <i>Bromus erectus</i> <i>Bromus sterili</i> <i>Cerastium fontanum</i> <i>Chenopodium album</i> <i>Dactylis glomerata</i>	262, with 2-h peaks of 524 twice per wk for 4 wks, then 262, with 3-h peaks of 786 3 times per wk for 3 wks, then	Total of 83 d	Solardomes (small greenhouses)		

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Ashenden <i>et al.</i> (1996) cont'd	<i>Dechampsia fleuosa</i>	262 with 3-h peaks of 786, 5 times per wk for 3 wks				Statistically significant responses: Total dry weight 18 species average decrease of 43% Root:shoot ratio 13 species average decrease of 36% Total leaf area 7 species average decrease of 40% Leaf area ratio 20 species average increase of 45
	<i>Desmazeria rigida</i>					
	<i>Digitalis purpurea</i>					
	<i>Epilobium hirsutum</i>					
	<i>Eriophorum vaginatum</i>					
	<i>Galium aparine</i>					
	<i>Helianthus annuus</i>					
	<i>Hieracium pilosella</i>					
	<i>Helianthemum</i>					
	<i>munularium</i>					
	<i>Hordeum vulgare</i>					
	<i>Leontodon hispidus</i>					
	<i>Lolium perenne</i>					
	<i>Origanum vulgare</i>					
	<i>Poa annua</i>					
	<i>Poa trivialis</i>					
	<i>Urtica dioica</i>					
	<i>Zea mays</i>					
	<i>Avenula pratensis</i>					Mean response of all 41 species: Total dry weight 25% decrease Root:shoot ratio 14% decrease Total Leaf area 10% decrease Leaf area ratio 23% increase
	<i>Brachypodium pinnatum</i>					
	<i>Briza media</i>					
	<i>Centaurea scabiosa</i>					
	<i>Chamaenerion</i>					No effects
	<i>angustifolium</i>					
	<i>Conyza canadensis</i>					
	<i>Dryas octopetala</i>					
	<i>Festuca ovina</i>					
	<i>Festuca rubra</i>					
	<i>Holcus lanatus</i>					
	<i>Koeleria macrantha</i>					
	<i>Lotus corniculatus</i>					
	<i>Plantago lanceolata</i>					
	<i>Rumex acetosa</i>					
	<i>Thymus precox</i>					

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Clapperton and Parkinson (1990)	<i>Phleum pratense</i> (timothy)	80	Emissions for 23 yrs prior to experiments	Field exposure Site 1 – 5 km downwind from source	VAM spore counts and VAM infection rate of roots of plants grown for 4 wks in soils from each site	714 spores/100 g DW soil Approx. 60% reduction (significant) VAM infection, relative to site 3
		27		Site 2 – 13 km downwind from source		872 spores/100 g DW soil approx. 24% reduction (significant) VAM infection, relative to site 3
		<27		Site 3 – 20 km upwind (control)		1,372 spores/100 g DW soil.
Clarke and Murray (1990)	<i>Eucalyptus rudis</i> Endl. (eucalyptus)	132 (ambient <13)	8 h d ⁻¹ , 17 wks	Open top chambers	Average leaf area	Increased by 37% significant
					Average leaf dry weight	Increased by 29% significant
					Leaf sulphur content	Increase not significant
					Height	Increase approx. 20% significant.
					Leaf abscission	Increased by 45% - significant
Coleman <i>et al.</i> (1990)	<i>Raphanus sativus</i> x <i>raphanistrum</i> (wild type radish) and <i>Raphinus</i> <i>sativus</i> cv. Cherry Belle (cultivated radish)	262, 629 and 1,048	24, 30 or 35 d	Growth chambers	Leaf sulphur content	Increased by up to 25% - significant
					Biomass production	Significant reduction in only one of five trials in Cherry Belle
					Variability in biomass production	Increased significantly as SO ₂ concentration increased
					Shoot dry weight, grain yield	No effect
Collis <i>et al.</i> (1992)	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	89 for 6 d, or 178 for 3 d, then ambient for 3 d, or 534 for 1 d, then ambient for 5 d	6 d cycle repeated 24 times over growing season	Open air fumigation system		
Julkunen-Tiitto <i>et al.</i> (1995)	<i>Salix myrsinifolia</i> (willow), six clones	300	7 h d ⁻¹ , 5 d wk ⁻¹ , for 3 wk	Fumigation chambers	Salicin and chlorogenic acid	Significant reduction of 15% to 70%

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Kropff (1990)	<i>Vicia faba</i> L. (broad bean)	165 (1985)	Growing season	Open air fumigation	Salicortin, 2'-O-acetyl-salicortin, other phenolics	No significant effect
					Soluble sugars in leaves (glucose, fructose, sucrose)	No significant effect
					Total DW	Significantly increased 14 to 48%
					Total dry matter	Decreased by 17%
Murray and Wilson (1990)	<i>Hordeum vulgare</i> L. cv Schooner (barley)	110, 317, 670 and 1,354	4 h d ⁻¹ (light), 79 d	Open top chambers	Yield (pods)	Decreased by 23%
					Total dry matter	Decreased by 9%
					Yield (pods)	Decreased by 10%
					Shoot height	Increased approx. 10% at 110 $\mu\text{g m}^{-3}$ SO ₂
					Shoot weight	15% decrease at 317 $\mu\text{g m}^{-3}$ SO ₂
					Shoot sulphur content	Linear increase: at 317 $\mu\text{g m}^{-3}$ SO ₂ >2.5 fold over control
Murray and Wilson (1991)	<i>Medicago truncatula</i> Gaerm. cv. Praraggio (barrel medic)	107 to 314	4 h d ⁻¹ , 7 d wk ⁻¹ , 72 d	Open top chambers	Head weight and number	Decreased 45 and 20%, respectively at 317 $\mu\text{g m}^{-3}$ SO ₂
					Shoot, stem, and leaf DW, shoot length, branch number, leaf number, flower number	No significant effects
					Shoot sulphur accumulation	Significant increase at 314 $\mu\text{g m}^{-3}$
					Shoot, stem, and leaf DW, flower number	Significant reductions (>40%)
					Shoot sulphur accumulation	Significant increase (approx 85%)
Qifu and Murray (1991)	<i>Solanum tuberosum</i> L. cv. Russet Burbank (potato)	288	4 h d ⁻¹ , 105 d	Top covered chambers		Well watered plants (significant effects at final harvest):
					Leaf area	20% decrease
					Leaf chlorophyll content	30 % decrease in 35 day old leaves
					Leaf sulphur content	> 100% increase
					All parameters measured	Water stressed plants no significant effects at final harvest

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result		
Qifu and Murray (1991) cont'd		786			Leaf area	Well watered plants (significant effects at final harvest): 40% decrease		
					Leaf dry weight	25% decrease		
					Tuber dry weight	35% decrease		
					Leaf chlorophyll content	40% decrease in 35 day old leaves		
					Leaf sulphur content	> 125% increase		
						Water stressed plants (significant effects at final harvest): 100% increase		
Qifu and Murray (1994)	<i>Glycine max</i> L. (soybean)	380	5 h d ⁻¹ for 3 wks followed by a 3-wk exposure to salinity stress	Top-covered chambers	Leaf sulphur content	No effect		
					Other measured parameters	No effect		
					Plant height, leaf number, and nodule number	No significant effects		
					Leaf area	Decreased 33% - significant		
					Chlorophyll content	Increased 19% - significant		
					Shoot dry weight	Decreased 16% - significant		
					Root dry weight	Decreased 25% - significant		
		786			Shoot:root ratio	Increased 18% - significant		
					Nodule fresh weight	Decreased 22% - significant		
					Plant height, leaf number and nodule number	No significant effects		
					Leaf area	Decreased 29% - significant		
					Chlorophyll content	Increased 35% - significant		
					Shoot dry weight	Decreased 16% - significant		
					Root dry weight	Decreased 25% - significant		
					Shoot:root ratio	Increased 23% - significant		
					Nodule fresh weight	Decreased 20% - significant		

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
		380, 786	5 h d ⁻¹ for 3 wks preceded by a 3-wk exposure to salinity stress		Low salinity pre-treatment	Reduced or eliminated SO ₂ effects on all growth variables
					High salinity pre-treatment	Severe injury, in high SO ₂ plants died
SO₂ Uptake and Plant S Content						
Agrawal and Singh (2000)	<i>Bougainvillea spectabilis</i> , (<i>Bougainvillea</i>) <i>Cassia siamea</i> (Cassod tree), <i>Delonix regia</i> (Flame- tree), <i>Eucalyptus</i> hybrid (<i>Eucalyptus</i>), <i>Mangifera indica</i> (Mango), <i>Psidium guajava</i> (Guava)	49 - 233 (seasonal avg - readings once every 10 d for 24 h at 2 h intervals) 22 - reference site	3 yrs	Transplanted trees - field conditions along a gradient from 2 coal-fired power plants	Total foliar sulphur content	24, 35, 27, 33, 41 and 25% increase - species as listed at most polluted site compared to reference site
Manninen and Huhtunen (1995)	<i>Pinus sylvestris</i> L. (Scots pine)	1980: 10 to 100 (mon mean), 56 to 1,700 (d mean) 1985: 8 to 61 (mon mean), 15 to 280 (d mean) 1989: 0 to 95 (m mean), 2 to 357 (d mean)	Long-term field exposures to SO ₂ from a point source	Natural sites selected at different distances from the SO ₂ source. Co- exposure to other pollutants (e.g., O ₃)	Needle sulphur accumulation, cuticular damage	Seven and 19 month old needles - Significant correlation between ambient SO ₂ concentrations expressed as monthly mean, or the sum of highest or second highest daily SO ₂ concentrations of each month Needles harvested from sites with higher ambient SO ₂ exposures exhibited a greater degree of cuticular damage

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Shaw and McLeod (1995)	<i>Picea sitchensis</i> Bong. Carr. (Sitka spruce), <i>Pinus sylvestris</i> L. (Scots pine), <i>Picea abies</i> (L.), Karst. (Norway spruce) (4 y old seedlings at start of treatments)	34 and 58	43 months (May 1987 to Dec 1990)	Open air field fumigation system	Foliar sulphur content	Increased in all species and at both SO ₂ concentrations
Liphook		plus 1.3 x ambient ozone			Foliar nitrogen content	Increased in both spruce species but not in the pine
					Measured parameters	No effects
Visible Injury						
Clapperton and Reid (1994)	<i>Phleum pratense</i> (timothy - SO ₂ tolerant and non-tolerant clones)	170	2 wks	Fumigation chambers	Visible injury	Experiment terminated at 1 st signs of visible injury – chlorotic areas, brown and dead leaf tissue
Photosynthesis						
Darrall (1991)	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	Ambient, low, medium, & high (100, 113 or 126 are averages for high SO ₂ tmt for each season)	3 growing seasons	Open-air fumigation system	Net photosynthesis, dark respiration, stomatal conductance, transpiration, internal CO ₂ concentration	No consistent effects of SO ₂ on any of the measured parameters
Germi <i>et al.</i> (1990)	<i>Zea mays</i> cv. Lenor G4441 (maize)	113 186 291 113	4 wks	Perspex chambers in a screenhouse	Grain yield, plant total weight	No effect
					Photosynthetic activity	Reduced by 20%
						Reduced by 20%
					Quantum yield	Reduced by 10%
						Decreased significantly (>15%)

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Montiel- Canobra <i>et al.</i> (1991) Littlehampton	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	186	Growing season	Open air fumigation system	Light saturated assimilation rate	Decreased <5%
		291				No significant effect
		113				Decreased significantly (approx 20%)
		186			Transpiration	Decreased significantly (>15%)
		291				Decreased significantly (approx 10%)
		113				Increased significantly (approx 40%)
		186			Stomatal conductance	Increased significantly (approx 30%)
		291				No significant effect
		113				Increased significantly (approx 20%)
		186		Water use efficiency		Increased significantly (approx 15%)
		291				No significant effect (increased <5%)
		113				Decreased significantly (approx 40%)
		186	Inter-cellular/ambient CO ₂			Decreased significantly (approx 35%)
		291				Decreased significantly (>15%)
		113				Increased significantly (approx 45%)
		186	Flag leaf size (area and dry weight)			Increased significantly (approx 35%)
		291				Increased <10%
		186				Significant reductions; area (20%) and weight (23%)
		291	Fructose-1,6-bisphosphatase activity			Significant reduction post-anthesis
		186				No significant treatment effects
		291				No significant treatment effects
		186	Phosphoribulokinase, NADP- dependent glyceraldehyde- phosphate dehydrogenase, phosphoglycerate kinase activity, chlorophyll content			Significant reductions; area (20%) and weight (21%) at 100 µg m ⁻³
		291				
		186				

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Lorenzini et al, (1995)	<i>Quercus cerris</i> L. (Turkey oak)	73, 160, 244	11 wks	Fumigation chambers	Fructose-1,6-bisphosphatase activity	Significant reduction post-anthesis at 100 $\mu\text{g m}^{-3}$
					Phosphoribulokinase, NADP-dependent glyceraldehyde-phosphate dehydrogenase, phosphoglycerate kinase activity, chlorophyll content	No significant treatment effects
					Photosynthetic activity	44% reduction (at 244 $\mu\text{g m}^{-3}$ SO ₂)
					Internal/ambient CO ₂	15% increase (at 244 $\mu\text{g m}^{-3}$ SO ₂)
					Foliar starch	15% increase (at 73 $\mu\text{g m}^{-3}$ SO ₂)
					Total S content	> 100% increase (at 73 $\mu\text{g m}^{-3}$ SO ₂)
					Plant height, leaf fresh weight	No effect
					Photosynthetic activity	5% reduction (at 73 $\mu\text{g m}^{-3}$ SO ₂)
					Water use efficiency	14% reduction (at 73 $\mu\text{g m}^{-3}$ SO ₂)
					Foliar starch	10% increase (at 73 $\mu\text{g m}^{-3}$ SO ₂)
Odasz-Albrigtsen et al. (2000)	<i>Betula pubescens</i> (birch), <i>Vaccinium myrtillus</i> (bilberry), <i>Empetrum hermaphroditum</i> (crowberry) Lichen species: <i>Hypogymnia physodes</i> L., <i>Parmelia olivacea</i> Cladonia spp, <i>Parmelia olivacea</i>	0 to 30 Avg ambient concentration from 1989 to 1992	Potentially since 1932 when the first smelter opened	Natural forest	Total S content	> 100% increase (at 73 $\mu\text{g m}^{-3}$ SO ₂)
					Plant height, leaf fresh weight	No effect
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased
					Photosynthetic efficiency	Decreased as SO ₂ concentrations increased

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
	<i>Pinus sylvestris</i> L. (Scots pine), <i>Betula nana</i> L. (dwarf birch), <i>Arctostaphylos alpinus</i> L. (piarmagin berry), <i>Salix lapponum</i> L. (lap willow), <i>Pleurozium schreberi</i> (Brid.) Mitt. (pine moss)				Photosynthetic efficiency	No significant effect
Panigrahi <i>et al.</i> (1992)	<i>Oryza sativa</i> L. cv. Jahati (rice) Plants 20, 40, 60, 80 and 100 d old <i>Phaseolus aureus</i> R. cv. Dhali (mung bean) Plants 15, 30, 45, and 60 d old	655 1,310 2,620 5,240	6, 12, 24 and 48 h.	Fumigation system	Chlorophyll content	Decreased as SO ₂ concentration increased, significant in all treatments Decreased as exposure duration increased in each SO ₂ treatment
Ranieri <i>et al.</i> (1999)	<i>Hordeum vulgare</i> L., cvs Panda and Express (barley)	210	75 d	Perspex exposure chambers in a greenhouse	Leaf area, leaf, stem and root fresh weight Photosynthetic activity Stomatal conductance Electron transport activity	Significant reduction (data not shown) Reduced: Panda by 29%; Express by 49% Decreased: Panda by 56%; Express by 58% Photosystem I - reduced: Panda by 7%; Express by 18% Photosystem II - reduced: Panda by 11%; Express by 24% Whole electron transport chain activity Reduced: Panda by 27%; Express by 29% Chlorophyll <i>a</i> Significantly decreased: Panda by 44%; Express by 10% Carotenoids Significantly decreased: Panda by 46%; Express by 10%

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Veeranjaneyulu <i>et al.</i> (1991)	<i>Acer saccharum</i> Marsh. (sugar maple, 5-7 yr old)	131, 262, 1,310, 2,619, 5,238	4 h	Fumigation chamber	β-carotene content	Reduced: Panda by 48%; Express by 14%
					VAZ pigments (violaxanthin, antheraxanthin, zeaxanthin)	Reduced: Panda by 50%; Express by 15%
					Quantum yield of O ₂	Increased 65% at 131 µg m ⁻³ Decreased 50% at 5,238 µg m ⁻³
					Energy used for photosynthesis (PES)	Increased 24% at 131 µg m ⁻³ Decreased 22% at 5,238 µg m ⁻³
Other Metabolic Processes						
Bernardi <i>et al.</i> (2001)	<i>Phaseolus vulgaris</i> L. cv. Groffy (bean seedlings)	79, 157 or 236	2, 4 or 7 days	Greenhouse fumigation chambers	Visible injury.	None
Borland and Lea (1991)	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	13 (ambient) 39, 73, & 100 season	Growing season	Open top chambers	Newly synthesized polypeptides (34.5 and 31 kDa)	Observed in all treated plants.
					FeSOD activity	Induced at 79 µg m ⁻³ SO ₂ 2 d exposure
					Nitrate reductase activity	Significantly decreased at 100 µg m ⁻³ Feb., Mar., Apr.
					Nitrite reductase activity	Significantly increased at 100 µg m ⁻³ Apr. & May
Chauhan (1990)	<i>Lycopersicon esculentum</i> Mill. (tomato)	262 524 262 524 262	2 h d ⁻¹ , 15 d 1 h d ⁻¹ , 15 d 2 h d ⁻¹ , 30 d 1 h d ⁻¹ , 30 d 2 h d ⁻¹ , 45 d	Closed-top chambers	Glutamine synthetase activity, glutathione reductase activity, and total glutathione content	No significant effects
					Glutamate dehydrogenase activity	Significantly increased at 100 µg m ⁻³ Dec, Jan & Jun
					Ethylene emissions (percent increase over controls)	100%
						100%
Chauhan (1990) cont'd		262	2 h d ⁻¹ , 15 d	Closed-top chambers		86.66%
						89.7%
						64%

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
		524	1 h d ⁻¹ , 45 d			81.65%
		262	2 h d ⁻¹ , 60 d			50%
		524	1 h d ⁻¹ , 60 d			71.42%
		262	2 h d ⁻¹ , 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d ⁻¹ , 15 d			Nil
		262	2 h d ⁻¹ , 30 d			100%
		524	1 h d ⁻¹ , 30 d			100%
		262	2 h d ⁻¹ , 45 d			100%
		524	1 h d ⁻¹ , 45 d			100%
		262	2 h d ⁻¹ , 60 d			88.88%
		524	1 h d ⁻¹ , 60 d			92.57%
		262 and 524	15, 30, 45 & 60 days		Acetaldehyde and Ethanol	100% increase over controls for both SO ₂ concentrations and all exposure times
	<i>Vigna radiata</i> L. Wilczek (mung bean)	262	2 h d ⁻¹ , 15 d	Closed-top chambers	Ethylene emissions (percent increase over controls)	100%
		524	1 h d ⁻¹ , 15 d			100%
		262	2 h d ⁻¹ , 30 d			66.85%
		524	1 h d ⁻¹ , 30 d			77.47%
		262	2 h d ⁻¹ , 45 d			40%
		524	1 h d ⁻¹ , 45 d			52%
		262	2 h d ⁻¹ , 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d ⁻¹ , 15 d			Nil
		262	2 h d ⁻¹ , 30 d			100
		524	1 h d ⁻¹ , 30 d			100
		262	2 h d ⁻¹ , 45 d			75
		524	1 h d ⁻¹ , 45 d			80.39
		262 and 524	15, 30, 45 d		Acetaldehyde and Ethanol	100% increase over controls for all exposure times

Chauhan
(1990) cont'd

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Gupta <i>et al.</i> (1991)	<i>Zea mays</i> L. (maize)	262	2 h d ⁻¹ , 15 d	Closed-top chambers	Ethylene emissions (percent increase over controls)	100
		524	1 h d ⁻¹ , 15 d			100
		262	2 h d ⁻¹ , 30 d			71.40
		524	1 h d ⁻¹ , 30 d			74.26
		262	2 h d ⁻¹ , 45 d			35.23
		524	1 h d ⁻¹ , 45 d			67.14
		262	2 h d ⁻¹ , 60 d			1.89
		524	1 h d ⁻¹ , 60 d			47.37
		262	2 h d ⁻¹ , 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d ⁻¹ , 15 d			Nil
		262	2 h d ⁻¹ , 30 d			Nil
		524	1 h d ⁻¹ , 30 d			Nil
		262	2 h d ⁻¹ , 45 d			100
		524	1 h d ⁻¹ , 45 d			100
		262	2 h d ⁻¹ , 60 d			64.28
		524	1 h d ⁻¹ , 60 d			67.46
		262 and 524	30, 45, 60 d		Acetaldehyde and Ethanol	100% increase over controls 30 to 60 d exposures
Gupta <i>et al.</i> (1991)	<i>Glycine max</i> L. Merr. (soybean)	131	1, 2, or 4 h	Plexiglas growth chambers	Visible injury	None
					ABA content of fully expanded leaves right after exposure	1 h – 28% higher than control (sig) 2 h – 87% higher than control (sig) 4 h – 141% higher than control (sig)
					ABA content after an 18 h recovery period	1 h – 23.6% higher than control 2 h – 17.2% higher than control 4 h – 42.8% higher than control
		524	1, 2 or 4 h		ABA content of fully expanded leaves right after exposure	1 h – 53% higher than control (sig) 2 h – 131% higher than control (sig) 4 h – 190% higher than control (sig)

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Madamanchi and Alscher (1991)	<i>Pisum sativum</i> L. cvs. Progress and Nugget (pea)	2,095	210 min	Continuously stirred tank reactors	ABA content after an 18 h recovery period	1 h - 50.5% higher than control 2 h - 57.5% higher than control 4 h - 46.7% higher than control
					Visible injury	Leaf curl and necrotic areas visible within 4 h of start of treatment
					ABA content of fully expanded leaves right after exposure	1 h - 86% higher than control (sig) 2 h - 153% higher than control (sig) 4 h - 229% higher than control (sig)
					ABA content after an 18 h recovery period	1 h - 64.8% higher than control 2 h - 68.5% higher than control 4 h - 73.7% higher than control
					Total glutathione (ratio of exposed/control)	Progress - significant increase - 1.11 to 2.04 Nugget - increased - 1.42 to 1.69
					Reduced glutathione (ratio of exposed/control)	Progress - significant increase - 1.11 to 1.93 Nugget - increased - 1.37 to 1.59
					Glutathione reductase activity	Progress - significant increase of 35% Nugget - increased by 21%
					Superoxide dismutase activity	Progress - significant increase of 90% Nugget - no effect
					Ascorbic acid, oxidized glutathione content	No significant effects
					Stomatal conductance	Decreased by 6 to 15%
Rao and Dubey (1990)	<i>Azadirachia indica</i> A. Juss (neem) <i>Mangifera indica</i> Linn. (mango) <i>Zizyphus mauritiana</i> Linn. (bet) <i>Syzygium cumini</i> L. Skeels. (jamun)	48 (monthly average)	Long-term field exposure. Study done for 12 months	Natural forest		
					Sulphate content in leaves	Increased by 26 to 48%
					Leaf protein levels	Decreased by 11 to 35%

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result			
69					Superoxide dismutase activity	Increased by 4 to 14%			
					Peroxidase activity	Increased by 10 to 22%			
					Stomatal conductance	Decreased by 12 to 21%			
					Sulphate content in leaves	Increased by 41 to 61%			
					Leaf protein levels	Decreased by 13 to 41%			
					Superoxide dismutase activity	Increased by 9 to 14%			
					Peroxidase activity	Increased by 15 to 24%			
					Stomatal conductance	Decreased by 19 to 22%			
					Sulphate content in leaves	Increased by 46 to 71%			
					Leaf protein levels	Decreased by 20 to 43%			
79					Superoxide dismutase activity	Increased by 12 to 17%			
					Peroxidase activity	Increased by 18 to 28%			
					Stomatal conductance	Decreased by 26 to 28%			
					Sulphate content in leaves	Increased by 65 to 92%			
					Leaf protein levels	Decreased by 26 to 52%			
					Superoxide dismutase activity	Increased by 16 to 20%			
					Peroxidase activity	Increased by 23 to 33%			
90									
Plant Resistance to Other Stresses									
Amnu-Kano et al. (1991) Littlehampton	<i>Triticum aestivum</i> cv. Rapier (winter wheat)	1984: 29 (ambient) 63, 120, 149	Growing season (Nov to July)	Open air fumigation system	Aphid numbers	Significant increase with increased SO ₂			
					Plant nitrogen	Significant increase in soluble nitrogen			
<i>Hordeum vulgare</i> cv. Igri (winter barley)		1985: 24 (ambient), 55, 84, 113 1986: 18 (ambient), 37, 76, 126	Growing season (Nov to July)	Open air fumigation system	Sugar content	No effects			
					Aphid numbers	1985: significant increase with increased SO ₂ 1986: no significant effects.			

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
Magan and McLeod (1991) Littlehampton	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	1986: 18 (ambient), 37, 76, 123 1987 13 (ambient), 37, 73, 100	Growing season (Nov to July)	Open air fumigation system	Pink yeast populations	Flag leaves: significant reductions, at highest treatment for both years Ears: significant reduction only in 1987 at highest treatment
Mansfield <i>et al.</i> (1991) Littlehampton	<i>Hordeum vulgare</i> L. cv. Igri (winter barley)	1984/85: 24 (ambient) 55, 84, 113 1985/86: 18 (ambient) 37, 76, 126 1986/87: 13 (ambient) 64, 73, 100	Growing season	Open air fumigation system	White yeast populations	Flag leaves: populations decreased Ears: same response as pink yeasts
					<i>Cladosporium</i> spp. populations	No consistent effect on populations on flag leaves or ears
					Powdery mildew	Increased infection with increasing SO ₂ (significant increases at some sample times)
					Leaf blotch	Decreased infection with increasing SO ₂ (significant decreases at some sample times)
Effects on Pollen	<i>Solanum nigrum</i> (Nightshade)	524	2h d ⁻¹ for 3, 7 or 11 d	Fumigation chamber	Eyespot, black ear moulds	Variable effects
					Brown rust, ear <i>Botrytis</i> , net blotch, <i>Fusarium</i> foot rot and sharp eyespot	No effect
					Chromosomal abnormalities	Diploid 19.67-26%, tetraploid 9- 17.99%, hexaploid 4.45-7% of total
					Pollen sterility	Diploid 19.5-21.6%, tetraploid 13- 15%, hexaploid 10-13% of total
Bosac <i>et al.</i> (1993)	<i>Brassica napus</i> L. cvs. Tapidor and Libravo (oilseed rape)	524	3 h	In vitro: petri dishes	Pollen germination and pollen tube growth – dry exposure	No effect

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
			6 h (pollen on anthers of intact plants)	In vivo: special chambers to only expose inflorescences	Pollen germination and pollen tube growth – unbuffered medium Pollen germination and pollen tube growth	Significant reduction in germination. Tube length reduced (too few germinated to do statistics) No effect
Multiple Pollutant (Interaction) Experiments						
Adaros <i>et al.</i> (1991a)	<i>Hordeum vulgare</i> L. cvs. Arena and Alexis (barley)	56	108 d (1988)	Open-top field chambers	Straw, ear, plant dry weights, ears/pot, grains/ear, ear length, grain yield 1000 seed weight	No significant effects. Arena: significant 5% decrease Alexis: no effect
		Plus: 47 O ₃ , 55 NO ₂ in all combinations	O ₃ - 8 h d ⁻¹ NO ₂ - 16 h d ⁻¹		All measured parameters	No consistent significant interactive effects
		63	99 d (1989)	Open-top chambers	Straw, ear, plant dry weights, ears/plant, grains/ear, grain yield Ear length	No significant effects Arena: significant 3% increase Alexis: no effect
		Plus: 121 O ₃ , 60 NO ₂ in all combinations	O ₃ - 8 h d ⁻¹ NO ₂ - 16 h d ⁻¹		1000 seed weight All measured parameters	Arena: significant 6% decrease Alexis: significant 8% decrease No consistent significant interactive effects
Adaros <i>et al.</i> (1991a) cont'd	<i>Triticum aestivum</i> L. cvs. Turbo and Star (wheat)	56	108 d (1988)	Open-top chambers	Straw dry weight, ear length	No significant effects
					Ear dry weight	Turbo: significant 8% decrease Star: no effect
					Plant dry weight	Turbo: significant 7% decrease Star: no effect

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Adaros <i>et al.</i> (1991b)	<i>Brassica napus</i> L. cv. Callypso (spring rape)	46 or 88 121 O ₃ , 60 NO ₂ in all combinations	O ₃ - 8 h d ⁻¹ NO ₂ - 16 h d ⁻¹ 98 d (1989)	Open-top chambers	Ears/pot	Turbo: no effect Star: significant 9% increase
					Grains/ear	Turbo: significant 4% decrease Star: no effect
					1000 seed weight	Turbo: significant 4% decrease Star: significant 4% decrease
					Yield	Turbo: significant 9% decrease Star: no effect
					All measured parameters	No consistent significant interactive effects for the two growing seasons
					Straw, plant dry weights, grains/ear	No significant effect
					Ear dry weight	Turbo: no effect Star: significant 10% increase
					Ears/pot	Turbo: no effect Star: significant 8% increase
					Ear length	Turbo: significant 4% increase Star: no effect
					1000 seed weight	Turbo: significant 5% decrease Star: no effect
					Yield	Turbo: no effect Star: significant 9% increase
					All measured parameters	No consistent significant interactive effects for the two growing seasons
		46 or 88	89 d (1987)	Open top chambers	Dry weights of stems, pods, whole plant, and 1000 seed DW	At 46 $\mu\text{g m}^{-3}$ small increases (<15%) reduced to control levels at 88 $\mu\text{g m}^{-3}$
					Pods/plant and seeds/pod	Decreased from controls
					Yield	Significant 12% decrease at 88 $\mu\text{g m}^{-3}$
		Plus: 44 or 85 O ₃		Measured parameters		No consistent significant interactive effects for the three growing seasons

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
		56	113 d (1988)	Open top chambers	Dry weights of stems, pods and whole plant, number of pods per plant, seeds/pod, 1000 seed weight and total yield	No significant effects
		Plus: 47 O ₃ , 55 NO ₂ in all combinations	O ₃ - 8 h d ⁻¹ NO ₂ - 16 h d ⁻¹		All measured parameters	No consistent significant interactive effects for the two growing seasons
		63	84 d	Open top chambers	Dry weight of stems, pods and whole plant, number of pods per plant, pod length, 1000 seed weight, total yield	No effects
		Plus: 121 O ₃ , 60 NO ₂ in all combinations	O ₃ - 8 h d ⁻¹ NO ₂ - 16 h d ⁻¹		Seeds per pod All measured parameters	Significant 5% increase No consistent significant interactive effects for the two growing seasons
Dueck <i>et al.</i> (1990)	<i>Pinus sylvestris</i> L. (Scots pine)	92	5 months	Open top chambers	Frost sensitivity as indicated by electrolyte leakage at -4, -7 or -10°C	Significant increase at -10°C.
		92 SO ₂ + 53 NH ₃			Frost sensitivity as indicated by electrolyte leakage at -4, -7 or -10°C	Significant increase at all 3 temperatures
					Visible injury	Needle tips brown (approx. 1/2 of needle)
Holland <i>et al.</i> (1995) Liphook	<i>Picea sitchensis</i> Bong. Carr. (Sitka spruce) (4-yr-old seedlings at start) <i>Pinus sylvestris</i> L. (Scots pine - 4-yr-old seedlings at start) <i>Picea abies</i> L. Karst. (Norway spruce - 4-yr-old seedlings at start)	10 (ambient control), 34 and 58	43 months (May 1987 to Dec 1990)	Open air fumigation system	Estimated extension growth of leading shoots	Increased 38 and 93% in low and high treatments, respectively (regression significant)
					Basal diameter growth, shoot elongation growth, height	No significant effect
					Basal diameter growth	Decreased at both treatment concentrations for 1988 and 1989 (regression significant)
					Height and estimated extension growth	No effect

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		Plus 1.3 x ambient O ₃ (approx 30 $\mu\text{g m}^{-3}$)			Measured growth parameters	No effect
Murray <i>et al.</i> (1992)	<i>Hordeum vulgare</i> L. cv Schooner (barley)	13, 144, 390, 686 and 1,424	4 h d ⁻¹ , 108 d	Open top chambers	<p>Foliar injury</p> <p>Vegetative shoot dry weight</p> <p>Above ground biomass</p> <p>Ear number per plant</p> <p>Ear DW per plant</p> <p>Grain number per plant</p> <p>Grain weight per plant</p> <p>Average kernel weight</p> <p>Leaf sulphur content</p> <p>Grain protein content</p>	<p>Results shown for barley are for 144 $\mu\text{g m}^{-3}$ treatment</p> <p>None</p> <p>Decreased by >10%</p> <p>Decreased by 15%</p> <p>Decreased by 10%</p> <p>Decreased by 15%</p> <p>Decreased by 15%</p> <p>Decreased by 25%</p> <p>Decreased by 10%</p> <p>Increased by >45%</p> <p>Decreased by 5%</p>
		Plus: 337 NO ₂			<p>Ear dry weight, number and weight of grain per plant</p> <p>Grain protein content</p>	<p>Increased at 144 $\mu\text{g m}^{-3}$ SO₂, then decreased as concentrations increased</p> <p>25% decrease at 144 or 390 $\mu\text{g m}^{-3}$ SO₂</p>
					Leaf sulphur content	No effect at 144 $\mu\text{g m}^{-3}$ SO ₂ , then increased as concentrations increased
	<i>Trifolium subterraneum</i> cv Trikkala (clover)	144	4 h d ⁻¹ , 108 d	Open top chambers	Longest branch length	Increased by 20%
		390			Total sulphur content	Increased by 55%
					Protein content	Decreased by 10%
					Longest branch length	Increased by 30%
					Total above ground biomass	Decreased by 20%
					Total sulphur content	Increased by 100%
		686, 1,425			Longest branch length, Visible injury	Decreased by >40% Severe

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Murray <i>et al.</i> (1994)	<i>Medicago turncatula</i> cv. Paraggio (barrel medic)	144, 390, 686 $\mu\text{g m}^{-3}$	4 h d ⁻¹ , 7 d wk ⁻¹ , 108 d	Open-top chambers	Total above ground biomass	>75% decrease
					Protein content	Increased by >15%
					Longest branch length	Approx. 20% decrease at 144 or 390 $\mu\text{g m}^{-3}$ SO ₂ , no effect at higher levels
					Total above ground biomass	>50% decrease at 144 or 390 $\mu\text{g m}^{-3}$ SO ₂ , no effect at higher levels
					Sulphur or protein content	No effect
					Stem length	Significant reduction in 2 highest treatments
					Shoot nitrogen content	Significant increase in highest treatment
					Shoot dry weight	Significant linear reduction
					Digestible dry matter, S content	Significant increase
					Shoot dry weight	Decreased about 50% in 144 and 390 $\mu\text{g m}^{-3}$ SO ₂ treatments
	<i>Medicago sativa</i> cv. Siriver (lucerne)	144, 390, 686 $\mu\text{g m}^{-3}$ NO ₂			Shoot nitrogen, S content	At 686 $\mu\text{g m}^{-3}$ SO ₂ , accumulation was reduced below that found in SO ₂ treatment alone
					Stem length, shoot dry weight	Significant decreases at 686 $\mu\text{g m}^{-3}$ SO ₂
					Leaf dry weight	Significant decrease at 2 highest SO ₂ levels
					Shoot S and nitrogen content	Significant increase at 2 highest SO ₂ levels
					Digestible dry matter	Significant increase at 686 $\mu\text{g m}^{-3}$ SO ₂
					Leaf dry weight	No reduction with increasing SO ₂
					S content, shoot dry weight, digestible dry matter	Decreased in comparison to SO ₂ treatment alone

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Peace <i>et al.</i> (1995) Liphook	<i>Pinus sylvestris</i> L. (Scots pine seedlings)	34, 58	43 months (May 1987 to Dec 1990)	Open air fumigation system	Activity of sucrose phosphate synthase	Significant reduction (>40%) in summer
					Soluble sugars	Significant reduction (>55%) in summer
		Plus 1.3 x ambient O ₃			Activity of sucrose phosphate synthase and soluble sugar content	No additional effects compared to SO ₂ alone
	<i>Picea abies</i> L. Karst. (Norway spruce seedlings)	34, 58			Activity of sucrose phosphate synthase	<20% reduction in summer
					Soluble sugars	>10% reduction in summer
		Plus 1.3 x ambient O ₃			Activity of sucrose phosphate synthase	No apparent interaction with SO ₂
					Soluble sugars	Significant reduction (>55%) in July
Pearce and McLeod (1995) Liphook	<i>Picea sitchensis</i> Bong. Carr. (Sitka spruce) <i>Picea abies</i> L. Karst. (Norway spruce seedlings)	34 and 58	2 yr	Open air fumigation system	Bark concentrations of astrigin and isorhapontin (natural antifungal agents)	No significant effect
					In vitro infection by <i>Heterobasidium annosum</i> (Fr.) Bref.	No significant effects
					Starch content – Sitka spruce	No effect
		Plus 1.3 x ambient O ₃	1 yr		Measured parameters	No interactive effects
Shaw <i>et al.</i> (1993) Liphook	<i>Pinus sylvestris</i> L. (Scots pine)	34	43 months, May 1987 to Dec 1990	Open air fumigation system	Number of trees showing needle necrosis	1988: 7 (2 in ambient control) 1989: 5 1990: 8 (1 in ambient control)
		58			Number of trees showing needle necrosis	1988: 34 1989: 7 1990: 33
		Plus 1.3 x ambient O ₃				No effects

Reference	Species	SO ₂ ($\mu\text{g m}^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	<i>Pinus sylvestris</i> L. (Scots pine - 4 yrs old at start)	655, 1310 or 2,619	4 h	Perspex growth chamber	Needle necrosis	No effect.
Slovik <i>et al.</i> (1996)	<i>Picea abies</i> (Norway spruce)	Ambient levels, 1984-1992 annual avg, approx. 1.5 to 50 19-24 NO ₂ 41-65 O ₃	Measurements on mature trees	Selected natural forest monitoring sites in Germany	Damaged canopy - needle loss and chlorosis	Significant correlation between damage and SO ₂ levels between 1984 and 1989.
Tripathi and Tripathi (1992)	<i>Phaseolus vulgaris</i> cv. Pusa parvati (white bean)	524 Plus 392 O ₃	6 h d ⁻¹ , 10 d	Fumigation chambers	Foliar injury	20.8% of leaf area injured
					Leaf diffusive resistance	Decreased slightly
					Foliar injury	16% of leaf area injured
					Leaf diffusive resistance	>4 fold increase over SO ₂ treatment alone
	<i>Oryza sativa</i> cv. Saket-4 (rice)	524 Plus 392 O ₃			Foliar injury	20.1% of leaf area injured
					Leaf diffusive resistance	Decreased slightly
					Foliar injury	28.6% of leaf area injured
					Leaf diffusive resistance	>5 fold increase over SO ₂ treatment alone
van Hove <i>et al.</i> (1991)	<i>Populus euramericana</i> L. cv. Flevo (poplar)	45 112	7 wks	Fumigation chambers inserted into growth chambers	Net CO ₂ assimilation, chlorophyll fluorescence, stomatal conductance, photochemical efficiency, quantum yield, cuticle integrity	No effect.
					Net CO ₂ assimilation, stomatal conductance	15% reduction from controls
					Chlorophyll fluorescence, photochemical efficiency, quantum yield, cuticle integrity	No effects

Reference	Species	SO ₂ (µg m ⁻³)	Exposure Duration	Experimental Method	Measurement	Result
		46 SO ₂ plus 69 µg m ⁻³ NH ₃			Net CO ₂ assimilation, chlorophyll fluorescence, stomatal conductance, photochemical efficiency, quantum yield, cuticle integrity	No interactive effects
Lichens						
Bates <i>et al.</i> (1996)	Epiphytic lichens - <i>Hypogymnia physodes</i> L. Nyl., <i>Lecanora</i> <i>conizaeoides</i> Nyl. ex Crombie, and <i>Evernia</i> <i>prunastri</i> L. Ach	11 (ambient), 32 or 53	May 1987 to Dec 1990	Field fumigation	Colonization of young trees quantified 5-7 months after fumigation	<i>E. prunastri</i> only in ambient SO ₂ <i>H. physodes</i> significantly reduced as SO ₂ increased <i>L. conizaeoides</i> increased as SO ₂ increased
		Plus 1.3 x ambient O ₃				No additional effects

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