Soil Salinity and Water Stress Modify Crop Sensitivity to SO_2 Exposure

By **Ma Qifu**
B. Agr. Sci., Zhejiang Agricultural University
M. Agr. Sci., La Trobe University

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Division of Environmental Science
Murdoch University
Murdoch, W. A. 6150
AUSTRALIA

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary educational institution.

Ma Qifu

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Publications Arising from This Thesis

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SUMMARY

Sulphur dioxide (SO_2) is a primary gaseous pollutant which has toxic effects on the growth, yield and quality of both agricultural and natural plant species. Although plant responses to SO_2 exposure have been extensively studied, much less is understood concerning the influences of other environmental stresses on the expression of effects of gaseous air pollutants. Evaluation of such interactions should be of an economic importance in agriculture and horticulture since plants growing in the field usually encounter air pollution and other stresses simultaneously. Soil water stress and salinity are the common environmental stresses and they have some physiological similarities. This thesis aims to investigate to what extent water stress and salinity modify or amplify the detrimental effects of SO_2 on foliar injury, plant growth and yield, and some physiological and biochemical changes in potato (*Solanum tuberosum* L. cv. Russet Burbank) and soybean (*Glycine max* L. cv. Buchanan) crops under field conditions.

SO_2 exposure induced growth reductions in well-watered potato plants but usually not in the water-stressed plants, indicating a protective function of soil moisture stress in the response of plants to SO_2. This could be caused by a reduced SO_2 uptake in water-stressed plants, as well-watered plants had much higher leaf sulphur concentrations than did the water-stressed plants at the same SO_2 fumigation levels. SO_2 also increased leaf sulphur concentrations in soybean, but simultaneous exposure to SO_2 and salinity significantly decreased leaf sulphur concentrations when compared with exposure

to SO_2 alone. As a consequence, SO_2-induced foliar injury was more severe in the well-watered or nonsaline plants than in the water-stressed or saline plants.

Exposure conditions can also be important in determining the response of a plant to stress interactions. Contrasts of sequential and simultaneous exposures to SO_2 and salinity were made in this project so as to examine stress compensatory mechanisms and predisposition characteristics. It was found that low salinity pretreatment (27 mM NaCl) ameliorated the detrimental effects of SO_2 on soybean growth probably by inducing stomatal closure. However, high salinity (48 mM NaCl) treated plants, which also showed high stomatal resistance, were severely injured by subsequent SO_2 exposure especially at high SO_2 concentrations (300 nl l^{-1}). It was likely that high salinity pretreatment decreased or even destroyed plant homeostasis due to direct injury of high ion concentrations. By comparison, plants pretreated with SO_2 became vulnerable to salt injury and those pretreated with high SO_2 were killed after 12 days of high salt stress. This was probably because SO_2 altered the patterns of assimilate allocation favouring shoot growth at the expense of root growth and induced other metabolic changes. As a consequence, the resistance of polluted plants to salinity stress was reduced.

SO_2 pollutant increased the shoot to root ratios by either reducing root growth or stimulating shoot growth, whereas soil moisture stress had the opposite effect. Exposure to 300 nl l^{-1} SO_2 under well-watered conditions induced an increase in the shoot to root (including tuber) ratios of potato plants early in the growing season. In contrast, water stress decreased the ratios in the control and 110 nl l^{-1} SO_2 treatments, but not at 300 nl l^{-1} SO_2 indicating that high SO_2 had disrupted this acclimatory response to soil moisture stress. SO_2-induced increase in the shoot to root ratios was also observed in the soybean experiments. However, it appeared that soil salinity did not significantly affect the ratios.

High SO_2 decreased the number and weight of root nodules, and suppressed nodule nitrogenase activity. Consequently, both shoot and root nitrogen concentrations were reduced. In combination with low salinity, however, the adverse effects of high SO_2 on nodule number, specific nodule activity and plant nitrogen concentrations were ameliorated. Biomass was usually not very sensitive to the interactive effects of SO_2 and salinity, probably because it is slower to respond to the stresses following physiological and biochemical processes. In the field, stress interactions may become even more complicated due to interactions with other environmental stresses.

In conclusion, moderate soil salinity and moisture stress can modify crop sensitivity to SO_2 exposure mainly through stomatal mechanisms. Such interactions, together with the knowledge of interactions of gaseous air pollutants and other environmental stresses (*e.g.* light, humidity and temperature), are important when we attempt to establish dose or concentration-response relationships for the development of predictive models for the effects of air pollutants on crops or native plants. Environmental factors may readjust the dose thresholds of air pollutants, above which detrimental effect are likely and below which insignificant effects or growth stimulations occur. Therefore, air quality standards designed to protect vegetation may need to consider variations in regional environmental conditions.

CHAPTER ONE

General Introduction

Sulphur dioxide (SO_2) is a primary gaseous pollutant, the product of the combustion of sulphur-containing fossil fuels and the smelting of sulphur-rich ores. It has been estimated that emissions of SO_2 in Europe in 1990 through industrial processes approximated 22 million tonnes (EMEP Report, 1991). In East Asia (China, Japan, South Korea, North Korea and Taiwan), the total SO_2 emission was 23.4 million tonnes in 1987 (Kato and Akimoto, 1992). Current SO_2 emissions in Australia are about 2 million tonnes per year (Murray, 1989). SO_2 can also be of natural origin as a result of entrainment of soil particles, fires, biological decay, volcanoes, fumaroles and hot springs, plant emissions and marine-derived sulphate salts. The natural emissions of SO_2 in the world are estimated to be 60 million tonnes per annum (Fowler and Cape, 1982). Volcanic activity represents a significant natural source of atmospheric SO_2. For example, the volcanic eruptions of Mt. St. Helens in Washington emitted 170,000 tonnes of sulphur (88% as SO_2, 12% as H_2S) during the period from March 1980 to March 1981 (Hobbs *et al.*, 1982).

SO_2 can influence the growth, yield and quality of many plant species which are components of agricultural or natural ecosystems (Bell, 1982; Godzik and Krupa, 1982; Murray, 1984*a*, *b*, 1985*a*, *b*, 1986; Murray and Wilson, 1989*a*, 1990*a*; Clarke and Murray, 1990; Wilson and Murray, 1990). The detrimental effects of SO_2 may fall into three categories: hidden (or metabolic), chronic and acute injuries. Metabolic injury is produced by variable, but usually low concentrations of SO_2 (Linzon, 1978) and the effects on growth and yield can take months to develop. Linzon (1978) and Heath (1980) characterised hidden injury using the following criteria: no foliar markings; measurable effects on physiological processes; reduction in growth, yield, quality or aesthetic value; no plant mortality; presence of pollutant and its by-products inside the leaf; increased susceptibility to disease, parasite or insect invasion.

In chronic injury, in addition to the hidden effects, there is yellowing of the leaves (chlorosis) due to slow bleaching of chlorophylls and carotenoids (Kozlowki and Mudd, 1975). Chronic damage occurs from long-term exposure to sublethal concentrations of SO_2.

Acute injury is caused by exposure to high SO_2 concentrations for short periods and is characterised by the collapse of marginal or intercostal leaf areas which initially appear water soaked and then dry and bleach to ivory or brown patches (Kozlowki and Mudd, 1975).

Although SO_2 is normally detrimental to crops and native plants, it may be beneficial under certain circumstances. Sulphur is a macronutrient essential for plant growth and consequently, as SO_2 can be metabolised by plants, low concentrations of SO_2 have been shown to have a beneficial effect on plant growth where sulphur has been growth-limiting (Murray, 1986). SO_2 also induces growth stimulations where plants are unlikely to have been nutrient deficient (Whitmore, 1985), or where plants have received high nitrogen supply as this may cause sulphur deficiency (Cowling and Koziol, 1982).

The response of plants to SO_2 is known to be influenced by other gaseous pollutants such as nitrogen dioxide (NO_2), ozone (O_3) and hydrogen fluoride (HF). Many studies have shown that the interactions of SO_2 + NO_2, SO_2 + O_3 or SO_2 + HF can be additive effects, greater than additive (synergistic) effects, or less than additive (antagonistic) effects, dependent upon such factors as pollutant concentration and exposure duration, as well as on the species and gases used (Ormrod, 1982; Neighbour *et al.*, 1988; Murray and Wilson, 1988*a*, *b*, *c*; 1990*b*).

The expression of effects of SO_2 on plants also depend to a large extent upon other environmental factors. As the predominant SO_2 uptake mechanism involves the stomata, factors which affect stomatal aperture, can affect SO_2 uptake rate and therefore the effective dose. The main influencing environmental factors include light intensity and air temperature (Jones and Mansfield, 1982), relative humidity (McLaughlin and Taylor, 1981), soil moisture and wind speed (Black, 1985; Roberts, 1989).

Although there have been numerous studies on the effects of SO_2 singly, or in combination with other gaseous pollutants, less is understood concerning the interactive effects of SO_2 and other environmental stresses, especially under field conditions. Evaluation of such interaction should be of an economic importance in agriculture and horticulture since plants growing under field conditions usually encounter air pollution and other stresses simultaneously. For example, relative humidity may influence plant responses to all pollutants and may account in part for the greater sensitivity of plants to air pollution in the humid eastern United States compared with the same species of plants grown in the

Southwest (Otto and Daines, 1969). McLaughlin and Taylor (1981) found that for the same exposure concentration, vegetation growing in humid areas may experience a significantly greater internal flux of pollutants than that in more arid regions.

Soil moisture stress (or drought) is considered as one of the most important environmental factors limiting growth and survival of vegetation throughout the world. The effects of water stress on crops and native plants have been studied more extensively than SO_2. However, little work has been done concerning the combined effects of SO_2 and water stress on plant growth, yield and quality characteristics. Krizek *et al.* (1986) found that SO_2 injury in *Euphorbia pulcherrima* was greatly reduced by soil moisture stress pretreatment. Similarly, Amundson *et al.* (1986) found that water stress tended to reduce the damaging effects of O_3 in soybean. The O_3 effects on soybean growth were greatest in the treatments having adequate soil moisture (Flagler, 1986). These findings indicate a protective function of soil water stress in response of plants to air pollution. However, combination of SO_2 and water stress following SO_2 predisposition caused the greatest decline of leaf photosynthetic capacity and high mortality in *Picea abies* (Cornic, 1987; Macrez and Hubac, 1988). Dotzler and Schutt (1990) suggested that plants weakened by air pollutants may succumb to natural stresses like drought or frost depending on their genetic susceptibility. It is widely believed that air pollution may play a major role in the rapidly increasing forest dieback observed in industrialized regions in Europe and North America, though there is inadequate evidence to fully support this hypothesis. Substantial data favour the possibility that drought may trigger or in some way be involved in forest decline (Johnson and Siccama, 1983).

Soil salinity has also been an important factor in agricultural or natural ecosystems. If there is limited rainfall and salt is not leached out of the soil column, crop growth and yield will be reduced as the salt concentration increases. This may be due to salt input by irrigation or other processes, or upward migration in the soil profile as a result of evapotranspiration. Plants are stressed in two ways in a high salt environment. In addition to the water stress imposed by the increase in osmotic potential of the rooting medium as a result of high solute content, there is the toxic effect of high concentrations of ions (Hale and Orcutt, 1987). Salinity can induce a suppression of nutrient absorption due to uptake of NaCl in competition with nutrient ions, specific toxic effects directly on the external plasma membrane or after penetration through the membrane into the protoplast, and a series of metabolic disturbances (Levitt, 1980). Although the effects of soil salinity on crop growth and yield have been extensively investigated, little has been known about salinity-pollutant interactions on plants particularly under long-term field conditions. Previous studies showed that salinity reduces ozone effects on injury and yield in alfalfa (*Medicago sativa* L.), pinto bean (*Phaseolus vulgaris* L.) and garden beet (*Beta vulgaris* L.) grown under laboratory conditions (Hoffman *et al.*, 1973, 1975; Maas *et al.*, 1973; Ogata and Maas, 1973; Bytnerowicz and Taylor, 1983). However, a field study found no overall interaction between ozone and salinity for alfalfa growth and yield (Olszyk *et al.*, 1988). The inconsistency between the laboratory and field studies may account for the different environmental conditions which can influence not only the response of plants to air pollutants but also the degree of salt injury.

Due to limited knowledge but importance of the stress interactions of air pollutants and other environmental factors on vegetation, this thesis aimed to investigate to what extent soil moisture stress and salinity modified the toxic effects of SO_2 on leaf injury, plant growth and yield, and some physiological and biochemical changes in potato and soybean crops. A series of experiments were undertaken in field chambers which provided similar climatic conditions to the ambient. Current scientific knowledge which is relevant to this thesis is reviewed in chapter two. Chapter three is a description of general materials and methods used for the whole research project. The experimental results are discussed in chapters four, five, six and seven respectively. Chapter eight summarizes the results of all the experiments and develops descriptive concepts for the interactive effects of SO_2, soil salinity and moisture stress on plant injury, growth and yield of the used crops, soybean and potato.

CHAPTER TWO

Literature Review

A general review of the effects of SO_2 on physiological processes, plant growth and yield are presented here. In accordance with the contents of the thesis, the effects of soil moisture stress or soil salinity on plants are briefly reviewed, but the emphasis is placed on the interactive actions of SO_2 and these two edaphic stresses. The influence of other environmental factors on the responses of plants to SO_2 exposure is also briefly reviewed. Finally, the importance of this work together with the experimental hypotheses are discussed.

In the literature, units of nl l^{-1} (ppb) or $\mu g\ m^{-3}$ are used to describe the concentrations of SO_2. To compare SO_2 concentrations as part of assessing comparisons of data, conversions of the units have been done at a common stated temperature (*i.e.* 20 °C), providing the original unit which is followed by nl l^{-1} in bracket. To convert from $\mu g\ m^{-3}$ to nl l^{-1}, multiply $\mu g\ m^{-3}$ by 0.375 (Unsworth and Ormrod, 1982).

2.1. SO_2 Uptake

2.1.1 The Chemistry of SO_2

The main oxide of sulphur found in the air is SO_2. It is highly soluble, and its dissociation in water depends upon pH and temperature. The pathway is as follows:

$$SO_2\,(g) \text{ -- } SO_2\,(aq) \text{ -- } H_2SO_3 \text{ -- } HSO_3\,(aq) + H^+ \text{ -- } SO_3\,(aq) + 2H^+ \text{ -- } SO_4$$

bisulphite sulphite sulphate

< pH 4 pH 4-5 <pH 5

Under normal cell conditions, sulphite and sulphate predominate, but they can be reduced in the chloroplasts to form sulphide (S^{2-}) which is then incorporated into methionine, cysteine and a number of other compounds (Hallgren, 1978; Malhotra and Khan, 1984). Sulphate is metabolised to produce glutathione which may be a form of sulphur storage in leaves, particularly in winter (Esterbauer and Grill, 1978).

2.1.2 SO_2 Pathway into the Plant

The uptake of SO_2 by leaves and vegetation surfaces begins with dispersion from a point source, dilution as a result of turbulent mixing in freely moving air, then penetration of the boundary layer surrounding the leaves. Once within the boundary layer, movement of SO_2 into the leaves is largely through the stomata by gaseous diffusion. Once inside the leaf, SO_2 either diffuses into the mesophyll cells directly or dissolves in the fluids exposed on the cell surfaces with subsequent uptake of the dissociation products, bisulphite, sulphite and sulphate ions, through the plasmalemma. Therefore, SO_2 uptake is closely related to atmospheric resistance, aerodynamic (leaf boundary layer) resistance, stomatal resistance and mesophyll resistance (Fig. 2.1)

Rates of diffusion of SO_2 across the leaf cuticle are usually regarded as negligible in contrast against the rates of diffusion across the stomatal aperture. Black and Unsworth (1979*b*) found that about 90% of the total flux of SO_2 to the leaves entered via the stomata, and the remaining 10% was either deposited onto the cuticle or diffused through it. They pointed out, however, that their experimental plants were not field-grown, and that the proportions

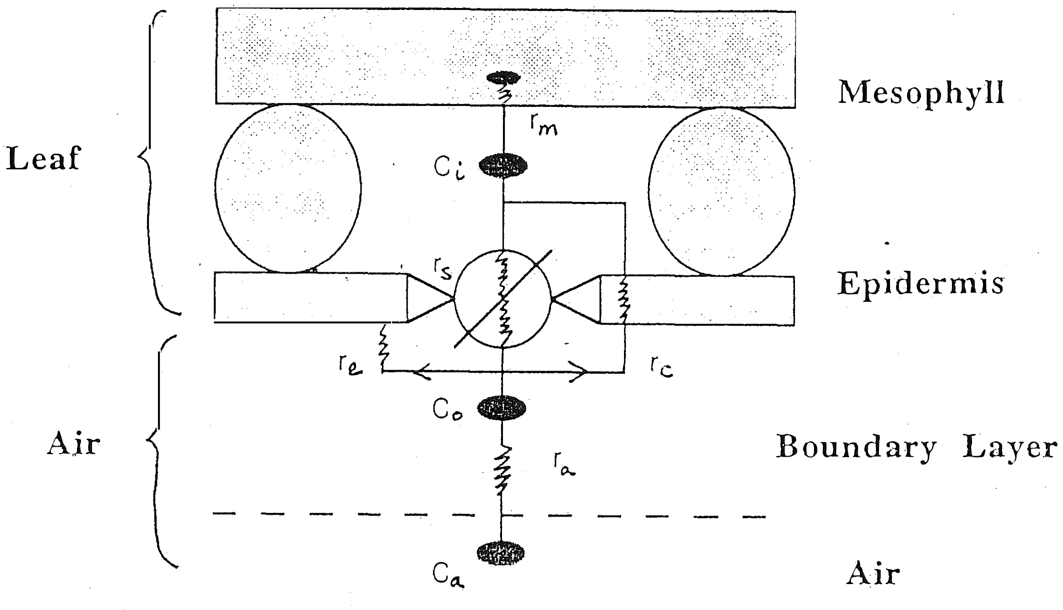


Fig. 2.1 A resistance analogue for SO_2 uptake by a model leaf showing possible paths. C_a, SO_2 concentration in the air; C_0, SO_2 concentration at the leaf surface; C_i, SO_2 concentration in the substomatal cavity; r_a, boundary layer resistance, boundary layer is indicated by a dotted line; r_e, epidermis or surface resistance; r_c, cuticular resistance; r_s, stomatal resistance; r_m, internal resistance (Adapted from Hallgren, 1984).

may be different after weathering of the surface in the field. In addition, as stomata are only partially open or closed for most of the time in the field, Fowler (1978) suggested that about half of the SO_2 influx is cuticular for some plants at least, although this will vary with plants and environmental characteristics. Hence, despite the importance of stomatal uptake, the cuticular uptake may account for the disparity between measurements of stomatal diffusion resistance and SO_2 uptake rates observed by a number of authors (Bressan *et al.*, 1978; Klein *et al.*, 1978; Taylor and Tingey, 1983).

2.1.3 Factors Affecting SO_2 Uptake

Plant uptake of SO_2 is affected by many factors which may be exogenous or endogenous. As the predominant uptake mechanism involves the stomata, those factors which influence stomatal opening have the greatest influence on the severity of the effects of SO_2 (Fig. 2.2).

2.1.3.1 Exogenous Factors

a. Light

Light intensity and quality are the primary factors controlling stomatal opening and thus SO_2 injury. In the absence of light, most stomata are partially or virtually closed in most species under normal conditions, and therefore gaseous exchange is low or minimal. At midday when stomatal opening is at a maximum in most species, SO_2 injury was found to be greater than during early morning or late afternoon (Tibbitts and Kobriger, 1983). Exceptions to this generalisation include plants with specialised metabolism and ecology, especially plants of arid environments which may

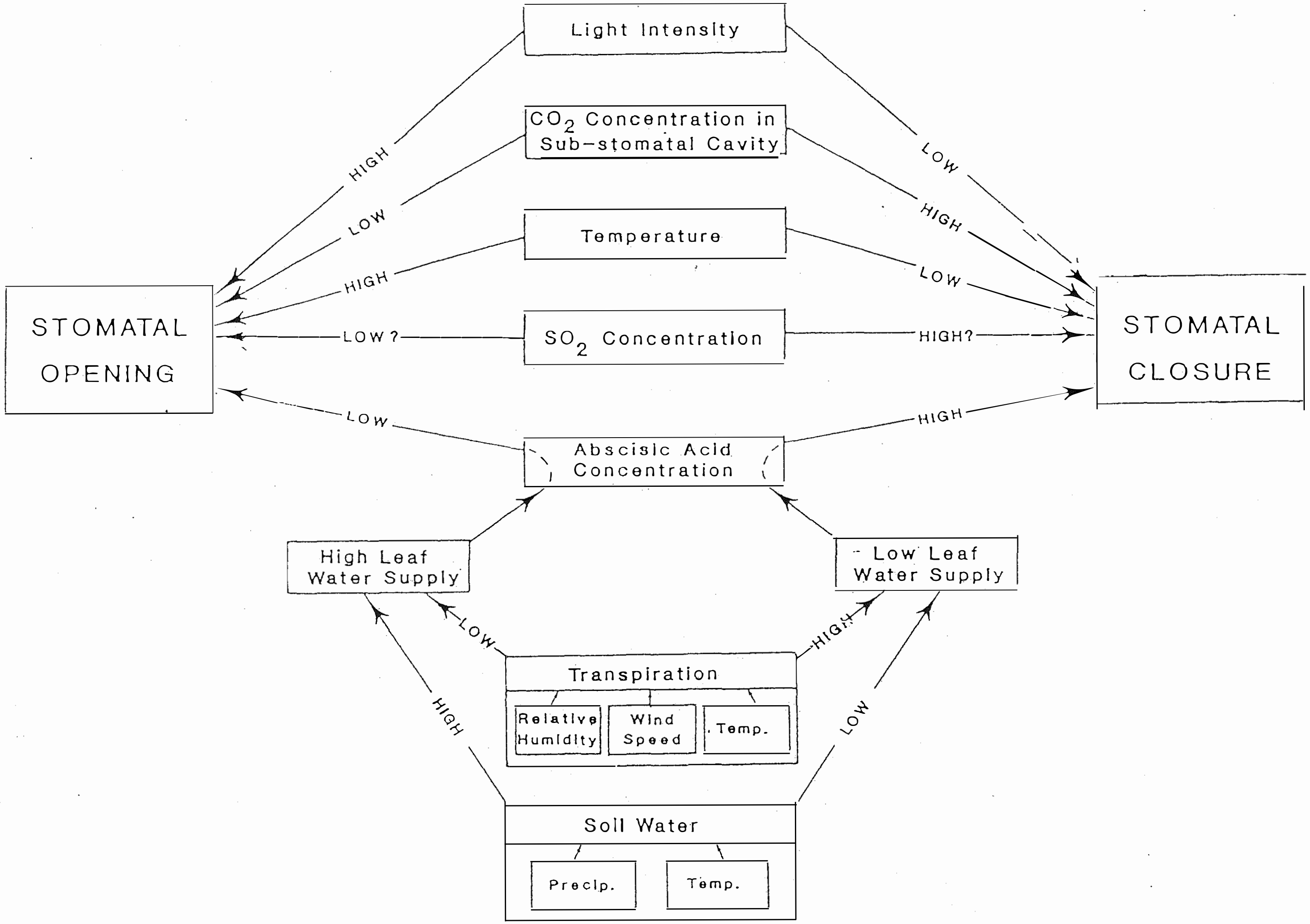


Figure 2.2 Factors which affect stomatal aperture.

normally close stomata during the day to conserve water, and open stomata at night for gaseous exchange. However, if plants have their stomata opened artificially by chemical means, SO_2 is considered to be more toxic in the dark than in the light (Olszyk and Tingey, 1984). This may be due to SO_2 detoxification by light-dependent mechanisms to non-toxic SO_2 derivatives, and due to light-dependent repair processes involving, *e.g.* ascorbic acid and glutathione (Murray, 1985*a*, *b*) or light-dependent reemission of S as H_2S (Sekiya *et al.*, 1982).

b. Humidity

Relative humidity (RH) also appears to be a significant factor regulating plant sensitivity to gaseous pollutants. High RH maintains plants in a turgid condition when adequate soil water is available, and hence favours the opening of stomata, and increases pollutant uptake and subsequent plant injury (Guderian, 1977; Kobriger and Tibbitts, 1985). This simple interaction may explain why the regional differences in average annual humidity are implicated in the greater sensitivity of vegetation to air pollution in the humid eastern United States compared with the same species of plants grown in the Southwest (Otto and Daines, 1969; McLaughlin and Taylor, 1981).

c. Temperature

Temperature plays only a minor role in affecting stomatal aperture and so pollutant uptake and is generally considered not to markedly affect plant response to SO_2 in the range of 18 - 40 °C, provided that other important factors are not changed (Lacasse and Treshow, 1978). Some studies have shown that low temperatures and SO_2 may interact to affect response at a biochemical level. SO_2 reduces the freezing resistance of ryegrass (Davison and Bailey, 1982) and spruce (Keller, 1981) and induces cold-stress injury in wheat plants (Baker *et al.*, 1982). A mechanism to explain the reduction in freezing resistance has been proposed which involves a SO_2-induced decrease in ascorbic acid concentration and the partial inactivation of the glutathione oxidation-reduction system. This leads to the accumulation of highly reactive oxygen radicals which cause protein aggregation upon freezing and thus tissue death (Murray, 1985*a*, *b*).

d. Wind Speed

Wind speed also affects the response of a plant to air pollutions by altering the leaf boundary layer resistance and hence the uptake rate (Ashenden and Mansfield, 1977). Unstirred air layers around plants represent significant barriers to the entry of gases (World Health Organisation, 1987). The thickness of these layers is dependent upon leaf size, shape and orientation, and upon wind speed. As wind speed increases, the boundary layer thickness is reduced, and so the resistance for pollutant uptake decreases. As a result, more molecules of the pollutant enter the leaf to cause damage (Unsworth *et al.*, 1985). For example, in a wind speed of 25 $m\ min^{-1}$, the leaf areas and total weights of perennial ryegrass were significantly reduced after 4 weeks exposure to 312 $\mu g\ m^{-3}$ (117 $nl\ l^{-1}$) SO_2, whereas at a wind speed of 10 $m\ min^{-1}$, SO_2 had no significant effects (Ashenden and Mansfield, 1977).

e. Soil Moisture

Soil moisture stress has an important role in stomatal function, as it directly influences the amount of water available to plants to maintain the cells in a turgid state. As stomata close under conditions of water deficit, plants are less susceptible to air pollutions due to less pollutant uptake (Reich *et al.*, 1985; Tingey and Hogsett, 1985). Treatment of water stress before exposure to SO_2 and O_3 decreased stomatal conductance and transpiration rate of the leaves and greatly reduced the pollutant injury (Tingey and Hogsett, 1985; Krizek *et al.*, 1986). Tingey and Hogsett (1985) used fusicoccin to induce stomatal opening in the water-stressed plants, which subsequently were as sensitive to O_3 as were the non-water-stressed plants, indicating that water stress protected plants from O_3 injury mainly through its influence on stomatal aperture rather than through anatomical or biochemical changes.

f. Nutrient Status

A number of studies have shown some relationship between the nutrition of plants and their response to air pollutants (Reviewed by Cowling and Koziol, 1982). Generally, plants with an adequate supply of nutrients are less sensitive to SO_2 injury than plants with a deficient supply. For instance, yield reduction of perennial ryegrass due to SO_2 (380 $\mu g\ m^{-3}$, *i.e.* 142 $nl\ l^{-1}$) exposure was less in plants grown with high nitrogen than with low nitrogen (Ayazloo *et al.*, 1980). An increase in leaf injury by SO_2 was observed with low but not with adequate addition of nitrogen to the plant culture (Cowling and Lockyer, 1978).

g. Exposure Conditions

Exposure conditions (concentration, duration and pattern) are also important in determining the response of a plant to air pollution. The term "dose", the product of the concentration of a pollutant and the duration of exposure, is commonly used to describe the level of exposure. This means that the same dose may result from short-term exposure to high concentrations, as from long-term exposure to low concentrations. However, in many cases plant response to such different exposure conditions is not equivalent (Roberts, 1984*a*). Lower concentrations over extended periods (chronic exposure) are less damaging than high concentrations over short periods (acute exposure). Continuous exposure to air pollutants is generally more harmful than intermittent exposure as there is no 'recovery period' in which the plant can metabolise, detoxify or store the pollutants (Maclean and Schneider, 1973).

2.1.3.2 Endogenous Factors

The biological factors which may affect plant susceptibility to pollutant-induced injury are ontogenic (developmental stage) or genetic. Seedlings are relatively resistant to SO_2 due to their lack of fully functional stomata and hence, their relatively low pollutant uptake (Halbwachs, 1984). Deciduous trees are more sensitive during their early growth periods and show increasing resistance with age (Guderian and Stratmann, 1968, as cited by Halbwachs, 1984).

Leaves and plants which have just reached physiological maturity suffer greater injury as they have higher SO_2 uptake rates than younger or older leaves and plants (Guderian, 1977). In some species there are critical growth stages at which plants are highly sensitive to SO_2. Bonte (1982) found that SO_2-induced yield loss was greatest in cereals when exposed at flowering stage, and in radish at full maturity.

Differences in resistance to SO_2 between various species, cultivars or individual plants can be attributed to differences in morphological and anatomical features relating to SO_2 uptake. Smaller stomata and lower stomatal densities reduce gaseous exchange rates. Higher trichome (leaf hair) densities increase boundary layer thickness, and smaller leaves reduce the surface area for uptake.

Slowly growing plants appear to be more sensitive than those with a faster growth rate with the exception of arid and semi-arid plants (Jones and Mansfield, 1982; Cowling and Koziol, 1982). It is likely that slower growth rates are associated with a reduced capacity for detoxification due to lower concentrations of substrates involved in detoxification pathways.

Figure 2.3 summarizes some of the factors affecting plant response to air pollutants.

2.2 Effects of SO_2 on Physiological Processes in Plants

2.2.1 Stomatal Responses

Stomatal changes induced by SO_2 can be variable in direction and magnitude. Stomata may be induced either to open or close under exposure to SO_2, depending on the species examined, the concentration and length of exposure to SO_2, and the prevailing environmental conditions. Some of the first measurements of

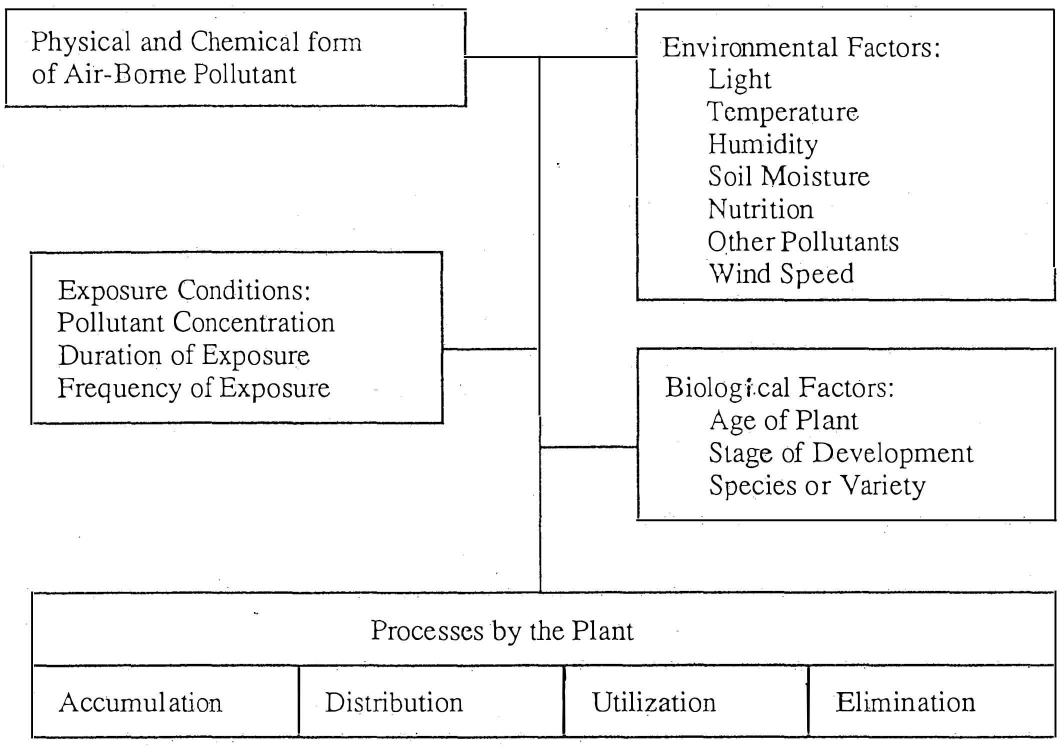


Fig. 2.3 Factors that determine the action and fate of air pollutant in plants (Adapted from Weinstein, 1977).

stomatal responses to SO_2 were made by Mansfield and Majernik (1970), who demonstrated an enhanced stomatal opening in *Vicia faba* plants exposed to concentrations of SO_2 greater than 250 ppb. Other species exhibiting a similar response include *Zea mays* (Unsworth *et al.*, 1972; Gerini *et al.*, 1990), pine (Farrar *et al.*, 1977), *Phaseolus vulgaris* (Ashenden, 1978; Rist and Davis, 1979), Pea and corn (Klein *et al.*, 1978), radish, sunflower and tobacco (Black and Unsworth, 1980), navy beans, cucumber, soybeans and white beans (Beckerson and Hofstra, 1979*a*, *b*) and *Atriplex triangularis* and *A. sabulosa* (Winner and Mooney, 1980*b*). Stomatal opening is the common response to low concentrations of SO_2.

In contrast, stomatal closure in response to SO_2 has been reported in many species. These include pinto beans (Sij and Swanson, 1974), pine (Caput *et al.*, 1978), deciduous and evergreen shrubs (Winner and Mooney, 1980*a*), peanut, tomato, radish, perilla and spinach (Kondo and Sugahara, 1978), wheat, corn, sorghum and bean (Kondo *et al.*, 1980), birch (Biggs and Davis, 1980), *Diplacus aurantiacus* and *Heteromeles arbutifolia* (Atkinson and Winner, 1987, 1989; Atkinson *et al.*, 1988). In general, when plants are exposed to high SO_2 concentrations, particularly for a long period, stomatal closure is usually observed.

The different responses of stomata are probably related to the differential SO_2 sensitivity of epidermal and guard cells (Unsworth and Black, 1981). Epidermal cells are more sensitive to SO_2 than guard cells, and low SO_2 concentrations may cause death of epidermal cells but not guard cells. Therefore, when the epidermal cells become flaccid, guard cell turgor results in further stomatal opening. However, high SO_2 concentrations can cause death of both epidermal and guard cells and result in stomatal closure.

2.2.2 Effects on Photosynthesis

The inhibitory effects of SO_2 on the photosynthetic CO_2 exchange of plants are documented in several reviews (Mudd and Kozlowski, 1975; Heath, 1980; Black, 1982; Hallgren, 1984; Darrall, 1989). In the majority of species, significant inhibition by SO_2 is first detected between 200 and 400 ppb. However, there are few reports of SO_2 effects at lower concentrations. In *Vicia faba* photosynthetic inhibition was detected upon fumigation with 35 ppb SO_2 for 2 hours (Black and Unsworth, 1979*a*). The inhibition was reported at 100 ppb SO_2 in *Hordeum vulgare* (Taniyama, 1972). Exposure to 43 and 71 ppb SO_2 induced a decrease in the apparent photosynthesis and quantum yield in comparison to the charcoal-air filtered controls (Gerini *et al.*, 1990).

Depressions in photosynthetic rates occurring within 30 min to a few hours after the start of exposure, are dependent on SO_2 concentration over a range of concentrations, and are readily reversible and usually not accompanied by visible injury, at least at low concentrations (Bull and Mansfield, 1974; Black and Unsworth, 1979*a*). At higher concentrations, responses are less reversible and appear to be associated with the breakdown of biochemical systems, tissues and appearance of visible injury.

The mechanisms of SO_2 effects on photosynthesis are largely unknown but many explanations have been proposed. Absorbed SO_2 can compete with CO_2 for binding sites on the carbon-fixing enzymes for both C_3 and C_4 types of photosynthetic systems

(Ziegler, 1972, 1973). This is consistent with the speed of photosynthetic inhibition and recovery, and the proportionality of the dose-response relationship. Explanations based on chlorophyll breakdown, chloroplast disorganisation, and protein and membrane disruption may account for chronic effects, but are too slow and insufficient to account for the rapid major effects observed after acute exposure (Black, 1982). In a recent study Gerini *et al.* (1990) found that SO_2-induced reductions in photosynthesis were mainly due to nonstomatal components, *i.e.* the direct effect of SO_2 on the mesophyll.

2.2.3 Effects on Respiration

Although the respiratory processes, *i.e.* dark respiration and photorespiration, are important components of the carbon budget, evidence for pollutant-induced modifications of respiration are less well documented than for photosynthesis. Both stimulation and inhibition of respiration in the absence of visible injury have been reported in plants exposed to SO_2. For example, it was reported that an inhibition of respiration resulted when lichens (Klee, 1970; Baddeley *et al.*, 1973) were exposed to high concentrations of SO_2. In contrast, when Baddeley *et al.* (1971, 1972) exposed lichens to a lower concentration of SO_2, a stimulation of respiration was observed. Respiratory rates may return to control values following exposure, as long as concentrations are relatively low and exposure short. This behaviour may reflect the capacity of plants to detoxify sulphite or repair damage incurred by exposure to toxic concentrations of SO_2 (Black, 1984).

2.2.4. Changes in Assimilate Partitioning

Much of the work concerning air pollution effects on the growth of plants has concentrated on aboveground biomass, largely because of the difficulty of recovering the whole of the root system from the soil, especially in field experiments. However, a number of studies have shown that air pollutants may alter the pattern of photosynthate allocation in plants. McLaughlin and McConathy (1983) found significant increases in foliar retention of ^{14}C-labelled photosynthate in *Phaseolus vulgaris* after exposure to SO_2, indicating a reduced rate of photosynthate export to roots. This is consistent with the reports by Noyes (1980), Jones and Mansfield (1982), Lorenc-Plucinska (1984) and Taylor *et al.* (1986). Changes in photosynthate allocation have been reported at pollutant concentrations below those causing inhibition of photosynthesis (Noyes, 1980; Freer-Smith, 1985). It is possible that the initiations of detoxification and repair processes which occur at concentrations below those causing inhibition of apparent photosynthesis act as a trigger to increase the retention of carbohydrates in the leaf tissues exposed to the pollutants (Darrall, 1989). In contrast, Cooley and Manning (1987) listed malfunctioning of the phloem loading processes, reduction in photosynthetic carbon fixation and greater demand for assimilate at the source as three possible responses causes that could lead to changes in carbohydrate distribution. The effects of SO_2 on assimilate partitioning in favour of shoot growth at the expense of root growth may result in an increase in shoot : root ratios (Ashenden and Mansfield, 1977; Norby and Kozlowski, 1981; Reinert and Gray, 1981; Freer-Smith, 1985; Murray, 1985*a*; Temple *et al.*, 1985).

The effects of SO_2 on assimilate distribution have been detected at concentrations ranging from 40 to 750 ppb (Jones and Mansfield, 1982; McLaughlin and McConathy, 1983; Lorenc-Plucinska, 1984; Taylor *et al.*, 1986). Freer-Smith (1985) reported an increase in the shoot : root ratio of silver birch after 9 weeks exposure to 40 ppb SO_2. Changes in shoot : root ratio were attributable to alterations in root weight alone in some cases (Freer-Smith, 1985; Murray, 1985*b*; Pande and Mansfield, 1985). However, in other studies, changes in both shoot and root weight occurred (Ashenden and Mansfield, 1977; Reinert and Gray, 1981; Temple *et al.*, 1985). The redistribution of dry matter in favour of the shoots may account for apparent growth stimulations reported at low concentrations of pollutants (Roberts *et al.*, 1984). The decreased allocation of carbohydrates to roots is likely to limit root growth, and may reduce water uptake under conditions of limited supply.

2.3 Effects of SO_2 on Plant Growth and Yield

2.3.1 Visible Injury

The extent of injury caused by SO_2 depends to a large extent on the exposure regime and plant species. First, the quantity of SO_2 required to cause injury depends on the flux per unit time. Guderian (1970) found that acute plant injury was more severe if the exposure concentration was increased and the time reduced. Secondly, there is the turnover of pollutant derivatives within the plant. Several processes have been identified, including translocation, dilution by new growth and losses through leaching, gaseous emission or exudation through the roots. Thirdly, environmental factors will modify the uptake of SO_2 and consequently influence the sensitivity of plants to the pollutant.

Fourthly, individual metabolic systems within different species may vary in their ability to tolerate the primary pollutant or its products (Garsed, 1984).

Acute injury due to the rapid absorption of SO_2 causes marginal and intercostal necrosis in dicotyledonous plants which at first have a dull, dark green, watersoaked appearance. On drying and bleaching, these areas become ivory/white in most plant species (*e.g.* garden pea, alfalfa) or brown or red may predominate (*e.g.* chrysanthemum and lettuce) (US Environmental Protection Agency, 1978). The necrotic areas extend through the leaf and are visible on both surfaces. The areas immediately bordering the veins are rarely injured as there are generally few stomata and limited intercellular spaces present. Leaf abscission may occur if a significant portion of the leaf is injured.

Plants exposed to sublethal concentrations of SO_2 may in time develop chlorotic symptoms. Yellowing of the margins and interveinal areas occurs with the affected areas remaining turgid. The normal lush green of the leaf is lost due to plasmolysis of the chloroplasts in affected mesophyll cells with the destruction of chlorophyll causing a bleached appearance. Under-surface yellowing of the leaf is common and is often extended to the upper surface. Silvering of the lower surface may occur and premature leaf senescence is not uncommon.

Monocotyledonous plants vary in their response to SO_2. Injury usually develops first at the leaf tips and then extends downwards with continued fumigation. Foliar injury by SO_2 ranges from chlorosis and necrosis to premature leaf senescence and abscission.

2.3.2 Growth and Yield Reductions

Earlier literature (Katz, 1949) insisted that plant growth remained unaffected by air pollution in the absence of visible injury. This has since been proven incorrect and it is now known that pollutant-induced growth reductions can occur with or without visible injury (Bell, 1982; Godzik and Krupa, 1982). A number of studies have shown the adverse effects of air pollutants on plant growth in the absence of visible symptoms with both ambient versus filtered air experiments (Bleasdale, 1973; Crittenden and Read, 1978; Awang, 1979) and fumigation with single (Ashenden, 1978; Bell *et al.*, 1979; Darrall, 1986; Murray and Wilson, 1989*b*) or combined pollutants (Bull and Mansfield, 1974; Ashenden and Williams, 1980).

The effects of SO_2 on physiological and metabolic processes are generally accepted to be the initial events in a series of changes which influence plant growth, yield and quality characteristics. In addition, the destruction of leaf area by necrosis and reduced photosynthetic activity due to chlorosis causes a reduction in growth and yield. However, the extent of these effects depends on several factors including: plant species, the plant part that is harvested, and prevailing environmental factors. This may explain that the effects of long-term exposure to SO_2 under realistic conditions are usually highly variable and controversial, even when results using the same cultivars but from different experiments are compared (Roberts, 1984*a, b;* McLaughlin and Taylor, 1985; Pande, 1985; Fowler *et al.*, 1988).

The growth stage is also considered to be important for effects of SO_2, and the concept of "critical growth stage" has been raised (Godzik and Krupa, 1982). For example, when cereals are exposed to SO_2 during emergence, flowering, and earing or maturation, flowering appears to be the most sensitive period for grain production (Bonte, 1982, Godzik and Krupa, 1982). Plants showing a "critical growth stage" apparently do not have sufficient time for recovery and compensation to the stress. It was found that SO_2 induced a reduction in pollen germination and pollen tube growth (Ma and Khan, 1976; Houston and Dochinger, 1977; Varshney and Varshney, 1981). Ma and Khan (1976) suggested that the inhibitory effect of SO_2 presumably resulted from physiological disturbance of the cell and severe damage to chromosomes of both generative and tube nuclei of the pollen tube.

However, low SO_2 exposure can induce a growth stimulation for plants (Laurence, 1979; Milchunas *et al.*, 1981; Murray, 1985*a*, 1986; Whitmore, 1985; Baker *et al.*, 1986; Clarke and Murray, 1990). This may be partly due to a fertilising effect as sulphur is an essential nutrient for plant growth. Studies in agricultural species with ^{35}S have clearly shown that sulphur from SO_2 can be translocated and metabolized (Garsed and Read, 1974, 1977*a*, *b*).

Growth analyses, with estimates of relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR), on plants fumigated with SO_2 are reported by Bell *et al.* (1979), Murray (1985*a*) and Coleman *et al.* (1989). RGR is a good measure of plant stress (Chapin, 1989). High RGR suggests that the plant has favourable conditions for growth; and low RGR can mean the opposite, *i.e.* limited resources and resource imbalances. Coleman *et* *al.* (1989) found that mean RGR in radish plants responded rapidly to changes in nitrate availability, and that SO_2 inhibited acclimation of radish plants to decreasing nitrate availability by impairing resource partitioning to nitrate uptake and root growth.

Significant reductions in RGR in *Lolium perenne* due to SO_2 exposure were reported by Bell *et al.* (1979). The effects of SO_2 on RGR were closely parallelled by proportionally similar fluctuations in NAR, with very little influence of the pollutant on LAR. Thus it appeared that SO_2 reduced growth by affecting some physiological process connected with photosynthesis or respiration, rather than a redistribution of assimilate from leaves to non-photosynthetic tissue. By comparison, Murray (1985*a*) found that plants exposed to high SO_2 (215 $\mu g\ m^{-3}$, *i.e.* 81 $nl\ l^{-1}$) had the largest RGR and NAR in the mid-season but later in the season, plants exposed to low SO_2 (78 $\mu g\ m^{-3}$, *i.e.* 29 $nl\ l^{-1}$) had the highest values for these parameters, and plants exposed to the high SO_2 treatment had the lowest. The data suggested that as the duration of exposure increased, there was an increasing stimulation of growth for plants in the low SO_2 treatment but a shift from stimulatory to an inhibitory role for the growth effects of SO_2 for plants in the high SO_2 treatment.

2.3.3 Dose-response Relationship

The establishment of dose-response relationships are necessary for the development of predictive models for the effects of air pollutants on plants. From these models threshold concentrations can be determined, which are levels above which detrimental effects are likely and below which insignificant effects or growth stimulations occur. This information provides a basis for decisions on the management of environmental quality.

The lowest concentrations of SO_2 that have been found to reduce the growth of higher plants were 16 nl l^{-1} over 173 days for perennial ryegrass (Bell *et al.*, 1979) and 21 nl l^{-1} over 28 days for tobacco and cucumber (Mejstrik, 1980). Lower concentrations which reduce growth or yield have been reported in the literature but the results have been obtained by using filtration experiments. For example, for perennial ryegrass in a filtration experiment, the lowest concentration of SO_2 at which growth reduction has been recorded is 13 nl l^{-1} over only 28 days (Awang, 1979). Buckenham *et al.* (1982) using similar techniques reported a yield reduction in barley due to ambient air containing SO_2 at 19 nl l^{-1}. However, many studies have shown that the interactive effects of SO_2 and other pollutants can be additive, greater than additive (synergistic) or less than additive (antagonistic) (Ormrod, 1982). Therefore, results need to be considered carefully when assessing dose-response relationships. It is important to consider the potential presence of several pollutants when establishing air quality criteria.

Roberts (1984*b*) provided one of the most comprehensive analyses of dose-response relationships by using a data base consisting of 125 exposures of 21 different species of grasses, horticultural crops and cereals to SO_2. The most appropriate functional form for the dose-response relation was a linear regression between SO_2 concentration and percentage yield loss. For instance, he examined various combinations of the data points which led to the establishment of two concentration-response curves, one based on SO_2 concentrations less than 50 nl l^{-1} from studies of 9 different species, and another based on the most extensively studied species *Lolium perenne* using only chamber studies with relatively high air exchange rates. The regression equation for the 9 species was marginally statistically significant ($P= 0.05$) and predicted zero yield loss at 21 nl l^{-1} SO_2 continuous exposure. The regression for *Lolium perenne* was significant at $P < 0.001$ and predicted zero yield loss at 35 nl l^{-1} SO_2 continuous exposure.

Murray and Wilson (1989*b*) grew five crops (wheat, soybean, peanut, navy bean and maize) from seedling to harvest in open top chambers. SO_2 was introduced into the chambers for 8 h/day at concentrations of about <0.4, 52 or 107 nl l^{-1}. The responses of the plants varied. Wheat and soybean were very sensitive with yield reductions of about 5% and 25% at 51 and 103 nl l^{-1}, respectively. Navy beans, maize and peanuts were less sensitive with navy beans and maize showing a yield increase of about 10% at 53 nl l^{-1} and unchanged yield at 107 nl l^{-1}. Peanuts showed yield reductions of about 5% and 10% at 53 nl l^{-1} and 105 nl l^{-1}, respectively. In another experiment, they (1990*a*) fumigated barley plants with 4, 42, 121, 256 or 517 nl l^{-1} SO_2, and found that the growth reduction was generally proportional to the exposure concentration at concentrations above 42 nl l^{-1}.

However, the attempt to establish one dose-response curve which can be applied universally is fraught with difficulties and is realistically unattainable. This is due to the larger number of factors that influence the effect a pollutant will have on a plant, such as the plant species and the conditions under which the plants are exposed, that is, light intensity, temperature, humidity, windspeed, nutrient availability and soil moisture.

2.4 Plant Growth, Drought and Salinity

Drought is a meteorological term that means a lack of precipitation over a prolonged period of time. Sometimes physiologists refer to the resulting effect on plants as water stress but water stress may also occur over relatively short periods of time (Hale and Orcutt, 1987). By comparison, plants are stressed in two ways in a high salt environment. In addition to the water stress imposed by the increase in osmotic potential of the rooting medium as a result of high solute content, there is the toxic effect of high concentrations of ions. Drought and salinity are the two most important environmental stresses affecting world food security and the condition and stability of the land source from which that food is derived (McWilliam, 1986).

2.4.1 Effects of Drought Stress

Water comprises 85% to 90% of the fresh weight of most living plants. In higher plants, water is absorbed by roots from soil and is translocated to the shoots as a result of pressure gradients developed either from root pressure or transpiration. Whenever the rates of water loss by transpiration exceed the rates of water absorption by roots, the plant is subject to a water deficit stress. The stress may produce a dehydration strain, which is elastic and completely reversible up to a point, beyond which it is plastic, irreversible, and therefore injurious (Hale and Orcutt, 1987). The different kinds of water stress injury and their relation to the stress-induced strains are indicated in Fig 2.4.

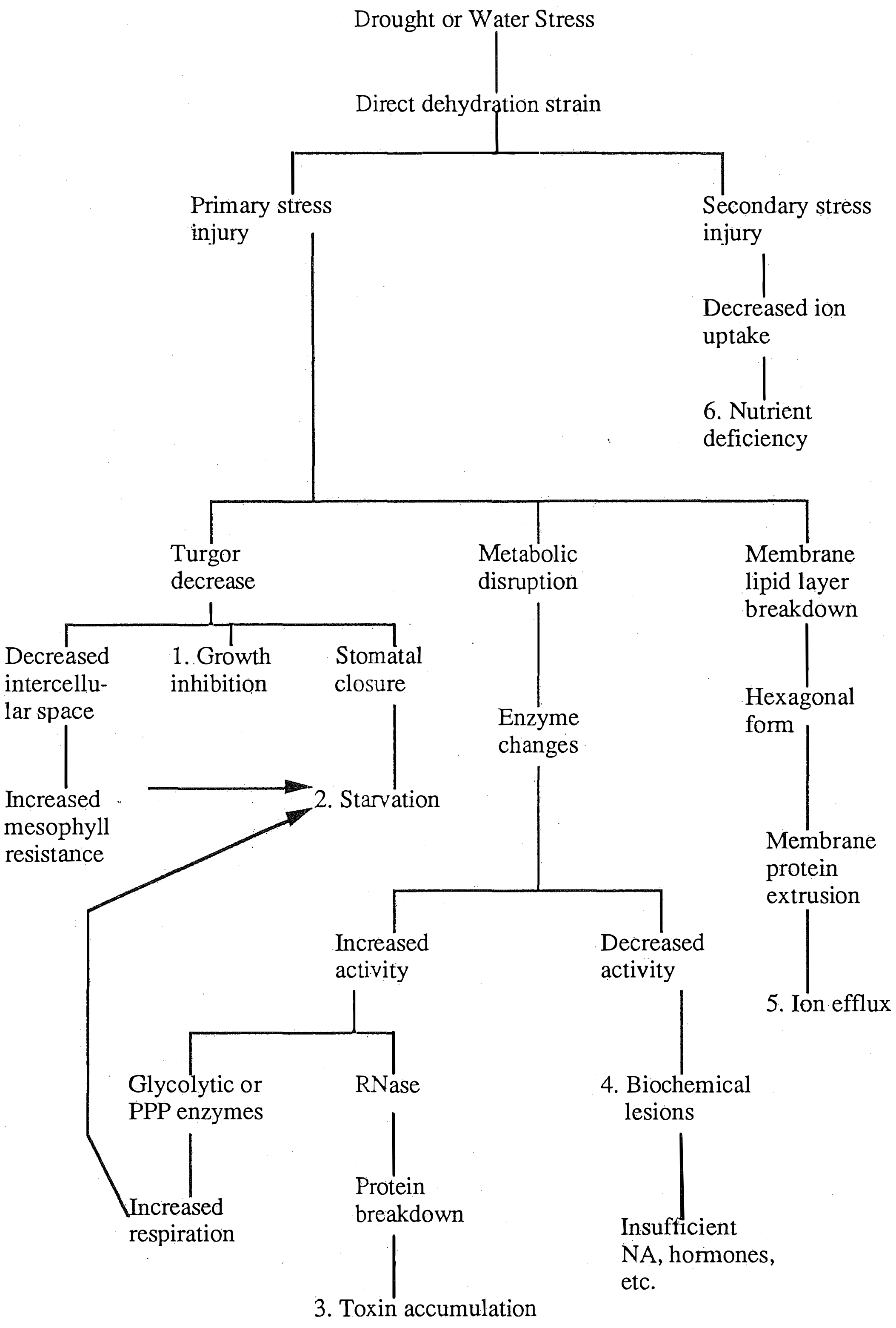


Fig. 2.4 Six different kinds of water stress-induced injury (Adapted from Levitt, 1980).

Decreased cell growth (enlargement) is the most sensitive response of the plant to water stress, since cell growth is quantitatively related to cell turgor, and cell turgor decreases with any dehydration-induced decrease in cell water potential (Levitt, 1980). Leaf enlargement is inhibited by dehydration earlier and more severely than photosynthesis or stomatal conductance (Wesselius and Brouwer, 1972). Loss of cell turgor causes stomatal closure, decreasing CO_2 and O_2 flow into and out of the leaf. As a result, both photosynthesis and respiration are reduced, although the reductive rates are much more rapid for photosynthesis than for respiration. Complete stomatal closure lowers the actual rate of photosynthesis to the level of the cell's respiration - zero net photosynthesis. If this occurs throughout the daylight period, plant respiration will result in a net loss of the reserves, and if for a long enough period, starvation may eventually lead to cell injury or death (Levitt, 1980).

The effect of dehydration on protein synthesis and breakdown is rapid. In *Zea*, free amino acids increased 20% within the first 6 hr of exposure to a water stress, and more than 250% after exposure for 48 hr, when the water potential of the leaf had dropped to -18 bars (Barlow *et al.*, 1976). The extent of free proline in plants with an optimum supply of water is usually very low (0.2-0.69 mg g^{-1}, dry matter). It rapidly rises to 40-50 mg g^{-1} dry matter during slow dehydration of tissues (Palfi *et al.*, 1973). It is suggested that the protein breakdown injures the plants, not simply due to a protein deficit, but to the accumulation of a toxic product of protein breakdown, probably NH_3. The NH_3 may be injurious not only by raising the pH of the cell and disturbing the metabolic balance, but also by inhibiting water uptake (Quebedeaux and Ozbun, 1973).

Dehydration also induces an increase in ribonuclease (RNase) activity. This increase must lead to a decrease in RNA content and therefore a decrease in protein synthesis (Levitt, 1980). The most consistently reported effect of water stress on nucleic acid components is a marked decrease in polyribosome content. Even under mild stress there is a shift from polyribosomes to monoribosomes that are inactive in protein synthesis (Hsiao, 1973).

Water deficit can also cause an increase in growth retardants and a decrease in growth promoters. One of the most apparent phenomena is the rapid increase in ABA concentration when plants encounter wilting. Two common responses to ABA are stomatal closure and inhibition of precocious germination. The role of ABA in the regulation of stomatal aperture is to prevent K^+ accumulation (and hence water uptake) and also to stimulate the efflux of K^+ and Cl^- with an accompanying net loss of water (Reviewed by Hetherington and Quatrano, 1991). The effect of ABA on preventing precocious germination and inducing desiccation tolerance in developing embryos includes expression of specific gene products, presumably with specific roles in these processes (Quatrano, 1987; Kermode, 1990).

The indirect primary injuries discussed above are all metabolic in nature. The effects of rapid dehydration may also be converted to the direct primary injury, such as plasmolysis, membrane damage, enzyme inactivation, ion efflux and permeability changes (Reviewed by Levitt, 1980). The water stress is also capable of leading to injury due to the induction of a secondary stress. For instance, it can induce a secondary heat injury in water savers by permitting the leaf temperature of nonirrigated plants to rise 9°-15°C above the ambient temperature (Sachs *et al.*, 1975), and induce a deficiency of mineral nutrients by decreasing ion uptake. The effect of water stress on phosphorus metabolism has received great attention. A reduced growth of tomato plants at a water stress of -10.4 atm is not due directly to the water stress, but to the marked decrease in phosphorus (Greenway *et al.*, 1969).

2.4.2 Effects of Salinity Stress

There is a direct and inseparable relation between the salt and water stresses. Since the addition of a salt to water lowers its osmotic potential, the salt stress must expose the plant to a secondary osmotic stress, or, as it has also been called, a physiological drought stress. Therefore, if a plant or plant part is transferred from a low salt to a high salt medium, it is immediately subjected to an osmotic dehydration, which is analogous to the evaporative dehydration (Levitt, 1980). There is another striking similarity between the injurious effects of the osmotic and evaporative dehydrations. Addition of NaCl to the root medium of barley plants markedly increased leaf RNase (ribonuclease) activity, parallel to the increase in leaf water-stress (Arad and Richmond, 1976). Since this rise in RNase activity is associated with water stress injury, it indicates that the salt stress is inducing a secondary (osmotic) water stress injury (Levitt, 1980).

An immediate osmotic effect of exposure of plants to a salt stress is a decrease in cell turgor and therefore in growth. For instance, the earliest response of a non-halophyte exposed to salinity is that its leaves grow more slowly (Munns and Termaat, 1986). Even if the salt-stressed cell eliminates the osmotic decrease in cell turgor and therefore in cell growth by the process of osmoregulation there may still be a significant decrease in growth. This is because the maintenance of a lower Na concentration in its protoplasm than in the surrounding soil in order for normal functioning of the cell requires the expenditure of energy that would otherwise be available for growth process (Gale, 1975). The decrease in growth and yield is quantitatively related to the salt concentration, and the growth inhibition may or may not be reversible, depending on the plant species, the salt concentrations, *etc*. (Levitt, 1980). Plant development is also affected by salt stress. NaCl salinity delayed the flower emergence in almost all the tested rice cultivars (Mercado *et al*., 1974), as well as in peach. In spite of this delay in flowering, salinity hastened the ripening of the peach fruit and the autumn defoliation (Ivanova, 1974).

The decreased growth due to salinization has also been explained by a suppression of nutrient absorption due to uptake of NaCl in competition with nutrient ions. Thus even when the osmotic stress was limited, growth of *Phaselous vulgaris*, *Pisum sativum*, and *Citrus aurantium* was decreased by the salt stress, and this inhibition was overcome by K (Giorgi *et al*., 1967). Even leaf slices of the halophyte *Atriplex* when placed in a solution of NaCl, took up the Na largely in exchange for K lost from the vacuole (Osmond, 1968). In addition, K is not the only element that may be deficient as a result of a salt stress. Both chlorides and sulfates caused a decrease in content of total and inorganic P in tomatoes (Zhukovskaya, 1973).

The salt-induced growth inhibition is accompanied by one or more metabolic disturbances. Na decreases both photosynthesis and respiration rates in many crop plants, although in some cases an increase of the rates has been reported (Reviewed by Levitt, 1980). Nitrogen supply could be lower after exposure to NaCl, as Cl^- inhibits NO^{3-} uptake (Deane-Drummond and Glass, 1982), and thus rates of protein synthesis are undoubtedly lower in NaCl-treated plants (Aspinall, 1986). Salt also causes an increase in hydrolysis of proteins, leading to an increase in the products of hydrolysis - the amino acids. The most commonly reported effect of salt stress on amino acid metabolism is an accumulation of proline. Many enzymes both increase and decrease as a result of salt stress. In some cases, the increase in activity occurs at low concentrations of the salt, the decrease at high concentrations. The difference in activity may also depend on the salt used (Levitt, 1980).

In opposition to the secondary salt injury, due to osmotic dehydration or nutrient deficiency, primary injury must involve specific toxic effects of the salt, (a) directly on the external plasma membrane or (b) after penetration through the membrane into the protoplast. In further contrast, osmotic stress injury is counteracted by salt absorption, and primary injury is increased by salt uptake (Levitt, 1980). High Na may result in increases in membrane permeability (Greenway and Munns, 1980). A rapid salt injury requires high concentrations of salt. The nature of this direct salt injury appears to be due to a salt-induced increase in permeability, and therefore an effect on the plasma membrane. It was also demonstrated that beans, maize and barley showed greater growth inhibition in NaCl than in isosmotic solutions of polyethylene glycol (PEG). Poorer growth in Cl^{-1} than in PEG is convincing evidence for ion excess because some PEG may be transported to the shoots and there exert its own adverse effects (Greenway, 1973; Greenway and Munns, 1980). Figure 2.5 summarizes the different possible kinds of salt injury.

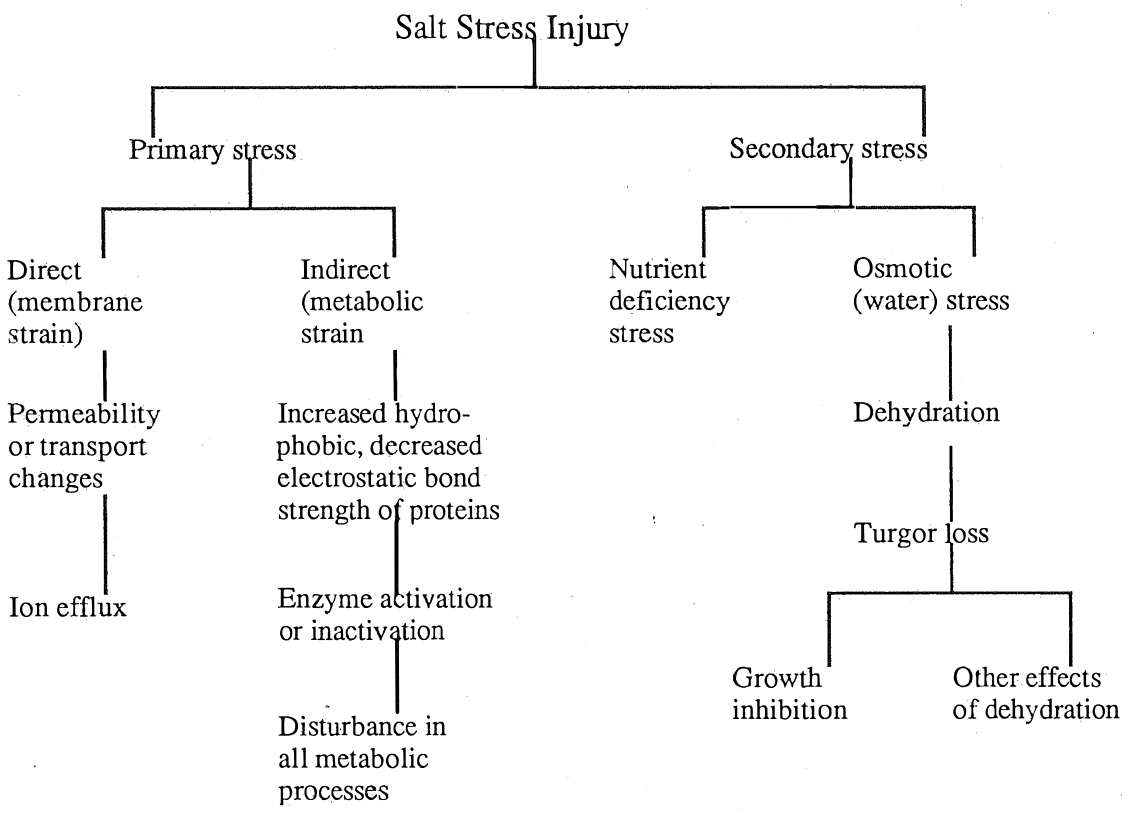


Fig. 2.5 Possible kinds of Na salt stress injury (Adapted from Levitt, 1980)

2.5 Interactive Effects of Air Pollutants and Drought

Plant susceptibility to air pollution stress is strongly influenced by other environmental factors. Drought stress has been recognized as one of the most important environmental factors which may interact with gaseous pollutants on plants. Conditions that minimize plant moisture stress, such as high soil moisture and high atmospheric humidity, generally increase plant susceptibility to foliar injury by air pollutants. In contrast, conditions with low soil moisture and low relative humidity normally modify the toxic effects of air pollution on plants. It is widely believed that water stress affects stomatal aperture, affects SO_2 uptake rate and therefore effective dose. On the other hand, air pollutants can cause changes in plants that are likely to increase susceptibility to drought (Mansfield *et al.*, 1988). These changes are of two basic kinds:

1) Changes in the epidermal layer caused by the deposition of pollutant molecules on the external surface of the cuticle, or into the interior of the cells after entry through stomatal pores.

2) Alterations in the growth characteristics of the plant as a result of interference with the process of translocations. The changes most often observed are reductions in root growth, and greater expansion of leaves relative to total dry mass of the plant.

2.5.1 Physiological and Biochemical Injuries

Several studies have demonstrated that prior exposure to air pollution may amplify the effects of drought stress on physiological and biological processes in plants. Cornic (1987) studied the interactive effects of SO_2 and drought stress on photosynthesis in 5-year-old *Picea abies* plants and found that the photosynthetic capacity of mature needles was not reduced by 3.1 μmol m^{-3} (75 nl l^{-1}) SO_2 for 5 weeks. However, subsequent drought stress induced the greatest decline of leaf photosynthetic capacity in the presence of SO_2. He suggested that the large decline in photosynthesis might be attributable to a greater dehydration - the lowest needle water content in the presence of SO_2. Another study with first exposure to SO_2 and NO_2 mixture and then to drought stress showed that birch leaves (*Betula* spp.) had an increased rate of water loss approximately corresponding to the pollutant concentrations to which they had been previously exposed. Under an electron microscope damaged epidermal cells were clearly visible on the polluted leaves and appeared to be responsible for areas of wide open stomata (Neighbour *et al.*, 1988). The reduced ability of polluted leaves to conserve water under conditions of severe water stress was also reported in Timothy grass (*Phleum pratense*) by Lucas (1990).

Air pollution is also known to amplify the effects of water stress on biochemical processes in plants. Pierre and Queiroz (1988) found that in watered *Picea abies* plants, SO_2 (3.1 μmol m^{-3}, *i.e.* 75 nl l^{-1}) had no significant effects, although sulphur accumulated in the needles. Water deprivation (1 or 2 weeks) of non-polluted plants decreased protein concentration and modified enzyme capacity, particularly for isocitrate and glucose-6-phosphate dehydrogenases. These effects were amplified in the polluted plants, indicating that vulnerability of cell metabolism to the effects of a drought period may be increased when water deprivation occurs under SO_2 pollution. Pierre and Savoure (1990) studied the effects of SO_2 and water stress on endoproteases using the same species and the same level of SO_2. They found that water stress produced a decrease in the endoprotease activity several days before the breakdown of total soluble proteins, and SO_2 amplified these effects in stress conditions producing a moderate rate of tissue dehydration.

There has been little consideration given to SO_2-induced changes in the pattern of stomatal behaviour in water-stressed plants. As discussed earlier, low concentrations of SO_2 may induce an opening of stomata attributable to a loss of turgor in the epidermal cells, whereas high concentrations of SO_2 may cause the closure of stomata due to direct injury to the guard cells, or following the inhibition of photosyntheses in the mesophyll. Mansfield *et al.* (1988) suggested that such changes may have a vital effect on the ability of leaves to survive in conditions of severe water stress. They fumigated *Betula pendula* and *B. pubescens* plants with SO_2 + NO_2, and leaves of equivalent age were excised after 30 days of fumigation. The leaves from the polluted plants dried out more quickly due to the sudden termination of the water supply than those from the control plants. They believed that the dynamics of water loss were affected by exposure to SO_2 + NO_2. It was clearly indicated that there was a lack of initial closure of stomata in heavily polluted leaves. Therefore, exposure to air pollutants may reduce the efficiency of water use, and the ability to regulate transpiration through stomatal mechanisms as the supply of soil water becomes limiting.

2.5.2 Visible Foliar Injury

Studies have shown that soil moisture stress can influence the injurious effects of air pollutants on plants. It was reported that soil moisture stress pretreatment greatly decreased stomatal conductance and greatly reduced SO_2 injury in 2 cultivars of poinsettia (*Euphorbia pulcherrima*) (Krizek *et al.*, 1986). Similarly, Davids *et al.* (1981) found that visible injury by SO_2 was severe on plants grown at -1/3 and -1 atm soil water potential, and negligible on plants grown at -3 and -5 atm water potential. Plant injury was highly correlated with percentage of soil moisture, and both the injury and soil moisture were highly correlated with stomatal conductance rate and water potential of the plants. Given the opposing environmental stress to water deficit, Norby and Kozlowski (1983) studied flooding and SO_2 stress interactions in *Betula papyrifera* and *B. nigra* seedlings. Stomatal conductance and SO_2 uptake in both seedlings were significantly reduced by flooding, and consequently SO_2 caused less visible injury and less growth inhibition in flooded than in unflooded seedlings.

In contrast, polluted plants appear to be more susceptible to drought stress than unpolluted plants. For instance, after 6 weeks of exposure to 230 $\mu g\ m^{-3}$ (86 $nl\ l^{-1}$) SO_2 *Picea abies* plants were simultanously exposed to SO_2 and water stress for 1 and 2 weeks, and then rewatered but maintained under exposure to SO_2 (Macřez and Hubac, 1988). As a result, the two simultaneous stresses were very damaging to the plants and 50% of the continuously SO_2 exposed plants died. More interesting, a recent study found that fumigation with low concentrations of O_3 and SO_2 16 months prior to drought stress still had a strong influence on the drought sensitivity of *Picea abies* plants (expressed in osmotic potential of cell sap and in percent survival) (Dotzler and Schutt, 1990).

2.5.3 Growth and Yield Reductions

Although the effects of SO_2 and other pollutants on plant growth and yield production have been extensively studied, the influence of drought stress on the expression of pollutant effects has received little experimental attention. Air pollution often occurs with environmental stresses. Therefore, extrapolation of the dose-response relationship derived from studies that use constant concentrations of the pollutant alone in chambers must take into account possible interactions with other stress factors, *e.g.* drought stress, and the occurrence of fluctuating concentrations in the field. Temple *et al.* (1985*b*) found that the effects of water stress and its interaction with O_3 stress appeared to depend on climatic conditions. During a hot and dry season in 1981, cotton yield in normally irrigated treatments was reduced 20% by ambient levels of O_3 and 45% by doubling ambient O_3 concentrations, whereas water-stressed plants showed almost no response to O_3. A repeat of the experiment during a cool and cloudy season in 1982 indicated no significant effects of water stress and no modification of the O_3 effect by water stress although doubling ambient O_3 concentrations reduced yields 65%. They suggested that the greater relative response of cotton yield to O_3 in 1982 may have resulted from the cooler, more humid growing conditions, which increased the susceptibility of cotton to O_3.

Miller *et al.* (1988) measured season-long growth of field-grown cotton that was exposed to a range of O_3 concentrations from 20 to 74 $nl\ l^{-1}$ at two levels of soil moisture, well-watered (WW) and water-stressed (WS). There were no significant O_3 x water interactions for the growth variables measured. Leaf area duration (LAD) was reduced by O_3 in both WW and WS treatments, but changes in the yield/LAD ratio and net assimilation rate indicated that reduced efficiency of leaves also was responsible for yield reductions in the WW plots. Heagle *et al.* (1988) found that moderate water stress (itself causing a 16% decrease in yield at low O_3 concentrations) reduced the sensitivity of the O_3 effect on cotton yield.

The effects of prior exposure to SO_2 and NO_2 on *Phleum pratense* growth under drought conditions were recently investigated by Lucas (1990). The plants were exposed continuously for 40 days to a range of mixture of SO_2 and NO_2, and were then transferred to the greenhouse for receiving water stress treatments - half the plants were watered each day and the rest received no further water during the following 23 days. After 40 days' exposure to SO_2 + NO_2, shoot and root dry weights had all been significantly reduced compared with the control plants. At the same time, shoot : root ratios showed that partitioning of dry weight to the above-ground parts in the polluted plants had increased from that of control treatments. Water stress treatments for 23 days after the fumigation showed a significant interaction between the effects of pollutant exposure and water stress, such that at the two high concentrations of 160 $\mu g\ m^{-3}$ (60 $nl\ l^{-1}$) SO_2 + 115 $\mu g\ m^{-3}$ (60 $nl\ l^{-1}$) NO_2 or 240 $\mu g\ m^{-3}$ (90 $nl\ l^{-1}$) SO_2 + 172 $\mu g\ m^{-3}$ (90 $nl\ l^{-1}$) NO_2 growth of the plants was reduced, with a greater reduction occurring in the unwatered group. However, this was apparently not caused by any changes in the reallocation of dry matter between the above- and below-ground parts of the plants as shoot : root ratios were similar.

It has been reported that excessive water may also influence plant responses to air pollution. Shanklin and Kozlowski (1985) grew *Taxodium distichum* seedlings under flooded or unflooded conditions for 8 weeks and then exposed them to 0, 0.5, 1.0 or 1.5 ppm SO_2 for 48 h. The results showed that exposure to SO_2 at 1.0 ppm or higher concentration reduced growth in both previously flooded or unflooded seedlings, but growth was reduced more in the unflooded seedlings which absorbed more sulphur.

2.6 Interactive Effects of Air Pollutants and Salinity

So far, very little work has been done in the area of salinity-pollutant interactions on plants. Those reported in the literature were mainly undertaken in a controlled environment instead of field conditions. As in the case of drought stress, soil salinity has also been known to play some protective roles in plant injury caused by gaseous pollutants. The protective mechanisms are also believed to be involved in the effect of salinity on stomatal aperture since salinity stress can induce a physiological condition with similarities to drought stress. As a consequence, soil salinity may induce stomatal closure reducing pollutant diffusion into the leaves.

2.6.1 Visible Foliar Injury

Oertli (1959) first demonstrated that plants grown in a saline environment were more resistant to injury from atmospheric pollutants. Later studies by Ogata and Maas (1973), and Maas *et al.* (1973) showed that foliar injury induced by O_3 and SO_2 occurred first in nonsaline plants, and with continued exposure the damaged areas became necrotic and desiccated. However, pollutant injury on plants grown under saline conditions developed later and advanced more slowly than that on nonsaline plants. Bytnerowicz and Taylor (1983) found that the amount of leaf injury decreased when salinity of the solutions increased.

2.6.2 Growth and Yield Reductions

Several laboratory studies showed that both O_3 and salinity stress reduced biomass production in alfalfa, garden beet and pinto bean (Maas *et al.*, 1973; Ogata and Maas, 1973; Hoffman *et al.*, 1975). Combination of these two stresses produced an apparent interactive effect, showing that salinity extended the tolerance thresholds for O_3 concentration and duration of exposure and reduced the deleterious effects of O_3. For instance, for nonsaline treatments, O_3 at 100, 150 and 200 ppb reduced forage yield of alfalfa by 16, 26 and 39%, respectively. As salinity increased, O_3 had less effect on the forage yield. Alfalfa exposed to 200 ppb of O_3 for 2 hr daily yielded 25% more at -200 kPa osmotic potential than at the nonsaline level, -40 kPa (Hoffman *et al.*, 1975).

Salinity-O_3 interactive effects on yield production in pinto bean were also significant in a controlled environment (Hoffman *et al.*, 1973). O_3 at 150 ppb decreased the number and weight of bean pods of nonsaline plants nearly 50%, and at 250 ppb and higher, no significant yield was obtained. The results were essentially the same for plants salinized to -2.0 bars. At -4.0 bars, the yield at 250 ppb was only reduced to half that of the O_3-free treatment. Although these early studies under laboratory conditions demonstrated a strong interaction on dry weight and yield production, a recent study under field conditions did not show an overall interaction between O_3 and salinity for alfalfa growth or yield (Olszyk *et al.*, 1988). The differences between the laboratory and the field studies were likely due to different environmental conditions including light intensity, temperature, relative humidity and wind speed, which had modified plant responses to air pollutants.

2.6.3 Effects of Air Pollutants and Salinity on Root Nodulation

Although interest in determining the effects of air pollution stress on plant productivity has been high, the effects of air pollutants on nodule formation and N_2 fixation in legumes have received only limited investigation (Tingey and Blum, 1973; Blum and Tingey, 1977; Jones *et al.*,1985; Satyanarayana *et al.*, 1985). It was found that nodule number was decreased in pigeonpea seedlings due to SO_2 exposure (Satyanarayana *et al.,* 1985). Similarly, O_3 impaired nodule number and weight per plant in soybean (Tingey and Blum, 1973). The possible mechanisms by which O_3 reduces root nodulation in soybean were investigated by Blum and Tingey (1977). They found that O_3 did not appreciably penetrate the plant growth substrates nor did it oxidize soil organic matter to form compounds inhibitory to *Rhizobium.* When O_3 was excluded from the plant foliage, but not from the soil, root nodulation was not reduced. However, when plant tops were directly exposed to O_3, root nodulation was reduced. So they suggested that the reductions in root nodulation resulted from an effect of O_3 on the plant foliage.

Total nodule activity (TNA, on a per-plant basis) in soybean cultivars was reduced after exposure to the combination of O_3 and SO_2, whereas specific nodule activity (SNA, on a per-gram fresh weight of nodules basis) was unaffected by the pollutant treatment (Jones *et al.*, 1985). Tingey and Blum (1973) found that nodule leghemoglobin content, which is positively correlated with N-fixing capacity, was significantly reduced by O_3 treatment. A major impact of O_3 and SO_2 exposures on root nodulation and N_2 fixation would likely be through photosynthate partitioning in favour of shoot growth at the expense of root growth (Brun, 1976). This reduction in the amount of photosynthate available for translocation to the root would decrease the energy available for root growth, nodule formation, and the carbon skeletons needed for N_2 fixation. For example, nitrogen contents in alfalfa plants grown in sand cultures supplied with minus N solutions were observed to be reduced by O_3 stress (Neely *et al.*, 1977).

Salinity is known to impair root nodulation in legumes mainly by affecting the infection process of Rhizobia since *Rhizobium* growth and survival are generally more tolerant *in vitro* to high osmotic pressures than their respective host legumes (Carr and Ballard, 1979; Singleton *et al.*, 1982; Rai and Prasad, 1983; Singleton and Bohlool, 1984). Singleton and Bohlool (1984) found that nodule number and weight in soybean were decreased by approximately 50% at 26.6 mM NaCl, and by more than 90% at 53.2 and 79.9 mM NaCl, and nodule development, as evidenced by the average weight of a nodule, was less affected by salt as was nodule number. Increased nodule size has been found in *Vicia faba* and chick pea under salt-stressed conditions (Lauter *et al.*, 1981; Yousef and Sprent, 1983; Elsheikh and Wood, 1990). Elsheikh and Wood (1990) observed that chickpea was more sensitive to salinity than *Rhizobium* Ch191, the roots were more sensitive than the shoots, and N_2 fixation was more sensitive to salinity than plant growth. The TNA was significantly affected by salinity stress, and the SNA, however, was less affected by salinity and was not significantly depressed until 79.9 mM NaCl in soybean (Singleton and Bohlool, 1984). Nodule function was relatively more resistant to salt stress than was plant growth (Singleton and Bohlool, 1984). By comparison, Yousef and Sprent (1983) found that both TNA and SNA were severely depressed by salinity stress.

2.7 What Questions Is This Work Trying to Answer

As discussed above, scientific research on the interactive effects of gaseous air pollutants and other environmental stresses has so far received little attention particularly under field conditions, and knowledge in this area is limited. However, studies on stress interactions are very important in agriculture and horticulture because air pollutants and other environmental stresses often co-occur in nature. Realistic pollutant dose-response relationships can be established only after the influences of environmental stresses on the expression of effects of air pollutants on plants are known. This work was initiated to examine the interactive effects of SO_2 and soil factors (water stress or salinity) on potato and soybean crops under natural climatic conditions. The following hypotheses were tested in a series of field experiments with top-covered chambers.

1) As it usually induces stomatal closure, soil moisture stress may reduce the adverse effects of SO_2 on foliar injury, growth and yield in crops compared with well-watered treatment, by decreasing SO_2 uptake.

2) Greater proportional allocation of carbon to roots in response to soil moisture stress may preclude an increase in carbon allocation to shoots often observed in response to SO_2 exposure.

3) Soil salinity may also have a protective function against SO_2 injury by inducing physiological drought stress in plants causing stomatal closure and thus decrease SO_2 uptake. As a consequence, SO_2-induced foliar injury and reduced growth may be less severe in the saline plants than in the non-saline plants.

4) Plant responses to sequential exposure to SO_2 and soil salinity may not be the same as those to the simultaneous exposure. In sequential exposure there may be compensatory mechanisms and predisposition characteristics of the prior stresses which could affect the expression of plant responses to the subsequent stresses. For instance, prior exposure to low SO_2 may modify plant sensitivity to subsequent salinity stress due to a common SO_2-induced growth stimulation. However, plants pretreated with high SO_2 may become vulnerable to salt injury due to their physiological weakness.

5) Root growth, nodule formation and nitrogenase activity may be more sensitive to the combination of SO_2 and salinity than to the single stresses. SO_2-induced increase in carbon allocation in favour of shoot growth relative to root growth may decrease root length and dry weight, and preclude nodule formation and nitrogenase activities. Likewise, soil salinity often has detrimental effects on these growth variables and physiological processes. For inoculated soybeans which are not nitrogen fertilized, the interactive effects of SO_2 and salinity on the root nodulations and nitrogenase activities may also induce a third stress, limited nitrogen resources for plant growth.

CHAPTER THREE

General Materials and Methods

3.1 Fumigation chambers

Six rectangular-shaped chambers were used for SO_2 fumigation (Figs. 3.1*a*, *b*). The fumigation chambers were top-covered to exclude rainfall, and consisted of a rigid aluminium frame covered by UV-treated PVC plastic. The front frame, with a 1.0-m-width door, was covered by a single layer of PVC plastic. The side panels were covered by a double thickness of the PVC envelope with the inner layer perforated by holes of 25 mm in diameter. The rear frame was also covered by a double thickness of the PVC, although with no holes in the inner layer, and connected to a fan via a duct. Dust-filtered air was fan-forced into the chamber through the holes and then out through the space between the roof and the chamber body. The output of the fan was 1 m^3 s^{-1}, enabling an air exchange rate of about 3.4 air changes per minute.

Dry air was mixed with bottled anhydrous SO_2 from a temperature-controlled cylinder and passed through a regulator and series of needle valves into the inlets of the fumigated chambers. SO_2 was sampled from the centre of each chamber for 12 minutes every 72 minutes using a timer-controlled electrical sequencer in conjunction with solenoid valves. The SO_2 concentrations were monitored using a Thermo Electron, Series 43 pulsed fluorescent ambient SO_2 analyzer, calibrated with a Thermo Electron, Model 145 calibrator, with NBS traceable certified permeation tubes. The SO_2 distribution in the chambers was relatively uniform (see Appendix 1). The SO_2

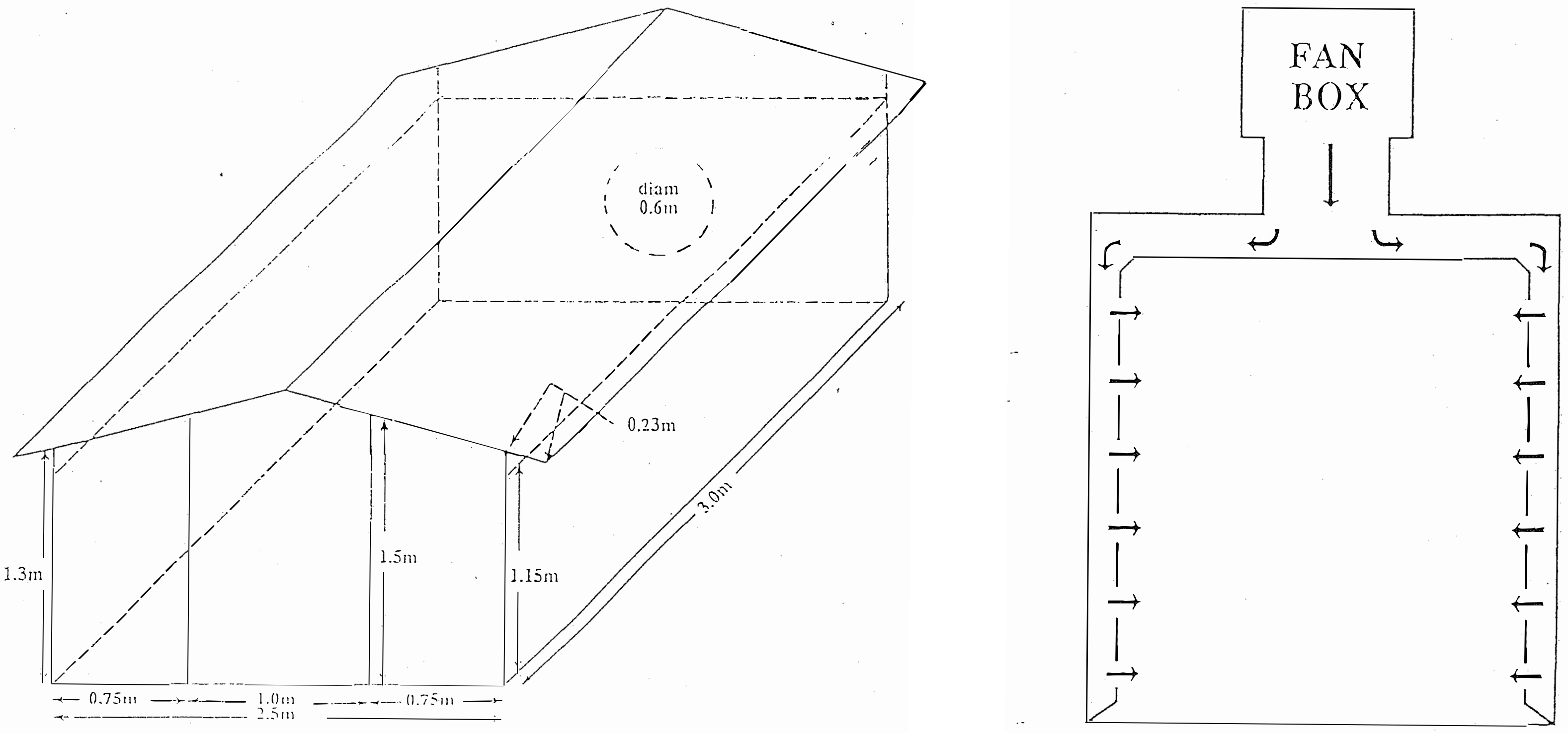


Figure 3.1a Structural drawing of the top-covered and rectangular-shaped fumigation chamber. A 1.0-m-width door is positioned at the front, and a duct in diameter of 0.6-m connected to a fan is positioned in the rear of the chamber.

Figure 3.1b SO_2 fumigation chambers are in the field.

concentrations from every position in the chambers were in the range of 98-111% of that at the central position.

3.2 Drip irrigation

This method was only used in the sand culture experiments. Six 200-litre drums were positioned 3 m above the ground for storage of nutrient and salt solutions. Drums were joined together in pairs, and connected to dripping rings in the pots of the chambers through plastic tubing. Flow rates of the solutions were controlled using solenoid valves in conjunction with an electrical timer. Irrigation was applied twice every day at 0900 h and 2100 h, giving 500-700 ml leachate per pot per day. Due to the large amount of irrigation, salts were hardly accumulated in the pots.

Nutrient components and concentrations (μM) were as follows: KH_2PO_4, 20; K_2SO_4, 600; $MgSO_4{\cdot}7H_2O$, 200; $CaCl_2{\cdot}2H_2O$, 600; H_3BO_3, 5; $Na_2MoO_4{\cdot}2H_2O$, 0.03; $ZnSO_4{\cdot}7H_2O$, 0.75; $MnSO_4{\cdot}7H_2O$, 1.0; $CoSO_4{\cdot}7H_2O$, 0.2; $CuSO_4{\cdot}5H_2O$, 0.2; FeNaEDTA, 20. Nitrogen (NH_4NO_3, 40 μM) was usually added during the first two weeks. High and low levels of salinity were achieved by dissolving 540 g or 320 g NaCl respectively in each 200 litres of irrigation water. No extra NaCl was added to the irrigation water of the control treatment. Electrical conductivities of the drum solutions and the pot leachates were measured every week using a laboratory conductivity meter (PW 9501/01, Philips). The mean (± standard deviation) conductivities varied slightly for the different experiments, and will therefore be discussed in each chapter.

3.3 Experimental design

Three of the four major experiments were factorially designed with simultaneous exposure to SO_2 and salinity (or water stress). The remaining experiment consisted of sequential stress exposure, *i.e.* prior exposure to SO_2 and then to salinity stress or *vice versa*. Three concentrations of SO_2 were used; control SO_2 (ambient air, 1-2 nl l^{-1}), low SO_2 (100 - 150 nl l^{-1}), high SO_2 (200 - 300 nl l^{-1}). Two levels of water stress and two or three levels of soil salinity were used, and each factorial combination with SO_2 was duplicated. The selected SO_2 concentrations were based on the higher extreme of monitored concentrations near an industrial area in the south of Perth, Western Australia (Department of Conservation and Environment, 1982; Environment Protection Authority, 1989 - see Appendix 2). Plants were fumigated every day usually between 1100h and 1600h. This was based on the common phenomenon in this climate with a high frequency of inversions that maximum concentrations of air pollutants near an emission source occur between late morning and early afternoon during inversion break-up. The SO_2 fumigation in some experiments was applied from seedling emergence to seed maturity, and in the others the fumigation lasted 3-7 weeks during the seedling periods. There were two levels of soil moisture treatment, well-watered and water-stressed. Soil salinity treatments were achieved by adding NaCl to the nutrient solutions. These details will be discussed in each chapter.

3.4 Growth conditions

Two crops were studied in this thesis, potato (*Solanum tuberosum* L. cv. Russet Burbank) and soybean (*Glycine max* L. cv Buchanan). Potatoes were grown in plastic pots filled with a mixture of pine bark, sand, vermiculite and perlite plus complete fertilizer (see details in Chapter 4). Soybeans were grown in pots filled with white sand (grain size of about 0.4 mm in diameter), plus nutrient solutions twice a day. The experimental site was located on the Murdoch University campus. During the entire period of SO_2 fumigations, temperature and relative humidity were measured using a thermohygrograph in a Stevenson screen. The mean daily maximum and minimum temperatures and relative humidities were recorded and are reported in each chapter. Air temperature was 2°C higher inside the fumigation chambers than outside and relative humidity was 5-10% higher inside the chambers compared with outside at midday on sunny days. Light intensity inside the chamber approximated 86% of that outside (see Appendix 3).

3.5 Harvesting procedure

Serial harvests were conducted for the experiments so as to examine changes in plant responses to SO_2 and the edaphic stresses singly and in combination as the growing season progressed. At each harvest, plants were divided into aboveground parts (stems, leaves) and belowground parts (roots, tubers). Leaf injury of the stressed plants was evaluated visually or using scanning electron microscopy. Leaf areas, which were green, were measured using an area meter (Delta T, Cambridge, UK). Plant material was dried in a forced-draft oven at 80°C, and dry weights were recorded.

3.6 Chemical measurements

Leaf sulphur concentrations were measured at each harvest to examine the effects of soil moisture stress and salinity on sulphur accumulation in plants exposed to SO_2. The leaf sulphur was brought into solution by nitric-perchloric acid digest (Johnson and Ulrich, 1959) and assayed for total sulphur by inductively coupled plasma emission spectrophotometry.

Leaf chlorophyll concentrations were measured at harvests using the second unfolded leaves. Leaves were collected from each treatment and placed in plastic bags in an ice box. Each leaf was finely chopped, a subsample of 0.1g taken, and infiltrated with phosphate buffer (0.1M, pH 7.0). Leaf chlorophyll was extracted with N,N-dimethyl-formamide (Moran and Porath, 1980), and measured by absorbance at wavelengths of 663 and 647 nm using a Varian Superscan 3 spectrophotometer.

3.7 Stomatal resistance

Diurnal changes of leaf stomatal resistance (s cm^{-1}) were measured four times a day on sunny days using an automatic porometer (Delta-T Devices, MK3). The measurements would implicate the effects of soil moisture stress and salinity on SO_2 uptake since gaseous pollutants enter plants mainly by diffusion through stomata. Five of the second unfolded leaves were chosen and only the adaxial surfaces were measured.

3.8 Statistical analysis

Each factorial treatment was duplicated so that chamber could be used as a factor in analysis. Preliminary statistical analysis using a 3-way analysis of variance (ANOVA) established that there was no significant interaction with the chamber factor, which justified pooling individual observations across duplicates. A 2-way ANOVA was used to test any significant effects of SO_2, drought or salinity singly and their interactions on the biochemicals, growth and yield parameters. Special contrasts were performed, using methodology outlined by Finn (1974), with the MANOVA procedure in the SPSS-X version 2.0 package. Homogeneity of variance was tested using Cochran's C-test; and if variances showed heterogeneity the data were log-transformed to achieve homogeneity. Other methods of statistical analysis will be discussed in individual chapters.

CHAPTER FOUR

Responses of Potato Plants to Sulphur Dioxide, Water Stress and Their Combination

4.1 INTRODUCTION

The effects of air pollution on potatoes have been documented in several studies. Petitte and Ormrod (1988) found that the plants had significantly reduced leaf area, and leaf, stem and root dry weight (DW) after exposure to 110 nl l^{-1} SO_2 and/or 110 nl l^{-1} NO_2 for 7 or 14 days. Small reductions in tuber yield and mean tuber size, but not in tuber number, were observed in potato plants when treated with 100 nl l^{-1} SO_2 for 255 hours (Foster *et al.*, 1983). It was reported by Pell *et al.* (1988) that SO_2 induced a stimulation and then decline of the number and DW of Grade One tubers, and O_3 induced a linear reduction of the same variables. No SO_2 x O_3 interaction was detected for any of the yield or quality functions measured.

SO_2 is known to shift resource partitioning to favour shoot growth (Jones and Mansfield, 1982; Murray, 1985; Darrall, 1989). In contrast, plants subject to water stress may favour root growth instead of shoot growth (Bloom *et al.*, 1985). Plants may respond to water stress by showing partial stomatal closure which will reduce uptake of air pollutants (Tingey *et al.*, 1982). Heagle *et al.* (1988) found that moderate water stress (itself causing a 16% decrease in yield at low O_3 concentrations) reduced the severity of the O_3 effect on cotton. However, Miller *et al.* (1988) reported that there were no significant interactions between O_3 and water stress in cotton on the growth variables measured. These differences are possibly related to the different exposure conditions of gaseous pollutant and water stress.

Plants are often exposed to multiple environmental stresses in nature. In this study, potato plants were subjected to SO_2 and soil water stress simultaneously under simulated field conditions. Since water stress can induce stomatal closure and resource allocation in an opposite direction from that by SO_2, it may modify the response of potato plants to SO_2 exposure. This modification was evaluated by examining the growth, yield and some biochemical parameters over a growing period of three and half months. To maintain two levels of soil moisture during the growing season, top-covered chambers were used.

4.2 MATERIALS AND METHODS

4.2.1 SO_2 Fumigation

The experiment was conducted using three SO_2 concentrations; high, low and control (non-filtered air). As exposures are rarely continuous, plants were fumigated 4 hours a day (1200 h -1600 h), 7 days a week from seedling emergence to the final harvest, lasting 105 days. The three seasonal mean (± standard deviation) SO_2 concentrations of the fumigation periods were 300 (± 60), 110 (± 30) and 2 (± 10) nl l^{-1}, respectively. The selected SO_2 levels were based on the higher extreme of monitored concentrations near an industrial area in the south of Perth, Western Australia (Department of Conservation and Environment, 1982).

4.2.2 Climate Conditions

During the growing season, the mean ambient maximum and minimum temperatures were 19.8 and 9.1 °C respectively, and the mean ambient relative humidity was 66%.

4.2.3 Plant Culture

Uniform tuber seeds of potato, which had an average size of 25 mm in diameter, were planted in 8-litre pots (diameter of 220 mm) with a mixture of pine bark, sand, vermiculite and perlite in a proportion of 4, 2, 3 and 3 respectively on the 8th of July, 1989. The planting rate was two seeds per pot, and later thinned into one plant per pot. A complete fertilizer was applied once a month. Within each chamber, 50 pots were used, and two soil moisture treatments were established by differential irrigation of the well-watered (WW) and water-stressed (WS) plots in combination with SO_2 treatments. This experiment was done in a single season.

Tensiometers (Irrometer, Riverside, California) were positioned 0.13 m deep in pots of each treatment to monitor soil moisture. Soil in the WW pots was irrigated to field capacity with tap water every two days early in the season and every day late in the season. The soil moisture tension was always below 10 kPa. Soil in the WS pots was irrigated to field capacity every four days early in the season and every two days in the late season, and a tension of 15 to 20 kPa was usually maintained at midday during the 3rd and 4th days after each irrigation.

4.2.4 Seasonal Harvests

Serial harvests were conducted on days 36, 63, 84 and 105 after planting (DAP). At each harvest, plants were divided into tubers, roots, stems and leaves. Leaf area was measured and the plant materials were dried in a forced-draft oven. Mean relative growth rates (mRGR) were calculated using $\log_e$-transformed biomass of the seasonal harvests (Hunt, 1978),

$$_{1\text{-}2}\text{mRGR} = (\log_{e2}W - \log_{e1}W) \,/\, ({}_2T - {}_1T)$$

where T is time, and W is total dry weight.

4.2.5 Chemical Observations

Leaf sulphur concentration of the plants was measured with six replicates per treatment at each harvest. To evaluate the effects of SO_2 concentration and duration on leaf chlorophylls under soil moisture stress, the youngest fully expanded leaves were tagged on DAP 50, as leaf age of 0, and measurements of the chlorophyll concentration were undertaken on DAP 58, 70, 79 and 85, as leaf age of 8, 20, 29 and 35 days old respectively. The methods of leaf sulphur and chlorophyll measurements are described in chapter three.

4.2.6 Statistical Analysis

A two-way analysis of variance (ANOVA) was used to test any significant effects of SO_2, water stress and their interactions on the growth variables. The mRGR's and leaf sulphur concentrations were analyzed using an ANOVA as described by Poorter and Lewis (1986). A trend analysis over time was conducted with the interaction sum of squares partitioned using orthogonal contrast. The linear component of the interaction sum of squares indicates differences in mRGR or leaf sulphur concentrations maintained throughout the seasonal experiment, while the quadratic component measures the extent to which differences in mRGR or leaf sulphur concentrations changed with time.

4.3 RESULTS

4.3.1 Plant Growth Responses

Visible foliar injury attributable to SO_2 toxicity was observed in the chambers fumigated with 300 nl l^{-1} SO_2 for 6 weeks and with 110 nl l^{-1} SO_2 for nine weeks (Fig. 4.1*a, b, c*). Defoliation occurred in the late season, especially under 300 nl l^{-1} SO_2 and well-watered conditions. SO_2 and water stress decreased leaf area and DW (Table 4.1). The SO_2 effects took longer to develop than the effects of water stress. Only 300 nl l^{-1} SO_2 suppressed the leaf growth. Interactions of SO_2 and water stress were significant from the second harvest especially at 300 nl l^{-1} SO_2 concentrations. As shown in Fig. 4.2, SO_2 exposure under well-watered conditions decreased leaf area and DW, whereas under water-stressed conditions there was usually no SO_2-induced reduction of the two growth variables.

Neither SO_2 nor water stress had any effect on stem number, but 300 nl l^{-1} SO_2 and water stress significantly decreased the stem DW. Tuber number was decreased by water stress but not by SO_2 (Table 4.2). Exposure to 300 nl l^{-1} SO_2 and water stress suppressed plant tuber yield over the seasonal harvests, while their interactions were significant at the second and final harvests but not at the first and third harvests. SO_2-induced reductions of stem and tuber DW were usually apparent under well-watered conditions, but were modified under water-stressed conditions (Fig. 4.2).

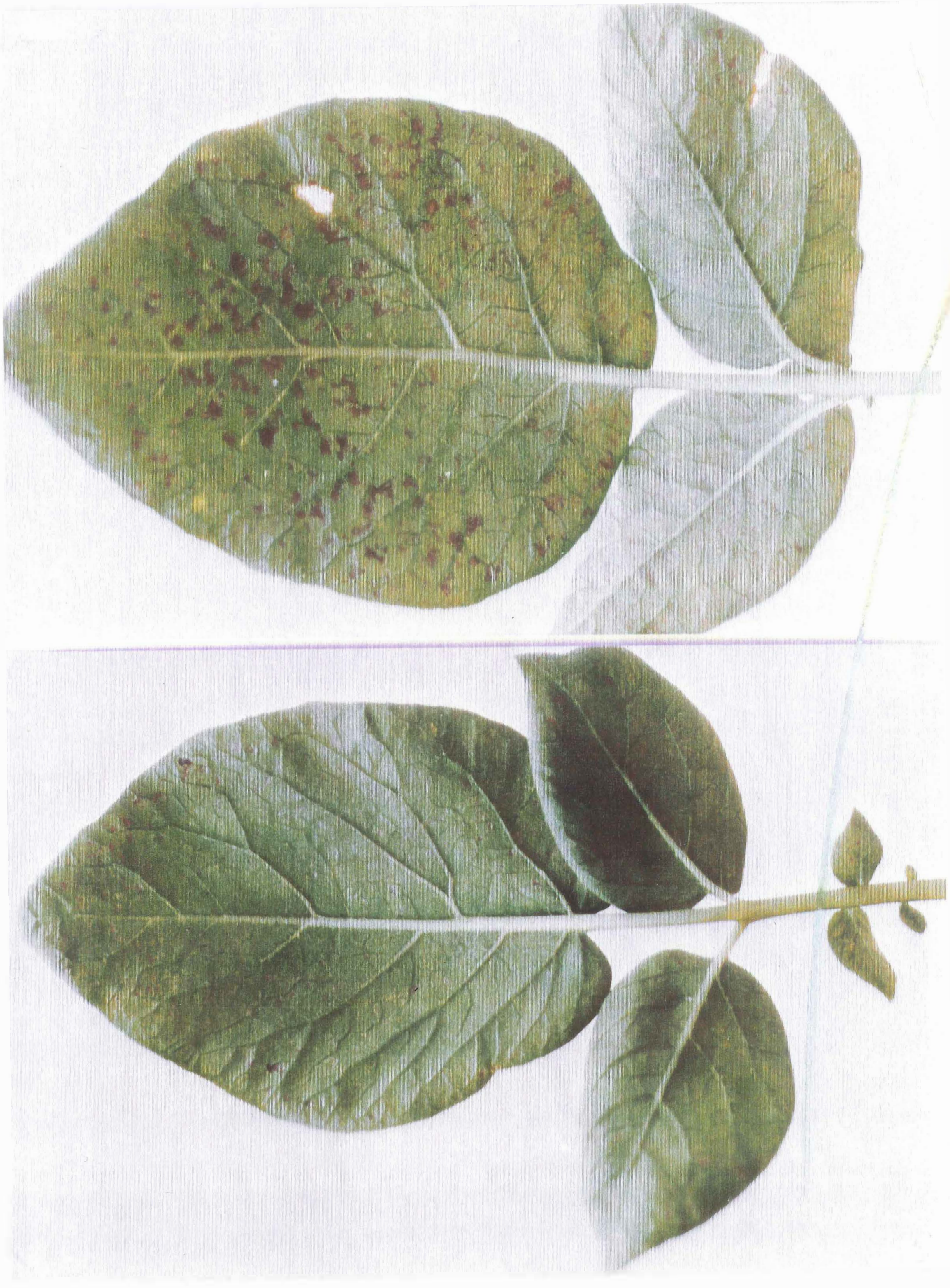


Figure 4.1a Apparent leaf injury caused by SO_2 was observed after 6-week exposure to 300 nl l^{-1} SO_2 under well-watered conditions (top). By comparison, there was only minor SO_2 injury by the same SO_2 concentration under water-stressed conditions (lower).

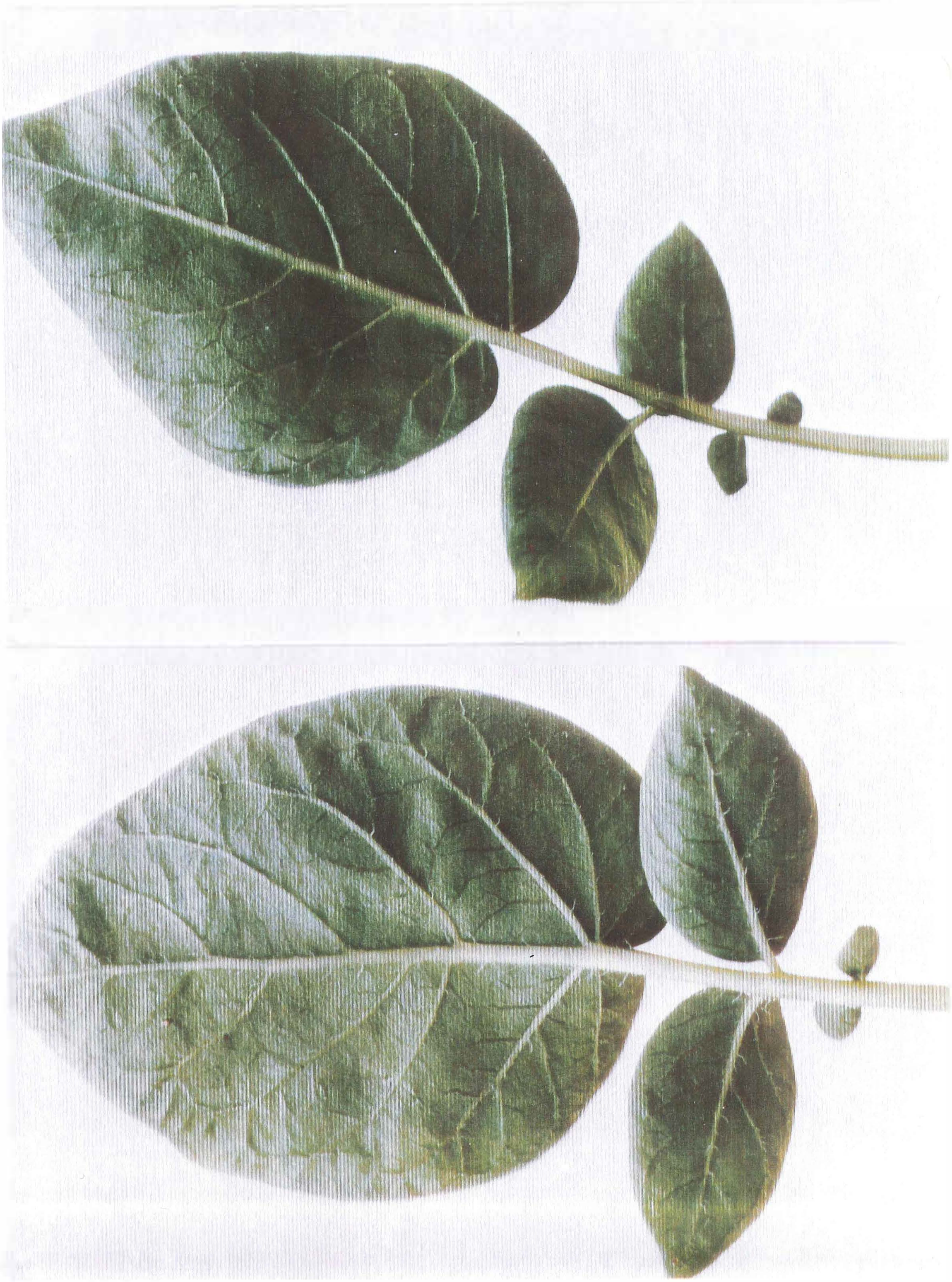


Figure 4.1b SO_2-caused leaf injury was not observed after 6-week exposure to 110 nl l^{-1} SO_2 under both well-watered (top) and water-stressed (lower) conditions.

Figure 4.1c The degrees of SO_2-caused leaf injury after 11-week exposure to 110 nl l^{-1} SO_2 under well-watered (top) and water-stressed (lower) conditions.

Table 4.1 Statistical summary of SO_2 and water stress effects on leaf characteristics from seasonal harvests

Treatments	Seasonal Harvests,			
	1st	2nd	3rd	Final
Leaf Area (m^2)				
SO_2	n.s.	**	***	***
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	**	***	***
Water stress	**	***	***	***
SO_2 x Water stress	n.s.	***	*	***
1st contrast	n.s.	**	n.s.	**
2nd contrast	n.s.	***	*	***
Leaf Dry Weight (g)				
SO_2	n.s.	**	**	**
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	**	**	**
Water stress	***	***	***	***
SO_2 x Water stress	n.s.	***	n.s.	**
1st contrast	n.s.	n.s.	n.s.	*
2nd contrast	n.s.	***	n.s.	**

Levels of significance: P=0.05(*), P=0.01(**), P=0.001(***).

For SO2 effects, the first contrast represents low SO_2 contrasted against control SO_2, and the second contrast represents high SO_2 contrasted against control SO_2. For interaction effects the first contrast represents the difference between control SO_2 and low SO_2 under well-watered conditions contrasted against the difference between control SO_2 and low SO_2 under water-stressed conditions. The second contrast represents the difference between control SO_2 and high SO_2 under well-watered conditions contrasted against the difference between control SO_2 and high SO_2 under water-stressed conditions.

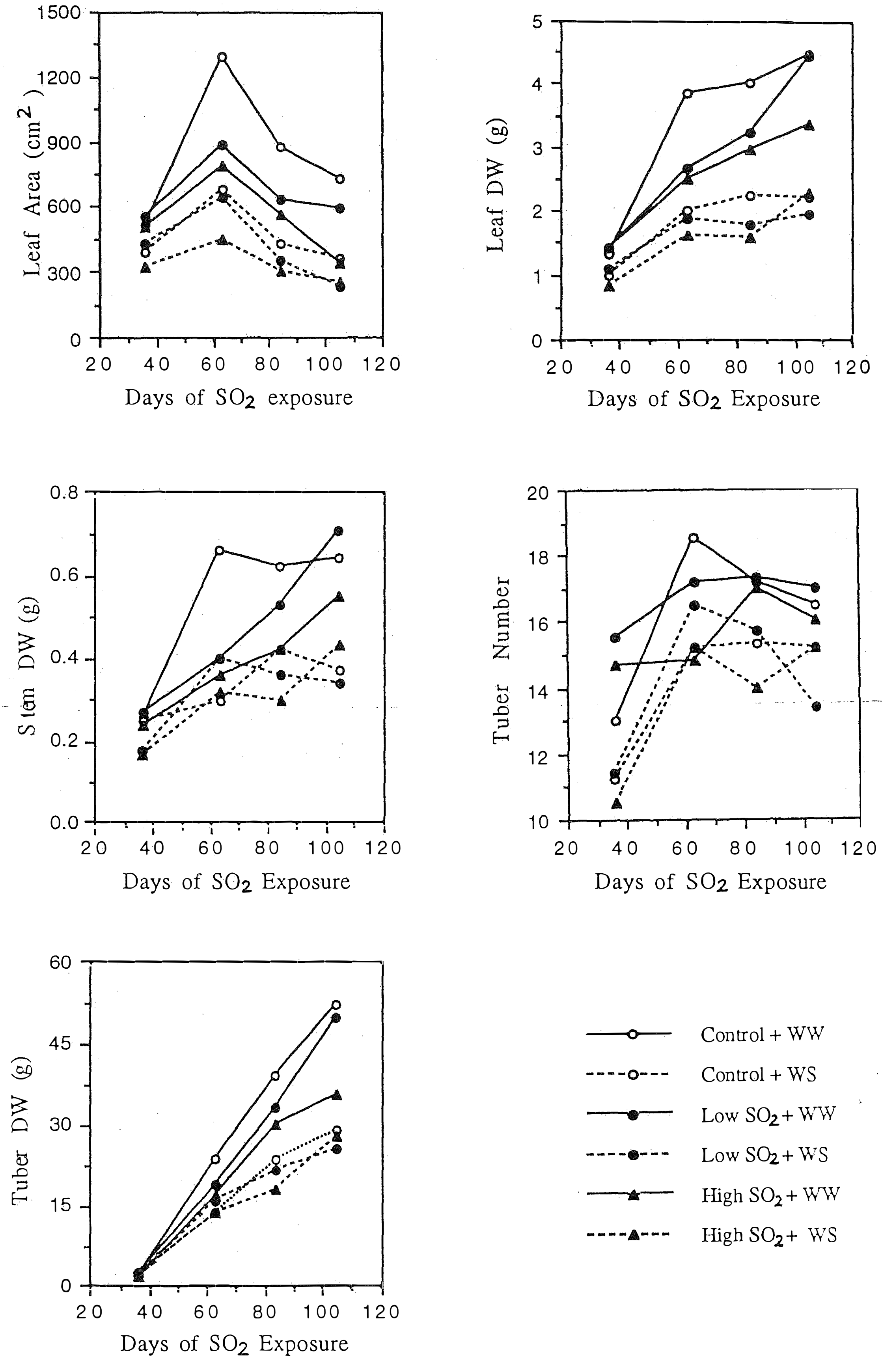


Figure 4.2 Joint action of SO$_2$ and water stress on leaf area, leaf dry weight (DW), stem DW, tuber number and tuber DW over the growing season. WW denotes well-watered, and WS denotes water-stressed.

Table 4.2 Statistical summary of SO_2 and water stress effects on stem, and tuber characteristics from seasonal harvests

Treatments	Seasonal Harvests, 1st	2nd	3rd	Final
Stem dry weight (g)				
SO_2	n.s.	***	***	n.s.
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	***	***	n.s.
Water stress	*	***	***	***
SO_2 x Water stress	n.s.	***	n.s.	***
1st contrast	n.s.	***	n.s.	**
2nd contrast	n.s.	***	*	*
Tuber number				
SO_2	n.s.	n.s.	n.s.	n.s.
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	n.s.	n.s.	n.s.
Water stress	***	n.s.	*	**
SO_2 x Water stress	n.s.	n.s.	n.s.	n.s.
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	n.s.	n.s.	n.s.
Tuber dry weight (g)				
SO_2	*	**	***	***
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	**	**	***	***
Water stress	**	***	***	***
SO_2 x Water stress	n.s.	***	n.s.	***
1st contrast	n.s.	*	n.s.	***
2nd contrast	n.s.	***	n.s.	***

Levels of significance: P=0.05(*), P=0.01(**), P=0.001(***).
See Table 4.1 caption for a description of the contrast terms.

Plant mRGR of all the treatments reached the highest level between the first and second harvests, and then continuously decreased. Exposure to 110 nl l^{-1} SO_2 under well-watered conditions and control treatments usually increased mRGR compared with the plants exposed to other treatments (Fig. 4.3). The ANOVA shows a lack of SO_2 X Water interaction, whereas SO_2 X Water X Time interactions were significant. Since the interactions were due to the quadratic component, the difference in mRGR changed with time (Table 4.3).

The shoot : root (including tubers) ratios were increased by 300 nl l^{-1} SO_2 under well-watered conditions early in the growing season (Table 4.4, Fig. 4.4). As the growing season proceeded, however, the SO_2 effects were negated and the effects of soil moisture stress became dominant. Water stress significantly decreased the ratios and modified the effects of SO_2. Similar results were observed for the shoot : root (excluding tubers) ratios except a stronger effect from the water stress.

4.3.2 Leaf Chlorophyll and Sulphur Concentrations

Leaf chlorophyll concentrations were significantly reduced by 300 nl l^{-1} SO_2, but 110 nl l^{-1} SO_2 had no effect (Table 4.5). Water stress appeared to protect leaf chlorophyll from high SO_2 injury. With development of leaf age, leaf chlorophyll concentrations decreased in all the treatments (Fig. 4.4).

SO_2 fumigation increased leaf sulphur concentrations (Table 4.3). Plants under SO_2 and well-watered conditions accumulated much more leaf sulphur than those under SO_2 and water-stressed

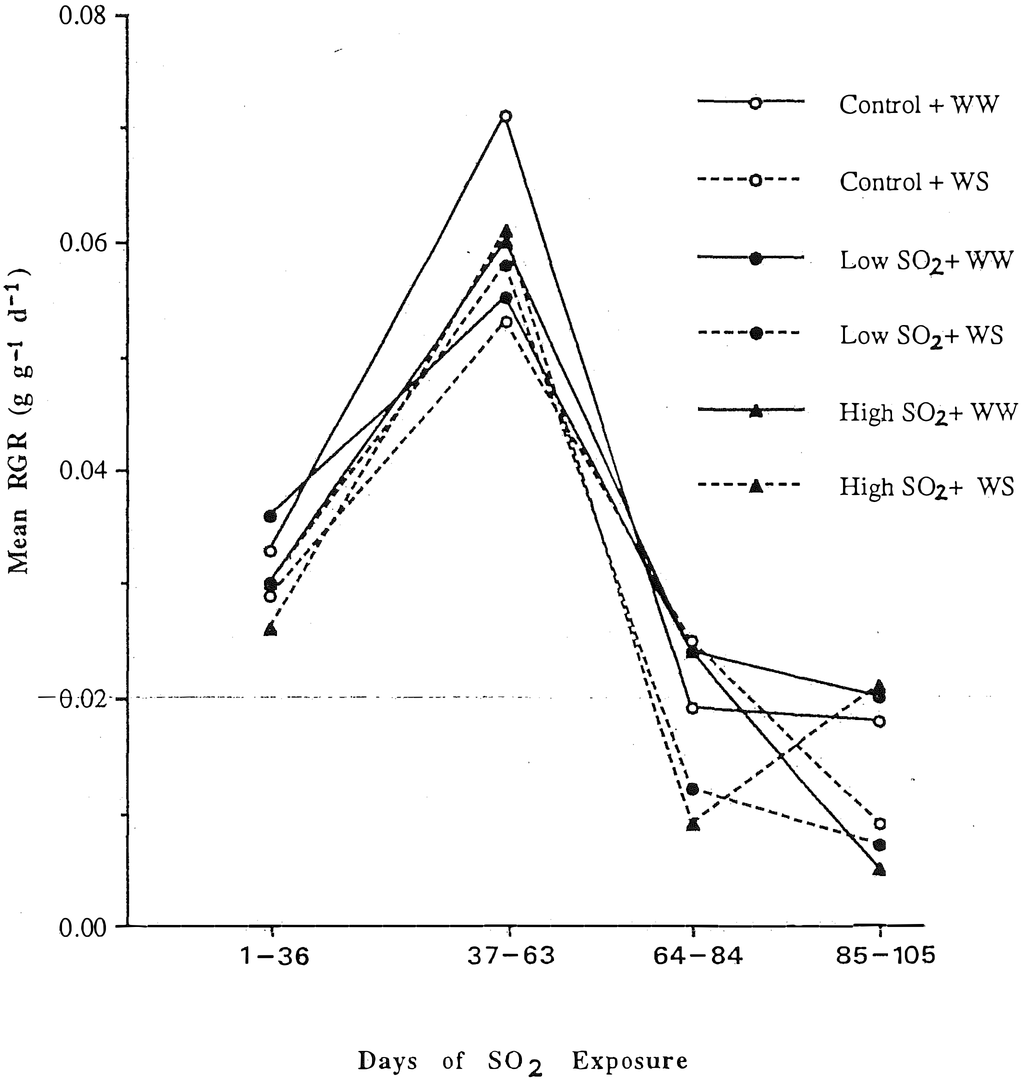


Figure 4.3 Joint action of SO_2 and water stress on mean relative growth rate over the harvest intervals. WW denotes well-watered, and WS denotes water-stressed.

Table 4.3 Effects of time, SO_2 X water stress on relative growth rate (g g^{-1} d^{-1}) and leaf sulphur concentration (%)

Source of Variation	SS*	*d. f.*	P
Relative Growth Rate			
Time	0.03	3	0.000
SO_2 X Water Stress	0.00	2	0.673
Time X SO_2 X Water Stress			
Linear	1.6E-4	2	0.091
Quadratic	9.7E-4	2	0.003
Residuals	0.00	30	
Leaf Sulphur Concentration			
Time	1.56	3	0.000
SO_2 X Water Stress	0.59	2	0.003
Time X SO_2 X Water Stress			
Linear	0.03	2	0.490
Quadratic	0.17	2	0.010
Residuals	1.26	30	

The linear component reflects differences in RGR or leaf sulphur that are maintained throughout the experiment. The quadratic component reflects the extent to which differences in RGR or leaf sulphur change with time (Poorter and Lewis, 1986).

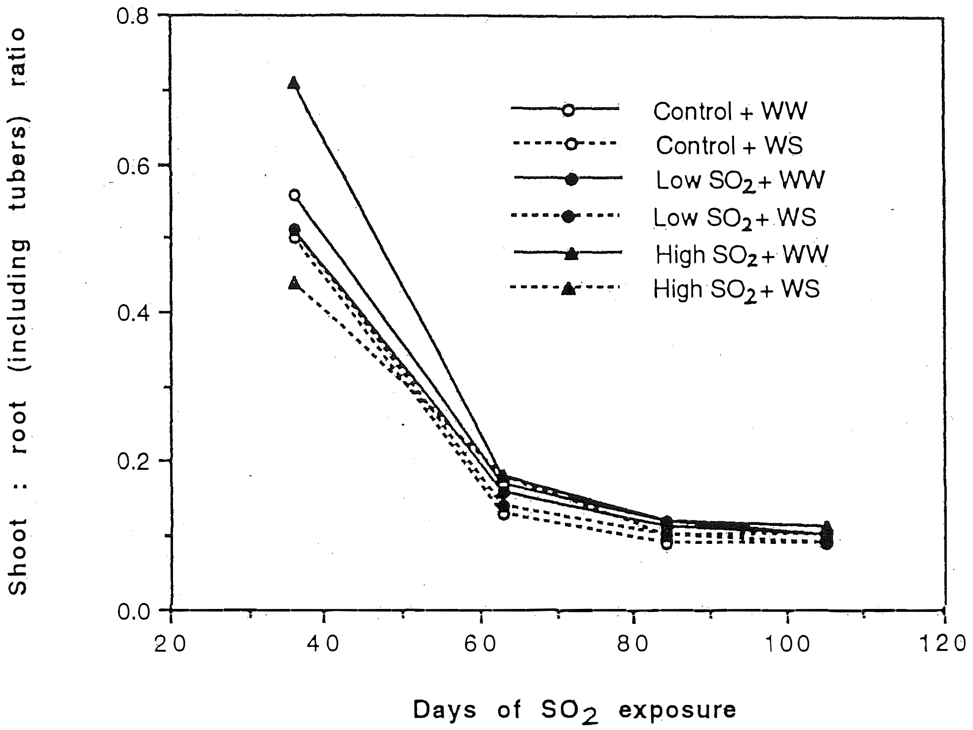
* SS = Sum of Squares.

Table 4.4 Statistical summary of SO_2 and water stress effects on the shoot to root ratios from seasonal harvests

Treatments	Seasonal Harvests, 1st	2nd	3rd	Final
Shoot : root (including tubers) ratio				
SO_2	*	n.s.	n.s.	n.s
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	*	n.s.	n.s.	n.s
Water stress	n.s.	**	*	**
SO_2 x Water stress	n.s.	n.s.	*	*
1st contrast	n.s.	n.s.	n.s.	*
2nd contrast	n.s.	n.s.	*	*
Shoot : root (excluding tubers) ratio				
SO_2	**	*	n.s.	n.s.
1st contrast	n.s.	*	n.s.	n.s.
2nd contrast	**	*	n.s.	n.s.
Water stress	*	**	**	***
SO_2 x Water stress	*	n.s.	*	*
1st contrast	n.s.	n.s.	*	*
2nd contrast	*	n.s.	*	**

Levels of significance: P=0.05(*), P=0.01(**), P=0.001(***).

See Table 4.1 caption for a description of the contrast terms.



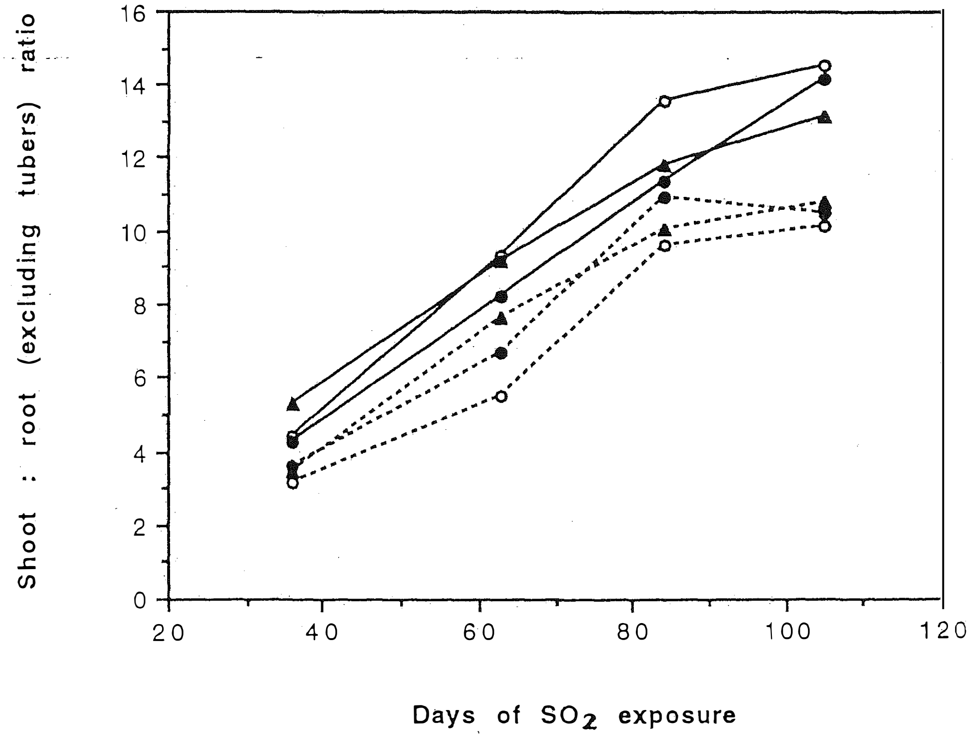


Figure 4.4 Joint action of SO_2 and water stress on the shoot : root ratios [including tubers (top figure) or excluding tubers (lower figure)]. WW denotes well-watered, and WS denotes water-stressed.

Table 4.5 Statistical summary of SO_2 and water stress effects on leaf chlorophyll concentrations (mg g^{-1}, fresh weight)

Treatments	Leaf age (days) 8	20	29	35
SO_2	**	**	***	***
1st contrast	n.s.	n.s.	n.s.	n.s.
2nd contrast	**	***	***	***
Water stress	n.s.	*	n.s.	n.s.
SO_2 x Water stress	n.s.	**	n.s.	**
1st contrast	n.s.	n.s.	n.s.	*
2nd contrast	n.s.	**	n.s.	**

Levels of significance: P=0.05 (*), P=0.01(**), P=0.001(***).

See Table 4.1 caption for a description of the contrast terms.

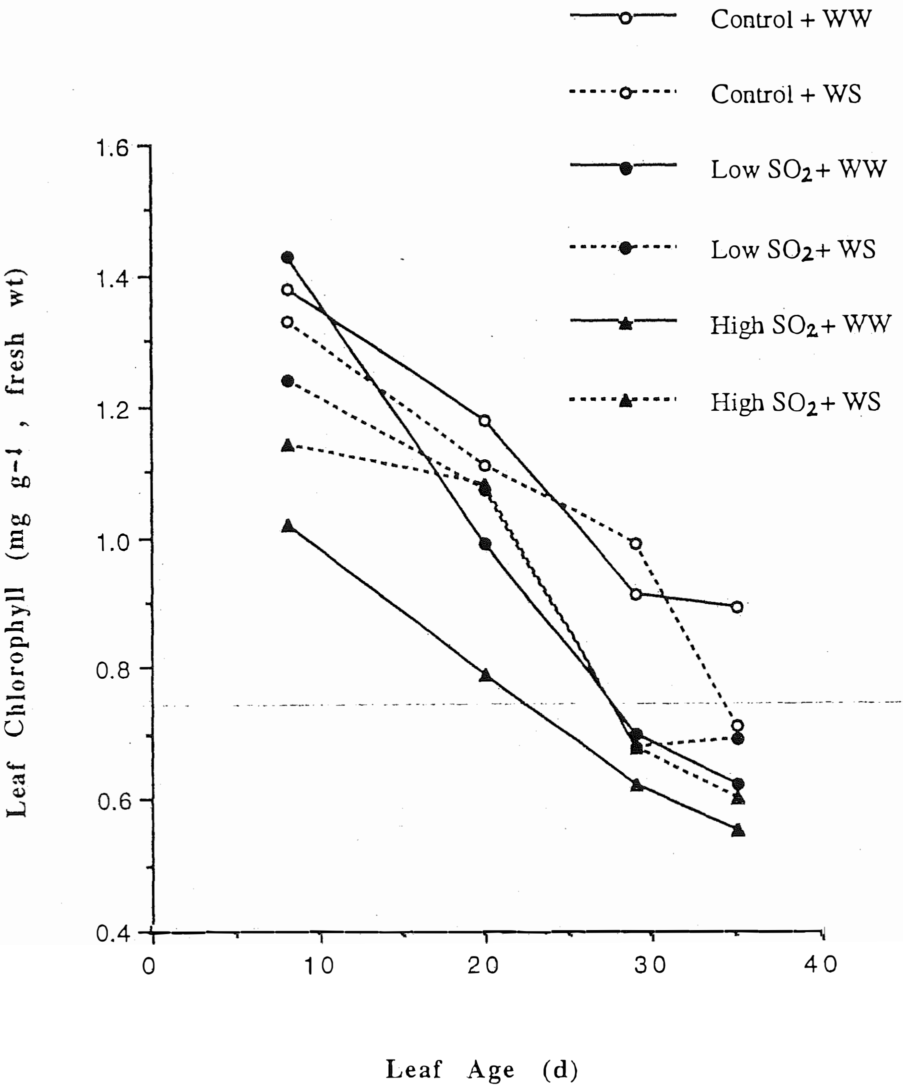


Figure 4.5 Joint action of SO_2 and water stress on leaf chlorophyll concentrations over the development of leaf age. WW denotes well-watered, and WS denotes water-stressed.

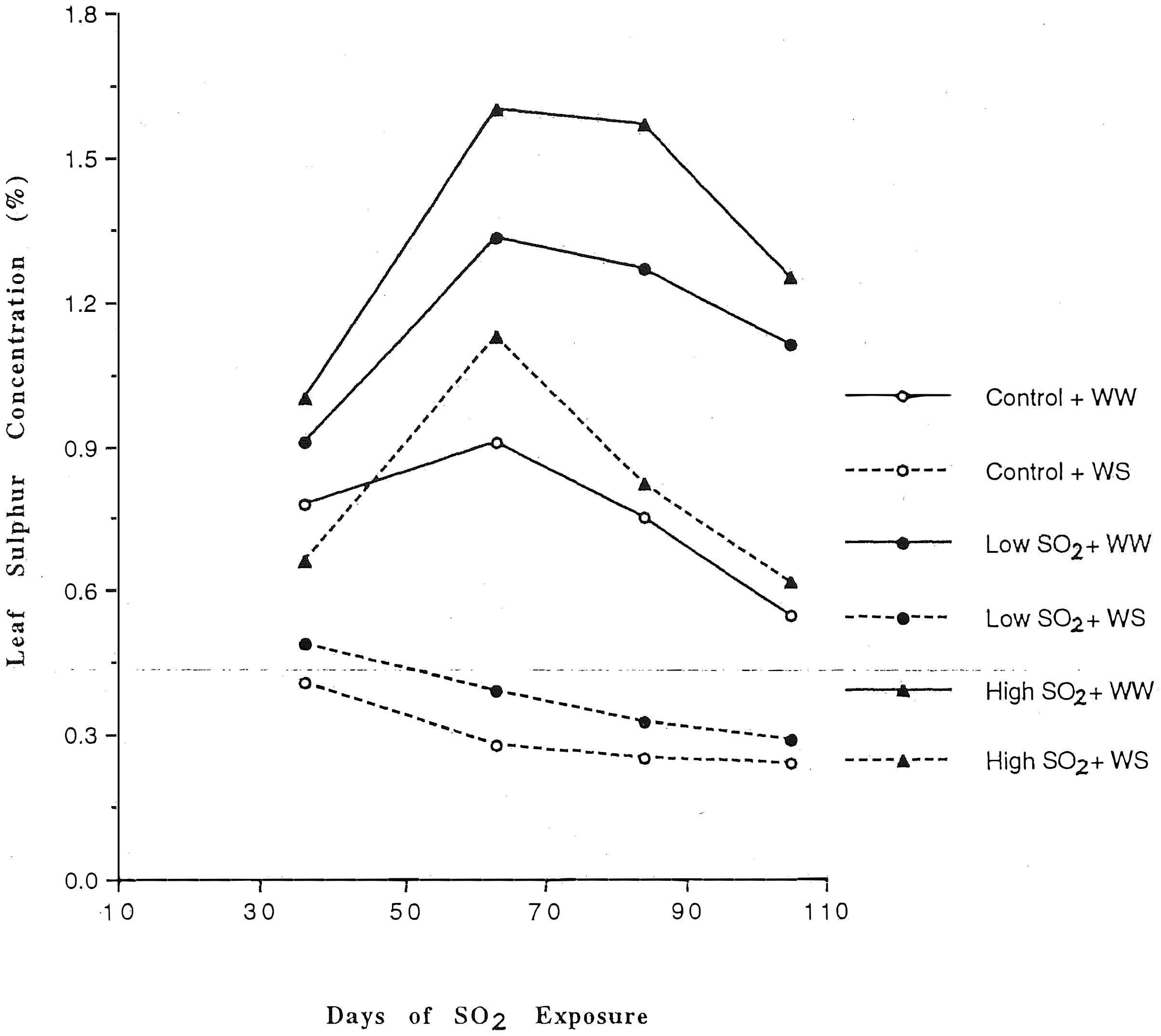


Figure 4.6 Joint action of SO_2 and soil water stress on leaf sulphur accumulation over the growing season. WW denotes well-watered, and WS denotes water-stressed.

conditions (Fig. 4.5). Leaf sulphur concentrations were also affected by SO_2 duration. They were significantly higher at the second harvest than that at the first harvest at both 110 and 300 nl l^{-1} SO_2 levels. However, it subsequently remained unchanged.

4.4 DISCUSSION

The objective of this study was to examine if SO_2 effects on potato growth were modified by soil water stress in the field. The results showed that exposure to 300 nl l^{-1} SO_2 under well-watered conditions produced defoliation and DW reduction of leaf, stem and tuber. By comparison, there was usually no DW reduction induced by 300 nl l^{-1} SO_2 under water-stressed conditions. This was consistent with the pattern of leaf sulphur accumulation; that is, plants exposed to SO_2 under well-watered conditions accumulated much more leaf sulphur than those exposed to SO_2 under water-stressed conditions.

4.4.1 SO_2 Effects

A number of studies have shown that SO_2 can alter the pattern of assimilate allocation favouring shoot growth at the expense of root growth (Whitmore *et al.*, 1982; Whitmore and Mansfield, 1983; Murray, 1985*a*). In contrast, water stress has been shown to have the reverse effect, favouring root growth at the expense of shoot growth (Bloom *et al.*, 1985). In this study, exposure to 300 nl l^{-1} SO_2 induced an increase in the shoot : root (both including and excluding tubers) ratios early in the growing season, but this increase was negated later probably due to the following two reasons. First, soil moisture stress significantly decreased the shoot : root ratios, which could counteract the effects of SO_2. The disruption of acclimatory response to SO_2 by soil water stress concerning resource allocation may be of importance in maintenance of physiological balance between the aboveground and belowground tissues, and may play some protective role against SO_2 injury. Secondly, with the progress of the growing season, the sink demand of potato tubers must have been increased. The tuber enlargement processes require a large translocation of assimilate from the aboveground tissues.

RGR is a good measure of plant stress (Chapin, 1989). High RGR suggests that the plant has favourable conditions for growth; and low RGR can mean the opposite; limited resources and resource imbalances. This study found that 110 nl l^{-1} SO_2 under well-watered conditions induced high mRGR relative to other treatments early in the season. Could this be a fertilising effect from SO_2 fumigation? In fact, the control plants had leaf sulphur concentrations of 0.3-0.7%, which reached the adequate levels of 0.3-0.5% sulphur in mid-stem leaves of potato reported by Piggott (1986). The growth stimulation in this case, therefore, could be attributable to some physiological and metabolic mechanisms. It is reported that low concentrations of SO_2 have a beneficial effect on plant growth by means of a number of different processes (Murray and Wilson, 1990*a*).

Although 110 nl l^{-1} SO_2 did not decrease leaf, stem and tuber DW, 300 nl l^{-1} SO_2 significantly suppressed the plant growth, which was in agreement with the effect of SO_2 on leaf chlorophyll concentration. It is known that SO_2-induced reductions in leaf chlorophyll concentration and photosynthesis are highly correlated (Saxe, 1983). This study showed that SO_2 exposure significantly increased leaf sulphur concentration. Similar results have also been demonstrated in broad bean by Adaros *et al.* (1988) and in wheat by Bytnerowicz *et al.* (1987). However, although leaf sulphur concentration increased significantly with total exposure duration early in the season, it stabilised later in the season possibly due to leaf death and abscission. Interestingly, Pierre and Queiroz (1982) fumigated bean plants with SO_2 and found that the level of sulphur accumulation stabilised after 15 days exposure, which occurred when variation in enzyme capacity attained its maximum, assumed to be an adaptive process.

4.4.2 Joint Action of SO_2 and Water Stress

The development of effects of gaseous pollutants on plants depends to a large extent upon other environmental factors. Krizek *et al.* (1986) found that soil moisture stress pretreatment resulted in greatly reduced SO_2 injury in *Euphorbia pulcherrima*. Macrez and Hubac (1988) reported that there were no differences between SO_2 exposed and non-exposed irrigated plants of *Picea abies* and water stress alone was not very injurious, but combination of the two stresses caused 50% death of the plants. The current experiment showed that significant interactions of SO_2 and water stress were expressed in most growth variables and some biochemical parameters measured. In general, water-stressed treatments modified the effects of SO_2 in comparison with well-watered treatments at the same SO_2 concentrations.

Undoubtedly, whether the SO_2-water stress interactions are synergistic, antagonistic or additive is related to different species used, different parameters measured and different environmental conditions under which the exposure to SO_2 and water stress takes place. Nevertheless, Murray (1985), Miller *et al.* (1988) and Lucas (1990) suggested that a relative increase in shoot tissue induced by

SO_2 at the expense of roots could have implications for reduced drought resistance of polluted plants due to the imbalance between the transpirational surface and the water absorptive surface. In contrast, moderate water stress could reduce the effects of SO_2 by increasing stomatal resistance and thus reducing SO_2 uptake (Mansfield and Freer-Smith, 1984), in addition to reducing the shoot : root ratio (Bloom *et al.*, 1985). In this study, well-watered plants accumulated significantly higher leaf sulphur than did water-stressed plants at the same levels of SO_2. Accordingly, SO_2-induced growth reductions of the well-watered plants were more severe than those of the water-stressed plants. This interactive effect increased with progress of the growing season, indicating a protective function of soil water stress in plant responses to SO_2 exposure.

In addition to water stress, salinity is another common stress in the soil which can expose the plant to a physiological drought (osmotic) stress. Therefore, if a plant grows in a saline medium, it will be subjected to an osmotic dehydration, which is analogous to the evaporative dehydration in the event of soil water stress. The current experiment indicated that water stress modified SO_2 effects probably by decreasing SO_2 uptake and sulphur accumulation in plants. Soil salinity may have a similar function in protecting plants from SO_2 injury due to the effects of osmotic stress on the plant. The following experiments were conducted to examine the influence of soil salinity on the expression of SO_2 effects by exposing the plant to SO_2 and salinity stresses simultaneously or in a sequential pattern.

CHAPTER FIVE

SO_2-Salinity Interactions on Foliar Injury, Plant Growth and Yield in Soybean

5.1 INTRODUCTION

Studies on the interactive effects of gaseous pollutants and soil water stress on plant growth and yield (Davids *et al.*, 1981; Krizek *et al.*, 1986; Heagle *et al*, 1988), and the study discussed in the previous chapter have shown that soil water stress can modify the detrimental effects of air pollution on plants. Salt stress is another very important edaphic factor which disturbs plant metabolism, suppresses plant growth and yield. It has a direct and inseparable relationship with water stress in the soil and can introduce a physiological drought stress in plants. As a consequence, soil salinity may also induce stomatal closure and decrease pollutant uptake by plants, and thus reduce plant sensitivity to air pollutants. However, salinity-pollutant interactions have so far received very little experimental attention, particularly under long-term field conditions (Olszyk *et al.*, 1988).

Studies conducted under laboratory conditions have shown that soil salinity can reduce ozone injury in alfalfa (*Medicago sativa* L.), pinto bean (*Phaseolus vulgaris* L.) and garden beet (*Beta vulgaris* L.) (Hoffman *et al.*, 1973, 1975; Maas *et al.*, 1973; Ogata and Maas, 1973; Bytnerowicz and Taylor, 1983). However, one field study showed no overall interaction between ozone and salinity for alfalfa growth and yield (Olszyk *et al.*, 1988). The differences between the laboratory and the field studies are likely to be caused by different environmental conditions, that can modulate stomatal aperture and thus pollutant uptake (Black, 1982; Tingey and Hogsett, 1985; Darrall, 1989). In addition, environmental factors also affect the degree of salt injury (Levitt, 1980).

Soil salinity is a common problem in semi-arid and arid regions of the world. Therefore studies on the interactive effects of air pollution and soil salinity under field conditions have an economic importance in agriculture and horticulture. So far, studies in this area have concentrated on ozone as the air pollutant and have usually been undertaken in a controlled environment. The objective of this experiment was to investigate whether soil salinity modified SO_2 sensitivity over a growing season in the field by measuring some responses of biochemical process, growth and yields of pot-grown soybeans to simultaneous SO_2 and Na salinity stress.

5.2 MATERIALS AND METHODS

5.2.1 SO_2 Fumigation

Soybean plants were fumigated using three SO_2 concentrations for 4 hours every day (1200 h-1600 h) from planting to seed maturity. The three mean (± standard deviation) SO_2 concentrations during the fumigation period were 189 (± 70.6), 99 (± 41.8) and an ambient air treatment 2 (± 5.1) nl l^{-1}.

The mean daily maximum and minimum temperatures outside the chambers were 28.5 and 15.6 °C respectively, and the mean daily relative humidity was 52%.

5.2.2 Plant Culture

Soybean seeds (*Glycine max* cv. Buchanan) were inoculated using *Rhizobium japonicum* strain USDA 110 and planted in 8-litre pots (diameter of 22 cm) filled with fine white sand on the 24th December, 1989. The planting rate was 2 seeds per pot. Within each chamber 30 pots were used, and evenly allocated for high, low and control salinities. When a pair of unifoliates were fully expanded, nutrient solutions were applied via a drip irrigation system. The drip irrigation, nutrient components and concentrations were as described in chapter three. After the 2nd trifoliate expanded, the seedlings were thinned into two plants per pot. Twenty-nine days after planting (DAP), NaCl was added to the nutrient solutions. This later application of salt stress aimed to decrease the effects of salinity on nodule initiation and development. High and low levels of salinity were achieved by dissolving 540 g or 320 g NaCl respectively in each 200 litres of nutrient solutions. No extra NaCl was added to the nonsaline treatment. Electrical conductivities of the solutions were 6.2 (± 1.3), 4.0 (± 0.9) and 0.6 (± 0.1) dS m^{-1} for the high, low and control salinities respectively. Electrical conductivity of the pot leachates was measured weekly over the growing season, and the mean conductivities were 7.0 (± 1.9), 4.9 (± 0.9) and 0.7 (± 0.1) dS m^{-1} for the high, low and control salinities respectively.

5.2.3 Seasonal Harvests

Serial harvests were conducted during the growing season on February 8, March 8 and April 9, 1990. Leaf area and plant dry weights were recorded. At final harvest yields and yield components of the different treatments were compared.

5.2.4 Biochemical Measurements

At each harvest four of the 2nd fully expanded leaves were collected from individual treatments for the measurements of leaf chlorophyll. Leaf sulphur concentration of the plants was also measured at each harvest to examine sulphur accumulation under different exposure conditions, using four replicates per treatment. The methods for assay of leaf sulphur and chlorophyll concentrations were the same as those described in chapter three.

5.2.5 Statistical Analysis

Response variables were subjected to a two-way analysis of variance (ANOVA) to identify significant effects of SO_2, salinity and their interactions. Contrasts were performed using methodology outlined by Finn (1974), with the MANOVA procedure in the SPSS-X version 2.0 package.

5.3 RESULTS

Both low and high SO_2 concentrations reduced leaf area and dry weight of the plants. In contrast, salinity-induced reductions of the two variables occurred only at the high salinity level. No significant stress interaction on these growth variables was observed over the growing season (Table 5.1).

Leaf chlorophyll concentrations were decreased by high SO_2 and high salinity, but there was no stress interaction on the variable. Leaf sulphur concentrations were tripled by the low and high SO_2 treatments but were reduced by the high salinity treatment. SO_2-salinity interactions on leaf sulphur accumulation were significant at

Table 5.1 Effects of SO_2 and salinity on leaf area and plant dry weight over the growing season. The data are the means of six plants per treatment.

	Salinity (dS m^{-1}) 0.7			4.9			7.0		
Days after planting --->	46	74	106	46	74	106	46	74	106
Treatment									
	Leaf area (cm^2)								
2 nl l^{-1} SO_2	839	5361	8299	709	3743	3168	458	2143	1099
99 nl l^{-1} SO_2	657	3856	6341	629	2082	4823	638	928	2810
189 nl l^{-1} SO_2	565	2795	3677	506	1247	689	402	1098	545
	Plant dry weight (g)								
2 nl l^{-1} SO_2	5.38	38.95	77.85	4.73	30.86	49.23	3.54	18.47	27.43
99 nl l^{-1} SO_2	4.49	29.98	48.72	4.16	17.13	42.83	3.91	11.59	36.15
189 nl l^{-1} SO_2	4.29	23.80	37.08	3.44	11.68	19.35	3.08	11.70	15.45

Statistical summary

	Leaf area 46	74	106	Plant dry weight 46	74	106
Days after planting	46	74	106	46	74	106
SO_2	*	***	***	n.s.	***	***
1st contrast	n.s.	***	**	n.s.	**	n.s.
2nd contrast	**	***	***	*	**	***
Salinity	*	***	***	*	***	***
1st contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2nd contrast	**	***	***	**	***	***
SO_2 X Salinity	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
1st contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
3rd contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4th contrast	*	n.s.	*	n.s.	n.s.	n.s.

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001.

For main effects of SO_2 and salinity, the first contrast represents control SO_2 or control salinity contrasted against low SO_2 or low salinity respectively, and the second contrast represents control SO_2 or control salinity contrasted against high SO_2 or high salinity respectively. For interactive effects, the first and second contrasts represent the differences between control SO_2 and low SO_2 under control salinity contrasted against that under low salinity and high salinity. respectively. The third and fourth contrasts represent the differences between control SO_2 and high SO_2 under control salinity contrasted against that under low salinity and high salinity, respectively.

the final harvest, as leaf sulphur concentrations in the SO_2-treated plants were decreased by the high salinity treatment (Table 5.2).

Production of pods per plant and seeds per pod was suppressed by high SO_2 and by high salinity, whereas average seed weight was mainly reduced by high salinity stress. Seed yields were reduced by high SO_2 and both the low and high salinities. SO_2 and salinity stress interacted on seed yield by affecting the number of pods but not the seeds per pod or average seed weight (Table 5.3).

Salinity caused foliar chlorosis and tip necrosis. With continuation of the stress, the necrotic areas expanded and the leaves became desiccated and finally defoliation occurred (Fig. 5.1). The salinity-caused injury was observed earlier and was more severe in the high SO_2 chambers than in the low SO_2 and control chambers. Foliar symptoms of SO_2 toxicity including chlorosis and stipple necrotic spots occurred after 7 weeks of SO_2 fumigation. The extent of SO_2 injury appeared to be reduced by the low salinity treatment.

5.4 DISCUSSION

This experiment was conducted to examine if the susceptibility of soybean plants to SO_2 toxicity was enhanced or reduced by soil salinity. The results showed that low soil salinity reduced plant sensitivity to SO_2 injury, but salt-induced injury was more severe in the high SO_2 chambers. Long term exposure of soybean to SO_2 or soil salinity reduced leaf chlorophyll concentration, leaf area and plant dry weight, but with the exception of affecting seed yield, number of pods produced and leaf sulphur concentration, stress interactions were usually not significant.

Table 5.2 Effects of SO_2 and salinity on leaf chlorophyll and leaf sulphur concentrations over the growing season. The data are the means of four plants per treatment.

	Salinity (dS m^{-1})								
	0.7			4.9			7.0		
Days after planting	46	74	106	46	74	106	46	74	106
Treatment									
	Chlorophyll concentration (mg g^{-1}, fresh weight)								
2 nl l^{-1} SO_2	2.17	2.35	2.37	2.21	2.06	1.85	1.64	2.04	1.34
99 nl l^{-1} SO_2	2.18	2.21	2.40	1.96	1.87	1.73	1.63	1.96	1.36
189 nl l^{-1} SO_2	1.93	2.18	1.88	1.87	2.26	1.36	1.55	1.82	1.34
	Sulphur concentration (%, dry weight)								
2 nl l^{-1} SO_2	0.33	0.23	0.21	0.25	0.24	0.19	0.27	0.24	0.22
99 nl l^{-1} SO_2	0.44	0.46	0.81	0.41	0.37	0.70	0.39	0.33	0.54
189 nl l^{-1} SO_2	0.68	0.54	0.83	0.63	0.56	0.58	0.59	0.49	0.63

Statistical summary

	Chlorophyll			Sulphur		
Days after planting	46	74	106	46	74	106
SO_2	n.s.	n.s.	*	***	***	***
1st contrast	n.s.	n.s.	n.s.	*	n.s.	***
2nd contrast	*	n.s.	**	***	***	***
Salinity	***	n.s.	***	**	n.s.	***
1st contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2nd contrast	***	*	***	**	*	***
SO_2 X Salinity	n.s.	n.s.	n.s.	n.s.	n.s.	*
1st contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2nd contrast	n.s.	n.s.	n.s.	n.s.	*	*
3rd contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4th contrast	n.s.	n.s.	n.s.	n.s.	n.s.	*

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001.

See Table 5.1 caption for a description of the contrast terms.

Table 5.3 Effects of SO_2 and salinity on seed yield and yield components. The data are the means of six plants per treatment.

	Salinity (dS m^{-1})			Salinity (dS m^{-1})		
Treatment	0.7	4.9	7.0	0.7	4.9	7.0
	Pod number			Seeds per pod		
2 nl l^{-1} SO_2	228	114	55	1.9	1.6	1.0
99 nl l^{-1} SO_2	147	112	88	1.7	1.4	1.1
189 nl l^{-1} SO_2	103	36	34	1.6	0.9	0.9
	Ave. seed weight (mg)			Seed yield (g)		
2 nl l^{-1} SO_2	58.0	47.5	29.5	25.17	8.32	1.70
99 nl l^{-1} SO_2	56.3	47.8	34.0	14.72	8.15	3.62
189 nl l^{-1} SO_2	52.0	42.[illegible]	42.5	9.93	1.42	1.50

Statistical summary

Treatment	Pod number	Seeds per pod	Ave. seed wt.	Seed yield
SO_2	***	**	n.s.	***
Salinity	***	***	***	***
SO_2 X Salinity	*	n.s.	n.s.	**

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001.

The significant effects of SO_2 or salinity on yield components are all due to the high SO_2 or high salinity treatments; the stress effects on seed yield are due to high SO_2 and both the low and high salinities; the interactive effects on pod number and seed yield are due to the combination of high SO_2 and high salinity (tested in the same way as shown in Tables 5.1 and 5.2).

Figure 5.1 Top: SO_2-caused leaf injury late in the season.
Lower: Salt-caused leaf injury late in the season.

Soil salinity can induce a physiological condition with similarities to drought (Levitt, 1980). Salinity-induced increases in stomatal resistance (Lewis *et al.*, 1989) are expected to decrease the uptake of SO_2 by a plant and result in less leaf injury caused by SO_2. Evidence in support of this hypothesis is provided by the current experiment. Salinity-SO_2 interactions on leaf sulphur accumulation were found at the final harvest, showing that salinity treatments significantly reduced leaf sulphur concentrations in the SO_2-treated plants when compared with exposure to SO_2 alone. Concomitantly, SO_2-induced leaf injury was decreased under the saline conditions late in the growing season. Similarly, some previous studies have demonstrated that the extent of leaf injury, caused by ozone, decreased with increasing levels of salinity (Ogata and Maas, 1973; Maas *et al.*, 1973; Hoffman *et al.*, 1975; Bytnerowicz and Taylor, 1983). Soil salinity extended the tolerance threshold to ozone exposure and lessened the deleterious effects of increasing duration of exposures (Maas *et al.*, 1973).

The current experiment also showed that high SO_2 induced an earlier and more severe salt injury to the leaves, indicating that the SO_2-polluted plants became more vulnerable to salt stress than non-polluted plants. This may be due to the effects of SO_2 on assimilate allocation in favour of shoots at the expense of roots (McLaughlin and McConathy, 1983; Whitmore and Mansfield, 1983; Murray, 1985) and the inhibitory effects of SO_2 on physiological and metabolic processes (Hallgren, 1984; Heath, 1984; Darrall, 1989). Elsheikh and Wood (1990) found that roots appeared to be more sensitive to salinity than shoots even at very low salinity levels of

1.0 dS m^{-1}. As a consequence, a combination of salinity and SO_2 could be very damaging to the roots and the whole plants. However, plant tolerance to the salt-induced osmotic stress is related to the synthesis of organic solutes for the maintenance of cell turgor - osmoregulation (Levitt, 1980). Since SO_2 can inhibit physiological and biochemical processes, the SO_2-treated plants may have developed a reduced efficiency in osmoregulation and consequently become more susceptible to salt injury. A number of studies have shown that exposure of a plant to SO_2 increases its vulnerability to water stress, such as photosynthetic capacity (Cornic, 1987), enzymes and total soluble proteins (Pierre and Queiroz, 1988) and even plant survival (Macrez and Hubac, 1988). These reports as well as the results described here indicate that plants, weakened by air pollutants, may succumb to natural stresses like drought or salinity.

SO_2 or salinity suppressed seed yield by reducing pod number, seeds per pod and average seed weight. The stress interactions also suppressed seed yield by reducing the number of pods produced. SO_2 is known to have direct effects on the processes of flowering and fruiting (Bonte, 1982), such as pollen germination and pollen tube length (Houston and Dochinger, 1977). Salinity can delay flower emergence and reduce the total number of flowers per plant (Levitt, 1980). The combination of SO_2 and salinity in the current experiment might amplify the detrimental effects on flowering and consequently result in an apparent reduction of pod number. By comparison, seed development, which follows pod initiation, appeared to be less sensitive to the interactive effects of SO_2 and soil salinity.

This experiment and the experiment discussed in chapter four were studying plant responses to simultaneous exposure to SO_2 and soil stresses, an exposure pattern which has been commonly used to examine the interactive effects of gaseous air pollutants and other environmental stresses. However, sequential exposure, *i.e.* first air pollutants and then other stresses or *vice versa*, is a recent exposure pattern which may provide some information about how polluted plants react to other stresses, biotic or abiotic, such as drought and salinity. Since the multiple stresses do not happen at the same time, the interactions may be different from that of simultaneous stress exposure. Next chapter will discuss the responses of soybean plants to sequential exposure to SO_2 and soil salinity.

CHAPTER SIX

Responses to Sequential Exposure to SO_2 and Salinity in Soybean

6.1 INTRODUCTION

In chapters four and five, the effects of simultaneous exposure to SO_2 and water stress on potatoes or to SO_2 and salinity on soybeans were discussed. The results showed that both water stress and salinity decreased SO_2-induced injury probably by decreasing plant sulphur accumulation. However, changes of environmental factors either prior to or after pollutant fumigation are also known to change the sensitivity of plants to pollutant toxicity. Krizek *et al.* (1986) found that soil moisture stress pretreatment greatly reduced SO_2 injury in poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzch.). In contrast, a high relative humidity prior to SO_2 exposure greatly increased subsequent plant injury from the pollutant in pea (*Pisum sativum* L.) (Kobriger and Tibbitts, 1985). Compared with the protective function of water deficit against air pollution, pre-polluted plants usually become more vulnerable to drought conditions. For example, Neighbour *et al.* (1988) exposed birch trees first to SO_2 and NO_2, and then to drought stress. They found that leaves of silver birch and downy birch were less efficient in the utilization of water after a period of exposure to the pollutants, and were less able to restrict water loss in a time of shortage when compared with the control plants. Dotzler and Schutt (1990) found that 16 months after fumigation with low SO_2 and O_3, strong influences of the pollutants on drought sensitivity of *Picea abies* plants were still apparent.

The effects of soil moisture stress or relative humidity on plant sensitivity to gaseous air pollutants are believed to be mainly the result of stomatal mechanisms. Stomata of water-stressed plants opened to a smaller degree, closed earlier during the day, and also closed more rapidly in the presence of O_3 (Dean and Davis, 1967; Rich and Turner, 1972). As stomata close, the plants become less susceptible to air pollution due to less pollutant uptake (Reich *et al.*, 1985). Tingey and Hogsett (1985) used fusicoccin to induce stomatal opening in the water-stressed plants, which subsequently were as sensitive to O_3 as were the non-water-stressed plants.

Effects of sequential exposure to SO_2 and soil salinity on injury and growth of soybeans are discussed in this chapter. Studies on the sequential stress exposure may help the understanding of how the compensatory mechanisms and predisposition characteristics of SO_2 and salinity affect plants. It is postulated that salinity pretreatment favours roots over shoots and decreases SO_2 uptake by inducing stomatal closure and thus results in less SO_2 injury. In contrast, SO_2 pretreatment favours shoots over roots and consequently increases salt injury. In practice, soybean plants were first exposed to salinity stress and then to SO_2 fumigation, or *vice versa* under simulated field conditions. Two experiments were conducted, one in the 1991 season and another in the 1992 season. Plant responses to the prior stresses and interactions with the sequential exposure were evaluated by measuring stomatal aperture, plant transpiration, visible injury, shoot and root growth, and root nodulations.

6.2 MATERIALS AND METHODS

6.2.1 The 1991 experiment

6.2.1.1 Plant culture

The drip irrigation, nutrient components and concentrations were as described in chapter three. Germinated soybean seeds were inoculated using *Rhizobium japonicum* strain USDA 110. Since this was a short term experiment, the seeds were planted in 3-litre pots (diameter of 16 cm) filled with fine white sand on the 21st October, 1990. The planting rate was 2 seeds per pot, and later 1 seedling per pot was retained. There were 30 pots in each fumigation chamber. Nutrient solutions were applied 2 days later and continued throughout the growing season. Before expansion of the 2nd trifoliate, NH_4NO_3 (40 μM) was added but stopped afterwards.

6.2.1.2 Stress exposure

During the episode of SO_2 treatment, plants were fumigated using three SO_2 concentrations for 5 h (1000 - 1500 h) every day. The exposure time was designed to match the closing movements of stomata. The three mean SO_2 concentrations ($\pm$ SD) during the fumigation period were 300 ($\pm$ 56.5), 145 ($\pm$ 25.1) and an ambient air treatment 1 ($\pm$ 3.7) nl l^{-1}. The mean daily maximum and minimum temperatures outside of fumigation chambers were 24.2 and 12.7 °C respectively, and the mean daily relative humidity was 56%.

Within each chamber, 30 pots were evenly divided for high, low and control salinity treatments. High and low salinities were achieved by dissolving 540 g or 320 g of NaCl in each 200 litres of nutrient solution, showing electrical conductivities (EC) of 6.0 and

4.0 dS m^{-1}, respectively. No extra NaCl was added to the control treatment showing an EC of 0.6 dS m^{-1}. The EC of the pot leachates was measured every week and the means (± SD) were 6.5 (± 1.1), 4.4 (± 0.8) and 0.7 (± 0.1) dS m^{-1} for the high, low and control salinities, respectively.

At seedling emergence, one-half the plants were submitted to salinity stress, and the remainings to SO_2 exposure. Three weeks later the salinity-treated plants were transferred to the SO_2-fumigated chambers, and the SO_2-polluted plants were subjected to salinity stress. The second stress episode also lasted 3 weeks.

6.2.1.3 Plant harvests

The first harvest was made at the end of salinity or SO_2 pretreatment, and the second harvest was at the end of subsequent SO_2 or salinity stress. Ten plants were randomly chosen and divided into leaves, stems, roots and nodules. Leaf area, total nodule number and nodule fresh weight were recorded. The materials were dried in a forced-draft oven at 80 °C for 48 hours for dry weight measurements.

6.2.1.4 Stomatal measurements

Stomatal diffusive resistance was measured using an automatic porometer (Delta T, Cambridge, UK) four times (900, 1100, 1300 and 1500 h) a day on the days prior to the first harvest and 3 days after subsequent exposure to SO_2 or salinity commenced. Five of the second fully expanded leaves from individual treatments were used and only the lower surfaces of the leaf blades were measured.

6.2.1.5 Statistical analysis

Response variables were subject to a one-way ANOVA followed by a Duncan's multiple range test to determine individual treatment effects after 3 weeks of Na salinity or SO_2 pretreatment. For the second part of the experiment, a factorial analysis (three levels of SO_2 by three levels of salinity) was used (Lucas, 1990). Estimates of the main treatment effects and their interactions were obtained using the SPSS-X version 2.0 package.

6.2.2 Effects of SO_2 and salt on plant transpiration in the 1992 season

In March and April, 1992, one more sequential stress exposure experiment was conducted to examine the effects of SO_2 and salt on plant transpiration. Twenty pots in one chamber were used and evenly divided into saline and nonsaline treatments. There were three fumigation chambers (control, low and high SO_2) for the experiment and thus a total 60 pots. Germinated seeds were sown on the 24th February, 1992, and 7 days later the plants were subjected to Na salinity (4.0 dS m^{-1}) and nonsaline (0.6 dS m^{-1}) treatments. Twenty days later, the plants were fumigated with three SO_2 concentrations (2, 108 and 235 nl l^{-1}) for 18 days. At the end of exposure to salinity and SO_2, the pots were thoroughly watered and allowed to drain to field capacity. Water loss through evaporation was prevented by covering the soil surface with aluminium foil. Plants were then submitted to drought stress by withholding irrigation solutions until showing wilt. The rate of pot weight loss through plant transpiration was recorded at noon on the following days. Seven days after exposure to drought stress (*i.e.* on the 17th April, 1992), all the plants were harvested and the effects of stress pretreatment on plant water conservation ability were estimated.

6.3 RESULTS

6.3.1 The 1991 experiment

6.3.1.1 Responses to salinity and subsequent SO_2

Although plant height and leaf number were not affected by exposure to the saline treatments after 3 weeks, leaf area and both shoot and root dry weights were significantly decreased (Table 6.1). The number of nodules per plant was decreased only by high salinity, whereas the decreases in nodule fresh weight occurred under both the low and high saline conditions. Salt stress also decreased leaf chlorophyll concentrations.

Pretreatment with low salinity significantly modified the adverse effects of high SO_2 on all the growth variables (Table 6.2). The combination of low salinity and SO_2 increased the number and fresh weight of root nodules. However, high salt-pretreated plants were severely injured by exposure to SO_2 especially high SO_2 which killed the plants.

Stomatal resistance was increased by salt stress, and 3 days after the plants were transferred to the SO_2 chambers, the effect of salinity on stomata remained apparent (Fig. 6.1).

6.3.1.2 Responses to SO_2 and subsequent salinity

Neither foliar injury nor decrease in plant height and leaf number was observed after 3 weeks of exposure to high SO_2. However, leaf area, shoot and root dry weight were significantly decreased (Table 6.3). The shoot : root ratio was increased by both the low and high

Table 6.1 Effects of 3 week pretreatment with salinity on selected growth variables, leaf chlorophyll (mg g^{-1}, fresh weight) and root nodulations

Treatment	Plant height (cm)	Leaf number	Leaf area (cm^2)	Total chlorophyll	Shoot DW (g)	Root DW (g)	Shoot:root ratio	Nodule number	Nodule fresh weight (g)
Nonsaline	10.6 a	4.1 a	91.5 a	1.33 a	0.42 a	0.29 a	1.41 a	69.8 a	0.42 a
Low saline	9.9 a	4.1 a	70.5 b	0.77 b	0.33 b	0.26 ab	1.38 a	67.0 a	0.32 b
High saline	9.9 a	4.0 a	69.4 b	0.64 b	0.28 b	0.22 b	1.30 a	44.0 b	0.20 c

The data for chlorophyll are the means of four plants per treatment, and the data for other variables are means of ten plants per treatment. Significant differences between the treatments are indicated by the absence of the same letter (Duncan's multiple range test, P = 0.05).

Table 6.2 Means of growth variables for plants after 3 weeks of SO_2 exposure following 3 weeks of salinity pretreatment

First		Salinity			Salinity			Salinity			Salinity		
		non	low	high	non	low	high	non	low	high	non	low	high
Then		Plant height (cm)			Number of leaves			Leaf area $(cm)^2$			Total chlorophyll (mg/g, fresh wt)		
SO_2	non	23.6	20.5	18.2	5.7	4.4	3.9	338	207	149	1.19	1.07	1.10
	low	22.1	21.9	17.1	4.6	4.6	3.7	216	228	113	1.78	1.25	0.74
	high	23.7	25.2	14.0	3.9	4.6	n.a.	139	243	n.a.	1.11	1.24	n.a.
		Shoot dry weight (g)			Root dry weight (g)			Number of nodules			Nodule fresh wt (g)		
SO_2	non	2.57	2.02	1.31	1.41	1.13	0.82	86.8	78.0	78.2	1.27	1.31	1.10
	low	1.96	1.79	0.89	1.14	1.15	0.60	85.2	79.5	38.0	1.35	1.76	0.66
	high	1.78	1.99	0.71	1.04	1.06	0.27	68.7	99.2	n.a.	1.16	1.66	n.a.

Statistical summary of treatment effects

	Plant height	Leaf No.	Leaf area	Chlorophyll	Shoot DW	Root DW	Nodule No.	Nodule wt
SO_2	n.s.	***	***	***	***	***	**	**
1st contrast	n.s.	***	n.s.	***	*	n.s.	n.s.	*
2nd contrast	n.s.	***	***	***	***	***	***	**
Salinity	***	***	***	***	***	***	***	***
1st contrast	***	***	***	n.s.	***	***	***	***
2nd contrast	***	***	***	***	***	***	***	***
Interaction	***	***	***	***	**	*	***	***

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001. n.a. = not available as the plants were dead.
The first contrast of SO_2 represents low SO_2 contrasted against control SO_2. The second contrast of SO_2 represents high SO_2 contrasted against control SO_2. The same explanations apply for the contrasts of salinity.

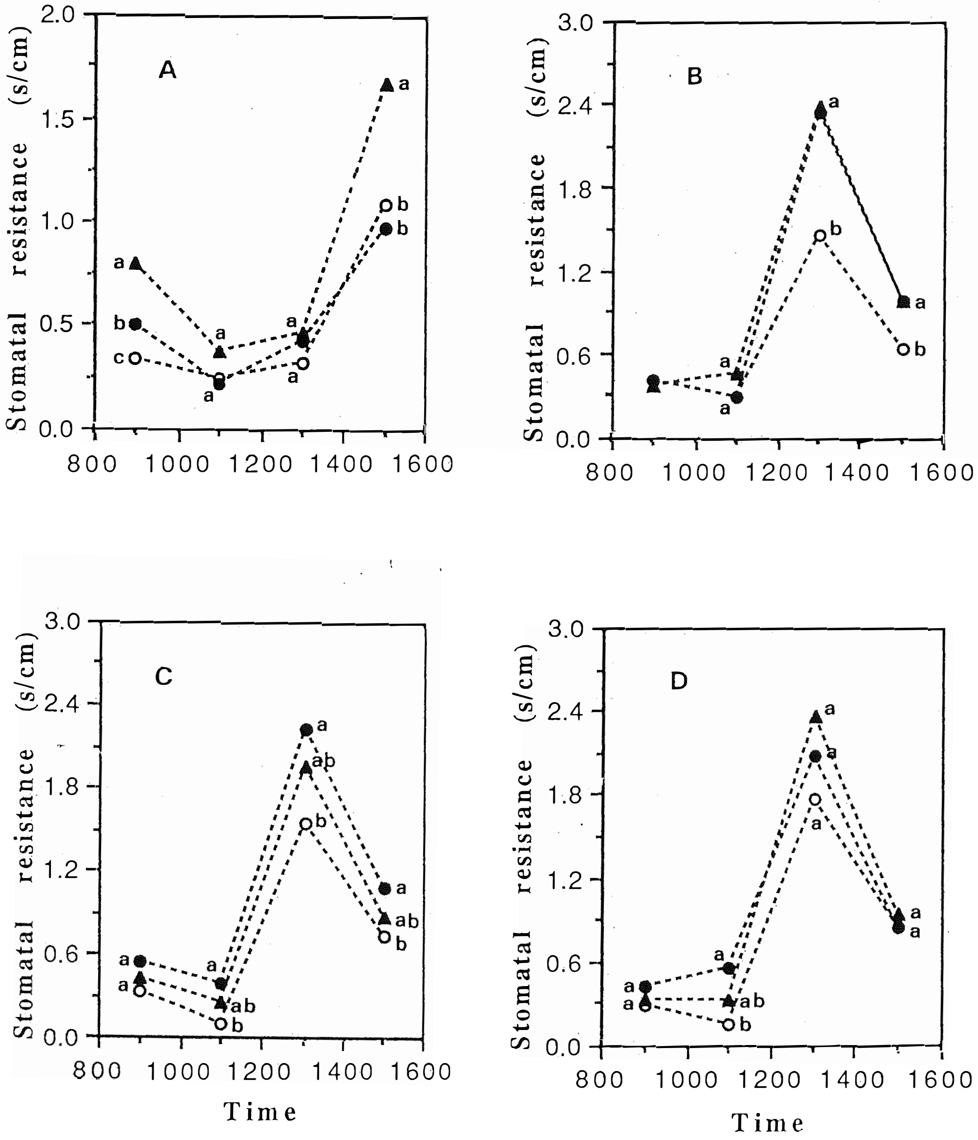


Fig. 6.1 The response of stomatal resistance to 3 weeks of salinity stress (A). Stomatal resistance was measured again 3 days after the plants were transferred to three SO_2 chambers; ambient (B), low SO_2 (C), or high SO_2 (D). Within each column, means without the same letter are significantly different at the P level of 0.05 (Duncan's multiple range test).

-- o --, nonsaline; -- ● --, low saline; -- ▲ -- high saline.

SO_2 treatments. SO_2 also induced an increase in leaf chlorophyll concentration. Although SO_2 had no effect on total nodule number, nodule fresh weights were significantly decreased.

The SO_2-polluted plants showed foliar injury after 5 days of high salt stress. All of the high SO_2-polluted plants were dead after 12 days of the high salinity treatment. In contrast, half the low SO_2-polluted plants died after 3 weeks of exposure to high salinity. Low SO_2 often moderated the growth depression caused by low salinity with a suggestion of slight stimulations in height, leaf number, leaf area and shoot dry weight (Table 6.4). However, SO_2 often amplified the reductive effects of high salinity on plant growth.

High SO_2 increased stomatal resistance early in the morning and late in the afternoon (Fig. 6.2). Three days after the SO_2 treatment ceased, SO_2-induced stomatal closure was still observed in the morning in the nonsaline plants but not in the saline plants.

6.3.2 Water loss by polluted plants in the 1992 season

The experiment conducted during the period of March-April, 1992 showed that soil salinity strongly induced stomatal closure, which persisted at the end of SO_2 exposure (Fig. 6.3). High SO_2 and salinity stress decreased both shoot and root growth, but their interactions were not significant (Table 6.5).

As shown in Figure 6.4, the rate of water loss through plant transpiration was greatly affected by salt stress but not much by SO_2 pollution. In the absence of soil salinity, control or SO_2-polluted plants were wilted 4 days after the irrigation was stopped. However, the salt-treated plants were not wilted until day 7. The

Table 6.3 Effects of 3 week pretreatment with SO_2 on selected growth variables, leaf chlorophyll (mg g^{-1}, fresh weight) and root nodulations

Treatment	Plant height (cm)	Leaf number	Leaf area (cm^2)	Total chlorophyll	Shoot DW (g)	Root DW (g)	Shoot:root ratio	Nodule number	Nodule fresh weight (g)
No SO_2	9.9 a	4.1 a	90.6 a	0.95 a	0.37 a	0.32 a	1.09 a	88.7 a	0.45 a
Low SO_2	10.2 a	4.0 a	60.3 b	1.13 ab	0.31 b	0.24 b	1.29 b	72.5 a	0.35 b
High SO_2	9.9 a	4.0 a	64.6 b	1.28 b	0.31 b	0.24 b	1.34 b	80.6 a	0.36 b

The data for chlorophyll are the means of four plants per treatment, and the data for other variables are means of ten plants per treatment. Significant differences between the treatments are indicated by the absence of the same letter (Duncan's multiple range test, P = 0.05).

Table 6.4 Means of growth variables for plants after 3 weeks of salinity stress following 3 weeks of SO_2 pretreatment

First			SO_2			SO_2			SO_2			SO_2	
		non	low	high	non	low	high	non	low	high	non	low	high
Then		Plant height (cm)			Number of leaves			Leaf area (cm)^2			Total chlorophyll (mg/g, fresh wt)		
	non	19.9	23.7	25.2	6.5	7.0	6.0	284	343	249	1.61	1.50	1.50
Salinity	low	19.9	21.0	19.6	2.6	3.6	2.6	95	144	94	0.94	1.03	1.41
	high	18.0	15.6	14.6	1.6	0.4	n.a.	50	6	n.a.	0.78	0.63	n.a.
		Shoot dry weight (g)			Root dry weight (g)			Number of nodules			Nodule fresh wt (g)		
	non	2.15	2.40	1.79	1.24	1.10	0.83	117.7	109.3	87.5	1.49	1.79	1.21
Salinity	low	1.00	1.25	1.08	0.61	0.59	0.57	88.8	68.3	74.8	0.83	0.85	0.73
	high	1.08	0.70	0.51	0.52	0.30	0.18	88.5	72.2	n.a.	0.60	0.49	n.a.

Statistical summary of treatment effects

	Plant height	Leaf No.	Leaf area	Chlorophyll	Shoot DW	Root DW	Nodule No.	Nodule wt
Salinity	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
1st contrast	n.s.	*	* *	* * *	* * *	*	n.s.	*
2nd contrast	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
SO_2	n.s.	*	* *	n.s.	* *	* * *	* * *	* * *
1st contrast	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	* * *
2nd contrast	n.s.	*	* *	n.s.	* *	* * *	* * *	* * *
Interaction	* * *	*	*	* * *	*	*	* * *	* * *

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001. n.a. = not available as the plants were dead.
See Table 6.2 note for a description of the contrast terms of SO_2 or salinity effects.

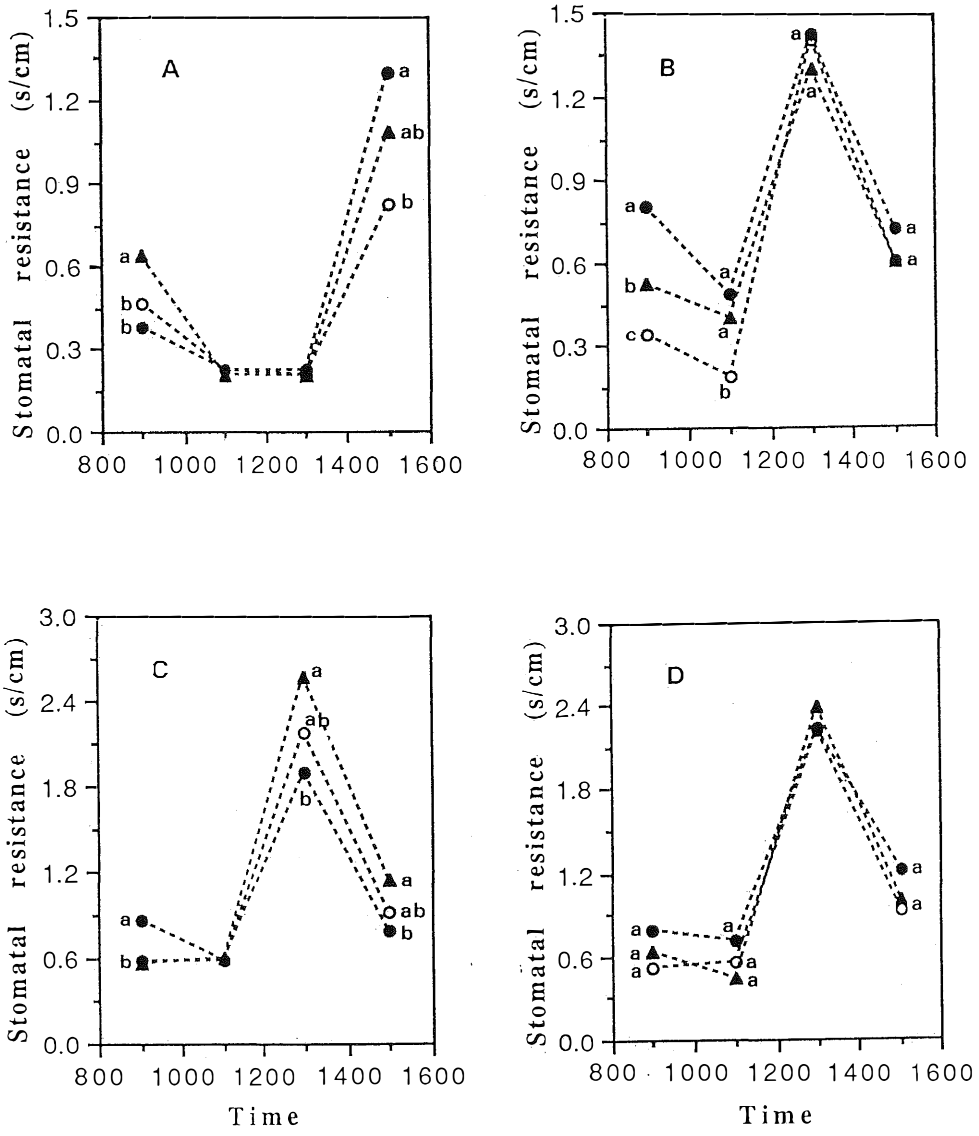
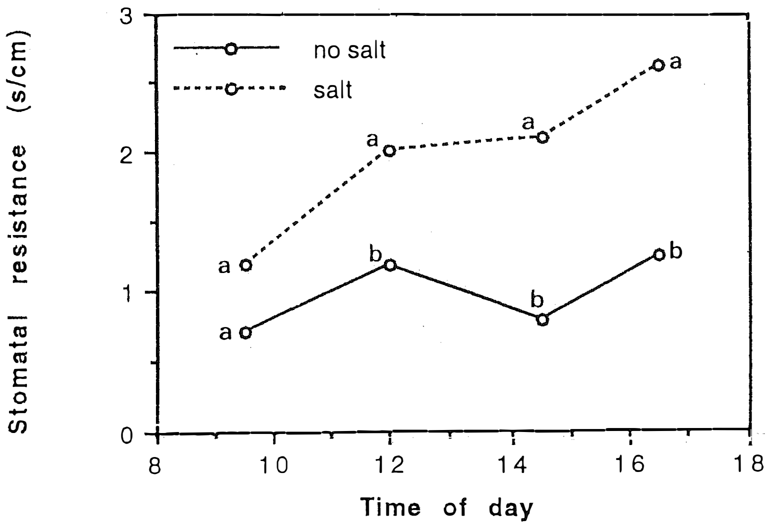


Fig. 6.2 The response of stomatal resistance to 3 weeks of SO_2 exposure (A). Stomatal resistance was measured again 3 days after the plants were transferred to three salinity treatments; nonsaline (B), low saline (C), or high saline (D). Within each column, means without the same letter are significantly different at the P level of 0.05 (Duncan's multiple range test).

-- o --, no SO_2; -- ● --, low SO_2; --▲ -- high SO_2.



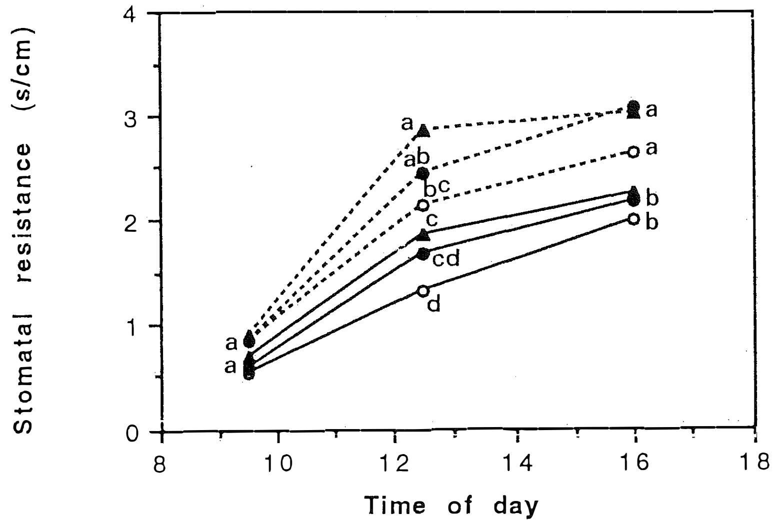


Figure 6.3 Responses of stomatal resistance to 3-week salinity pretreatment (top) and subsequent 18-day exposure to SO_2 and salinity combination (lower). Within each column, means without the same letter are significantly different at the P level of 0.05 (Duncan's multiple range test).

——o——, no SO_2 + no salt; ---o---, no SO_2 + salt;

——●——, 108 nl l^{-1} SO_2 + no salt; ---●---, 108 nl l^{-1} SO_2 + salt;

——▲——, 235 nl l^{-1} SO_2 + no salt; ---▲---, 235 nl l^{-1} SO_2 + salt.

Table 6.5 Responses of shoot and root growth to 3-week salt pretreatment and subsequent 18-day exposure to SO_2 and salt combination

Source of Variation	*d.f.*	Shoot dry weight (g)	Root dry weight (g)
SO_2 (A)	2	*	*
1st contrast	1	n.s.	n.s.
2nd contrast	1	*	* *
Salinity (B)	1	* *	*
A x B	2	n.s.	n.s.
2 nl l^{-1} SO_2, nonsaline		3.62	1.82
" saline		2.97	1.54
108 nl l^{-1} SO_2, nonsaline		3.78	1.84
" saline		2.77	1.68
235 nl l^{-1} SO_2, nonsaline		3.02	1.52
" saline		2.70	1.46

n.s., not significant; * P=0.05; ** P=0.01.

See Table 6.2 note for a description of the contrast terms of SO_2.

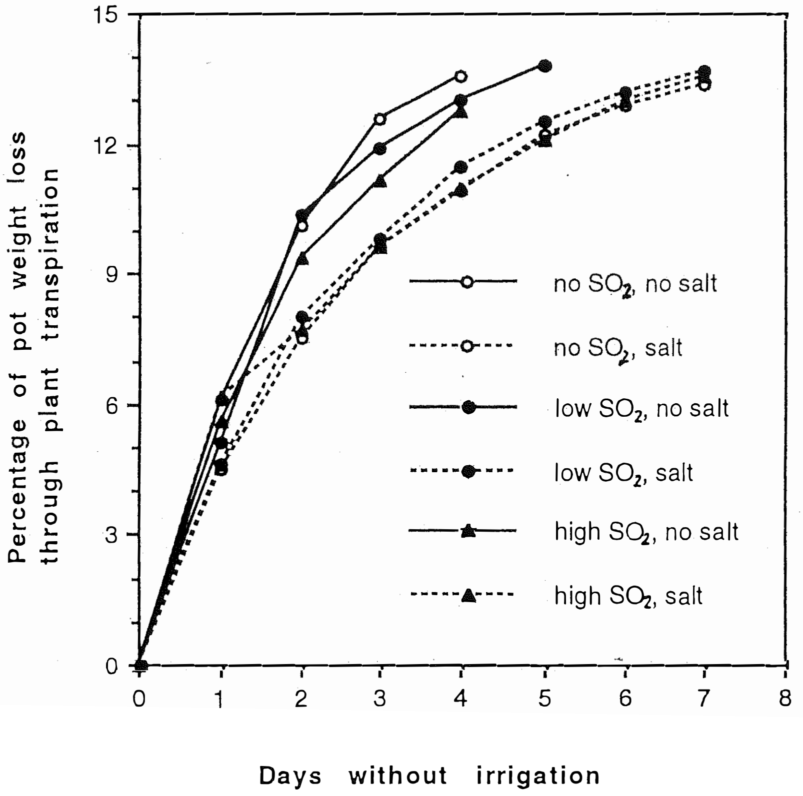


Figure 6.4 Rates of pot water loss through plant transpiration after salinity and SO_2 pretreatment. The daily rates were recorded until plants wilted.

improved ability of saline plants to conserve water seemed to be largely due to their closed stomata and thus decreased transpiration rate.

6.4 DISCUSSION

6.4.1. Responses to salinity and subsequent SO_2

Salinity or SO_2 exposure decreased the leaf area and dry weight of the plants. However, in the sequential exposure, low salinity protected the plants from SO_2-induced reductions of all the growth variables measured. This could be attributable to the salinity-induced stomatal closure causing a decrease in SO_2 uptake. In addition, this stomatal closure, as demonstrated in the 1992 experiment, was able to reduce plant water loss through transpiration and improve the ability of plants to resist drought stress. Similarly, soil moisture stress prior to SO_2 exposure greatly reduced SO_2 injury with greatly decreased stomatal conductance (Krizek *et al.*, 1986). Simultaneous exposure of soybeans to SO_2 and salinity showed that SO_2-induced leaf injury was more severe in the nonsaline plants than in the saline plants, and leaf sulphur concentrations significantly decreased by soil salinity (Qifu and Murray, 1991*b*). SO_2-induced growth reductions of water-stressed potato plants were less than those of the well-watered plants, and the water-stressed plants accumulated significantly less leaf sulphur than did the well-watered plants exposed to the same concentrations of SO_2 (Qifu and Murray, 1991*a*). Tingey and Hogsett (1985) demonstrated that soil water stress protected Pinto bean plants from ozone injury mainly through its influence on stomatal aperture rather than through biochemical or anatomical changes.

Although the pretreatment with low salinity decreased the toxicity of high SO_2, high salinity followed by SO_2 stress (especially high SO_2) was very damaging to soybean plants despite also showing salt-induced stomatal closure. All the plants were dead at the end of the high SO_2 fumigation. The toxic pretreatment of high concentrations of salt ions, which caused sufficient metabolic injury, must have impaired plant resistance to the subsequent SO_2 exposure. It is known that salt stress can induce specific toxic effects, directly on the external plasma membrane or after penetration through the membrane into the protoplast, and disturb photosynthesis, respiration and protein metabolism (Levitt, 1980). The disturbance of cell metabolism may finally decrease or destroy the homeostatic safety margin in term of energy and enzyme capacity (Atkinson, 1977; Queiroz, 1983) required to counter the detrimental effects of SO_2. Therefore, the compensatory mechanisms and predisposition characteristics of salinity stress against subsequent SO_2 depend largely upon the stress levels used.

The decrease of nodule formation at high salinity might be attributed to shrinkage of root hairs (Tu, 1981). Nodulation in soybean is sensitive to NaCl, as nodule number and weight were decreased by approximately 50% at 26.6 mol m^{-3} NaCl (3.1 dS m^{-1}) in the rooting medium under glasshouse conditions (Singleton and Bohlool, 1984). By comparison, under field conditions of this study, high salinity (6.5 dS m^{-1}) decreased both nodule number and nodule fresh weight per plant, but low salinity (4.4 dS m^{-1}) only decreased the nodule fresh weight. It was also found that the combination of low salinity and SO_2 increased both the number and fresh weight of root nodules. This increase can be important for nitrogen resource since no NH_4NO_3 was applied late in the season, and may thus make some contribution to the protective function of low salinity from SO_2-induced decrease in leaf area and plant dry weight.

6.4.2. Responses to SO_2 and subsequent salinity

SO_2-induced growth impairments were accompanied by a decrease in leaf area and nodule fresh weight without changing the number of leaves and nodules. Plants exposed to SO_2 had significantly higher shoot : root ratios than control plants, indicating a shift in assimilate allocation. This SO_2-induced impairment of assimilate partitioning to root growth might account for the decrease in nodule fresh weight to some extent.

The SO_2-induced increase in shoot : root ratio may also harm the water relations of the plant when transferred to a high salt medium because of not only the imbalance between the transpirational area and the absorptive area for water, but also the salt-induced osmotic dehydration. In addition, SO_2, like other air pollutants, can weaken plants by inducing a series of physiological and metabolic changes. For example, SO_2 clearly has access to the cytosol where the metabolism of the cell is modified in response to elevated concentrations of the ionic species. If the anion concentration exceeds the metabolic capacity of the cell, deleterious reactions ensue which may include damage to cell membranes (Mudd *et al.*, 1984). Ultrastructural studies have shown that exposure to SO_2 can also disrupt the structure of the thylakoids and grana within the chloroplasts, and such disruptions are likely to have important consequences on the activities of PSI and PSII and on the light-modulated enzymes of photosynthesis (Huttunen and Soikkeli, 1984). As a result, pre-polluted plants may not be able to maintain cell turgor by a sufficient increase in cell solutes to compensate for the external osmotic stress (*i.e.* osmotic adjustment) in a high salt medium, and subsequently become more susceptible to salt injury. Evidence in support of this hypothesis was provided in the present study. Although prior exposure of plants to high SO_2 did not cause any visible injury, subsequent high salinity resulted in severe leaf injury. As the high salinity treatment continued, the SO_2-polluted plants suffered high mortality. This is largely consistent with the previous finding that salinity-induced leaf injury occurs earlier and is more severe in the high SO_2 and saline-treated plants than in the low SO_2 and non-fumigated saline-treated plants (see Chapter Five). It appears that the adaptive mechanisms that achieve tolerance to the effects of high SO_2 or high salinity are not capable of balancing the effects of the two stresses in combination.

CHAPTER SEVEN

Effects of SO_2 Fumigation on Nodule Activity and Plant Growth in Soybean under Saline Conditions

7.1 INTRODUCTION

In the previous three chapters, discussion is mainly focused on the effects of SO_2 on the above-ground components of crop plants under water-stressed or salt-stressed conditions. However, the effects of SO_2 on the below-ground components are also important. Limited investigations have shown that air pollution can affect root nodulation and nitrogen fixation in legume crops. Tingey and Blum (1973) found that ozone decreased nodule number, nodule weight per plant and leghaemoglobin content in soybeans, which is positively correlated with N-fixing capacity. These reductions were reported to be an indirect result of an effect of ozone on the plant foliage (Blum and Tingey, 1977). Exposure of soybean plants to 6.7 $\mu mol\ m^{-3}$ (161 $nl\ l^{-1}$) ozone and 11.1 $\mu mol\ m^{-3}$ (267 $nl\ l^{-1}$) SO_2 for 5 successive days resulted in a lower rate for total nodule activity expressed as per-plant basis, but had no effect on specific nodule activity expressed as per-gram nodule fresh weight (Jones *et al.*, 1985).

Salinity is known to suppress nodulation in legumes by affecting the infection process of rhizobia (Rai and Prasad, 1983; Singleton and Bohlool, 1984). A reduction of root hairs in lucerne and the shrinkage of root hairs in soybean were observed under saline conditions (Lakshmi-Kumari *et al.*, 1974; Tu, 1981). Nodule number and total nodule weight per plant were decreased by salinity, but the average weight per nodule was increased by the higher levels of salinity. Salinity caused severe depression of both total and specific nodule activities (Balasubramanian and Sinha, 1976; Yousef and Sprent, 1983; Elsheikh and Wood, 1990).

In legume crops, SO_2 may affect not only root growth but also root nodulations which largely determine nitrogen supply to plant growth. In my experiments, soybean plants were subjected to SO_2 and salt stress simultaneously under simulated field conditions. It was postulated that SO_2 and salinity may interact to decrease root growth, nodules and N-fixation and impair nitrogen resources in the plants, and finally plant growth. These responses were examined by measuring root nodules, nodule fresh weight, nitrogenase (acetylene reduction) activity, shoot and root nitrogen concentrations and plant dry weight. Stomatal resistance was measured and scanning electron microscopy was used to investigate the effects of SO_2 and Na salinity on stomata.

7.2 MATERIALS AND METHODS

7.2.1 Plant culture

Two separate experiments were conducted, one in 1991 and another in 1992. The 1991 experiment was run from March 30 to May 6, a late growing season having relatively low temperatures. In contrast, the 1992 experiment was run from February 19 to March 20, a mid-growing season having higher temperatures. Germinated soybean seeds (*Glycine max* L. cv. Buchanan) were inoculated using *Rhizobium japonicum* strain USDA 110 and planted in 3-litre pots (diameter of 16 cm) filled with white sand. The sowing rate was 2 seeds per pot, and later was thinned into one plant per pot. Thirty pots were used in each chamber and evenly divided for nonsaline and saline treatments. Nutrient solutions were applied through a drip irrigation system twice each day at 0700h and 1900h The nutrient components and concentrations were as described in chapter three, but NH_4NO_3 was not used.

7.2.2 Stress exposure

In the 1991 experiment, plants were subjected to two levels of SO_2 concentrations (1 and 45 nl l^{-1}) and two levels of Na salinity (0.6 and 4.0 dS m^{-1}) in a factorial combination at seedling emergence. Each SO_2 treatment was duplicated. Use of this low SO_2 concentration aimed to examine the sensitivity of root nodulation to the pollutant. The SO_2 exposure was conducted from 1100h to 1600h every day for 37 days. The mean daily maximum and minimum temperatures outside of the chambers were 26.5 and 13.4 °C respectively, and the mean daily relative humidity was 59% during the period of experiment.

In the 1992 experiment, plants were subjected to three levels of SO_2 (2, 110 and 250 nl l^{-1}) and two levels of Na salinity (0.6 and 4.0 dS m^{-1}) in a factorial combination 13 days after planting. Each SO_2 treatment was duplicated. Use of high SO_2 concentrations aimed to investigate whether the effects of SO_2 on root nodulation would affect plant growth by impairing nitrogen resources within a short period of time. The SO_2 exposure was also conducted from 1100h to 1600h every day. The episode of SO_2 and salinity stress was for 16 days. The mean daily maximum and minimum temperatures outside of the chambers were 28.6 and 17.3 °C respectively, and the mean daily relative humidity was 61.4%.

7.2.3 Nitrogenase activity assay

In the 1991 season, three sequential samplings were made on the days 23, 30 and 37 after planting. In the 1992 season, one sampling was made at the end of SO_2 and salinity exposure. The plants were randomly chosen and the sand was shaken from the roots. Three intact plants were placed directly into a Fowlers No. 31 jar with four replicates per factorial treatment. Lids were firmly clamped onto the jars and 70 mls of air were removed to create slight negative pressure. The jars were then placed in a waterbath maintained at 28 °C for 30 min to allow temperature equilibration. The reaction was started by injection of 44 ml of 5% acetylene. Gas samples of 0.5 ml were withdrawn from the jars at intervals of 10, 25 and 40 min and were analysed for ethylene and acetylene using gas chromatography. Nodules were removed from the roots and their fresh weights were recorded. Nitrogenase activities were expressed as total nodule activity (TNA) on a per-plant basis and specific nodule activity (SNA) on a per-gram fresh weight of nodules basis.

7.2.4 Nitrogen measurement

After analyses of acetylene reduction by nodules, plants were divided into shoots and roots and were dried in a forced-draft oven at 80 °C for 48 h for dry weight, total nitrogen measurements. Plant material was digested using the Kjehdahl method (McKenzie and Wallace, 1954), and total nitrogen was determined using a Technicon Auto-Analyzer II with four replicates for each treatment.

7.2.5 Root Length Measurement

In the 1992 season, six plants per factorial treatment were randomly chosen at the end of stress exposure for root length measurements using a root length scanner (Comair, Australia).

7.2.6 Scanning electron microscopy

In the 1992 season, 12 days after exposure to SO_2 and salinity, leaf segments of 10 mm long were fixed in 2.5% glutaraldehyde in 0.025 M phosphate buffer, dehydrated with acetone series and then subjected to critical point drying in solid carbon dioxide. The dried specimens were coated with gold-palladium and examined in a Philips (Eindhoven, The Netherlands) 505 scanning electron microscope at 25 kV.

7.2.7 Statistical analysis

Response variables were subject to a two-way analysis of variance (ANOVA) to identify significant effects of SO_2, salinity and their interaction. Special contrasts were performed, using methodology outlined by Finn (1974), with the MANOVA procedure in the SPSS-X version 2.0 package.

7.3 RESULTS

7.3.1 The 1991 experiment

By increasing average nodule size, salt stress (6.0 dS m^{-1}) decreased the number of nodules 30 days after planting (DAP) but not nodule fresh weight. Both total nodule activity and specific nodule activity were suppressed by soil salinity over 3 successive harvests (Table 7.1, Fig. 7.1). By comparison, the effects of 45 nl l^{-1} SO_2 on root nodulation and nodule activity were varied and inconsistent over the harvest intervals. Measurements of plant total

Table 7.1 Summary of analysis of variance for 45 nl l^{-1} SO_2 and salinity effects on root nodules, nodule activities and plant growth from the 1991 experiment

Variable and Treatment	Days after planting 23	 30	 37
Nodule number per plant			
SO_2	n.s.	**	n.s.
Salinity	n.s.	**	**
SO_2 X salinity	n.s.	n.s.	n.s.
Nodule fresh weight (g plant^{-1})			
SO_2	n.s.	**	n.s.
Salinity	n.s.	n.s.	n.s.
SO_2 X salinity	n.s.	n.s.	*
Total nodule activity (nmol plant^{-1} min^{-1})			
SO_2	n.s.	**	n.s.
Salinity	*	***	***
SO_2 X salinity	n.s.	*	*
Specific nodule activity (nmol g^{-1} fresh wt min^{-1})			
SO_2	*	**	n.s.
Salinity	**	***	**
SO_2 X salinity	n.s.	n.s.	n.s.
Leaf area (cm)^2			
SO_2	n.s.	n.s.	n.s.
Salinity	n.s.	*	*
SO_2 X salinity	n.s.	n.s.	n.s.
Shoot biomass (g)			
SO_2	n.s.	n.s.	n.s.
Salinity	*	**	***
SO_2 X salinity	n.s.	n.s.	n.s.
Root biomass (g)			
SO_2	n.s	**	*
Salinity	*	n.s.	**
SO_2 X salinity	n.s.	n.s.	n.s.

n.s. = not significant; * P = 0.05; ** P = 0.01; *** P = 0.001.

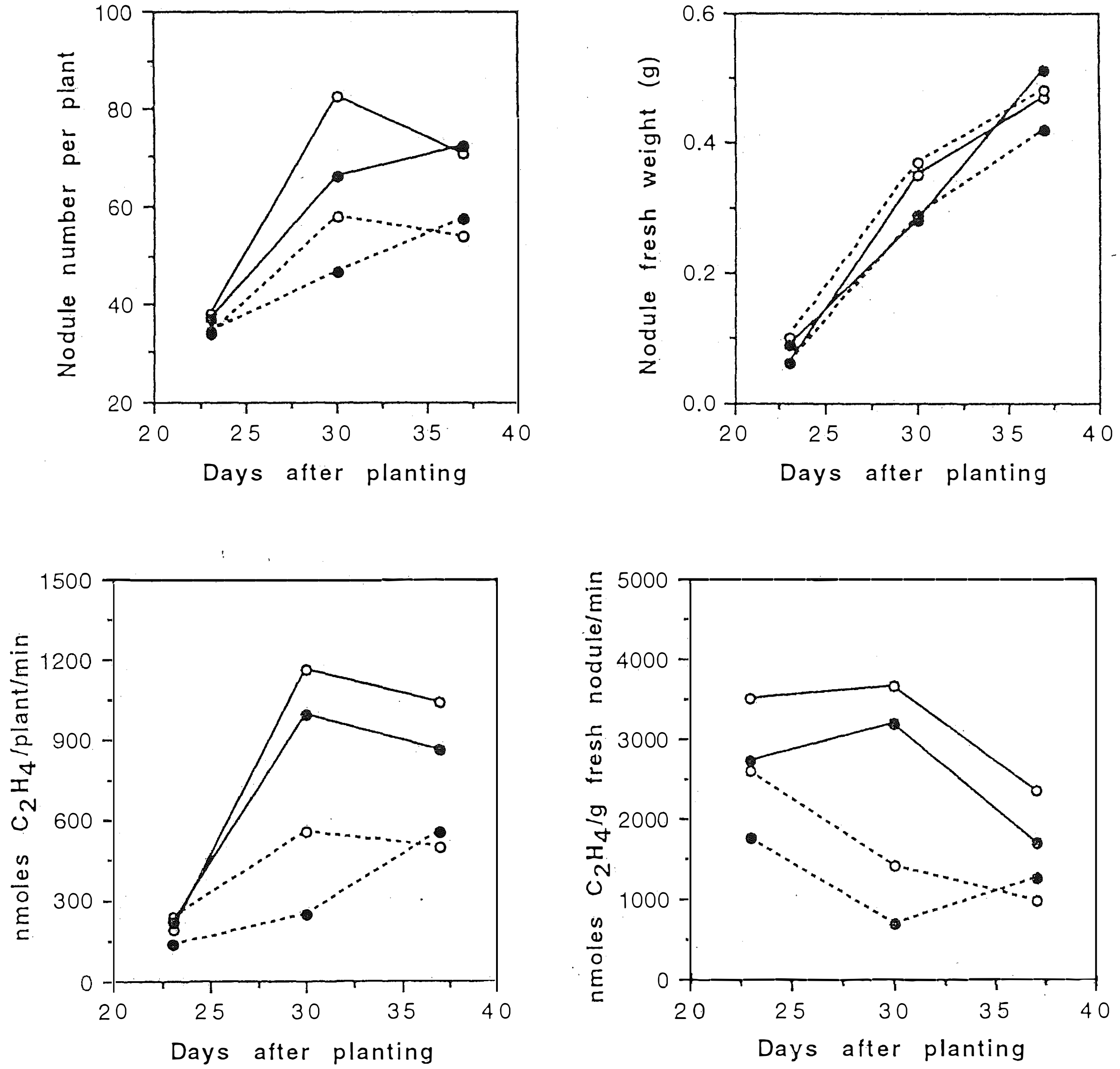


Figure 7.1 Responses of root nodules and nodule activities to low SO_2 and soil salinity over three weekly sampling intervals of the 1991 experiment.

— o —, no SO_2 + no salt; -- o --, no SO_2 + salt;

— ● —, 45 nl l^{-1} SO_2 + no salt; -- ● --, 45 nl l^{-1} SO_2 + salt.

Table 7.2 Total shoot and root nitrogen concentrations after 37 days of 45 nl l^{-1} SO_2 and salinity treatments of the 1991 experiment

ANOVA Summary		Combination treatment	
	Total shoot nitrogen (mg g^{-1})		
SO_2	n.s.	No SO_2 + No salt	39.5
Salinity	* *	No SO_2 + Salt	34.9
SO_2 X Salinity	n.s.	SO_2 + No salt	37.8
		SO_2 + Salt	34.5
	Total root nitrogen (mg g^{-1})		
SO_2	n.s.	No SO_2 + No salt	19.6
Salinity	* *	No SO_2 + Salt	14.9
SO_2 X salinity	n.s.	SO_2 + No salt	18.5
		SO_2 + Salt	14.5

n.s. = not significant; ** P = 0.01.

nitrogen on DAP 37 showed that both shoot and root nitrogen concentrations were decreased by salinity (Table 7.2).

Exposure to 45 nl l^{-1} SO_2 did not affect leaf area or shoot biomass, but it decreased root biomass on DAPs 30 and 37. In contrast, salt stress usually decreased leaf area, both shoot and root biomass through the three weekly harvests. However, SO_2-salt interactions on the growth parameters were not significant (Table 7.1, Fig. 7.2).

7.3.2 The 1992 experiment

7.3.2.1 Nodulation and nitrogenase activity

After 16 days of SO_2 exposure, nodule number was decreased by 250 nl l^{-1} SO_2 but not by 110 nl l^{-1} SO_2. Exposure to 250 nl l^{-1} SO_2 decreased nodule fresh weight, both total and specific nodule activities of the plants, whereas 110 nl l^{-1} SO_2 had no effect and even increased specific nodule activity (Table 7.3). Salt stress suppressed the root nodulations and nodule activities. The toxic effects of 250 nl l^{-1} SO_2 on nodule number and specific nodule activity were modified by soil salinity relative to that of 250 nl l^{-1} SO_2 only under nonsaline conditions.

The plants were not nitrogen-fertilized and the stress effects on the root nodulations and nodule activities impaired nitrogen resources in the plants, as both shoot and root nitrogen concentrations were significantly decreased by 250 nl l^{-1} SO_2 or salinity stress (Table 7.4). By comparison, 110 nl l^{-1} SO_2 did not induce any significant changes in plant nitrogen concentrations. Antagonistic effects of SO_2 and salinity occurred in the 250 nl l^{-1} SO_2 chambers.

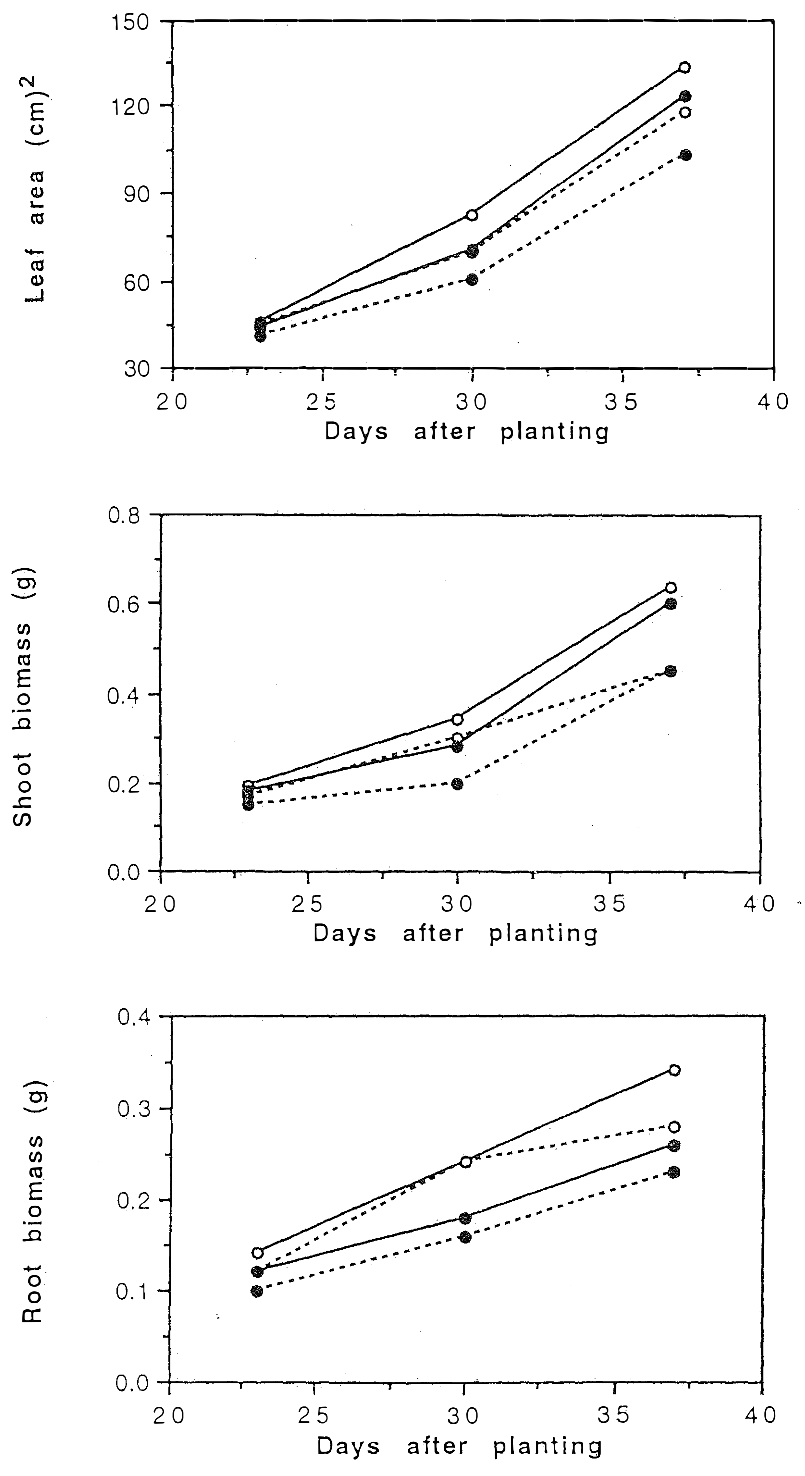


Figure 7.2 Responses of leaf area, shoot and root biomass to low SO_2 and soil salinity over three weekly sampling intervals of the 1991 experiment.

— o —, no SO_2 + no salt; -- o --, no SO_2 + salt;

— ● —, 45 nl l^{-1} SO_2 + no salt; -- ● --, 45 nl l^{-1} SO_2 + salt.

Table 7.3 Effects of SO_2, salinity and their combination on nodule number, nodule fresh weight, ethylene production expressed as total nodule activity (nmol $plant^{-1}$ min^{-1}) and specific nodule activity (nmol g^{-1} fresh wt min^{-1}) of the 1992 experiment

Source of Variation	*d.f.*	Nodule number	Nodule fresh weight (g)	Total nodule activity	Specific nodule activity
SO_2 (A)	2	* * *	* *	* * *	* *
1st contrast	1	n.s.	n.s.	n.s.	*
2nd contrast	1	* * *	* *	* * *	* *
Salinity (B)	1	* * *	* * *	* * *	* * *
A x B	2	n.s.	n.s.	n.s.	*
1st contrast	1	n.s	n.s.	n.s.	*
2nd contrast	2	*	n.s.	n.s.	*
Non SO_2, nonsaline		33.5	0.53	148.6	303.0
" saline		24.3	0.41	87.6	203.3
Low SO_2, nonsaline		29.3	0.46	146.3	317.6
" saline		21.0	0.43	93.0	206.0
High SO_2, nonsaline		23.3	0.42	114.6	238.6
" saline		19.1	0.35	70.0	199.6

n.s., not significant; * P= 0.05; ** P=0.01; *** P=0.001.

For SO_2 effects, the 1st contrast represents low SO_2 contrasted against control SO_2 and the 2nd contrast represents high SO_2 contrasted against control SO_2. For interaction effects, the 1st contrast represents the difference between control SO_2 and low SO_2 under nonsaline conditions contrasted against the difference between control SO_2 and low SO_2 under saline conditions. The 2nd contrast represents the difference between control SO_2 and high SO_2 under nonsaline conditions contrasted against the difference between control SO_2 and high SO_2 under saline conditions.

Table 7.4 Effects of SO_2, salinity and their combination on total nitrogen concentrations (mg g^{-1}) in shoots and roots from the 1992 experiment

Source of Variation	*d.f.*	Shoot	Root
SO_2 (A)	2	**	*
1st contrast	1	n.s.	n.s.
2nd contrast	1	**	*
Salinity (B)	1	***	***
A x B	2	***	n.s.
1st contrast	1	n.s.	n.s.
2nd contrast	2	***	*
Non SO_2, nonsaline		32.7	20.0
" saline		22.1	14.9
Low SO_2, nonsaline		29.0	16.9
" saline		23.8	13.6
High SO_2, nonsaline		24.6	15.6
" saline		23.6	14.7

n.s., not significant; * P=0.05; ** P=0.01; *** P=0.001.
See Table 7.3 caption for a description of the contrast terms.

7.3.2.2 Plant growth

Shoot biomass was significantly greater than controls in 110 nl l^{-1} SO_2-treated plants but not in 250 nl l^{-1} SO_2-treated plants (Table 7.5). Root biomass was only decreased by 250 nl l^{-1} SO_2. As a result, exposure to both SO_2 levels increased the ratios of shoot to root biomass. Salt stress decreased shoot growth and the shoot to root ratios. Stress interaction on the ratios was observed in the plants exposed to 110 nl l^{-1} SO_2 and salinity.

Root length was decreased by 250 nl l^{-1} SO_2 but not 110 nl l^{-1} SO_2 (Table 7.5). Salinity caused significant reductions of root length but modified the detrimental effects of 250 nl l^{-1} SO_2.

7.3.2.3 Stomatal responses

Stomatal resistance was measured 8 and 10 days after salt stress and SO_2 fumigation commenced. The results clearly indicated that soil salinity strongly induced stomatal closure in comparison to the control treatment. The combination of salt stress and 250 nl l^{-1} SO_2 caused even more closure of the stomata than other treatments on the cloudy day but not on the sunny day (Fig. 7.3), which may be due to an interaction with irradiance.

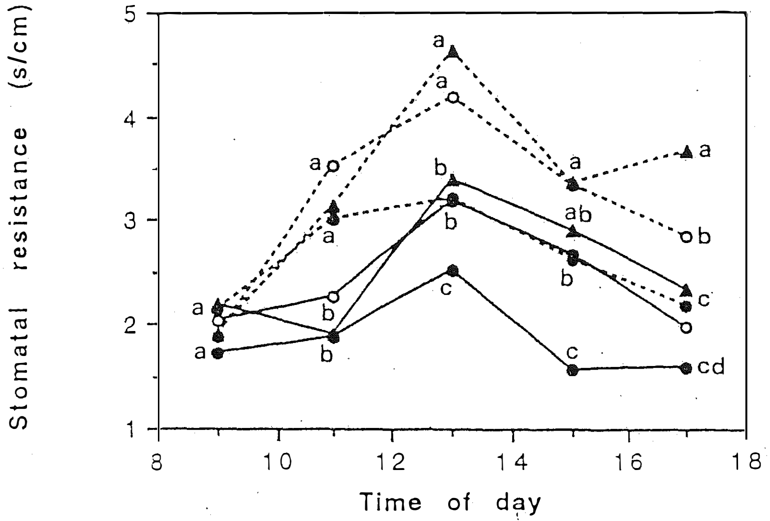
7.3.2.4 Foliar injury

Twelve days after SO_2 fumigation commenced, SO_2-caused leaf injury was apparent in the treatment of 250 nl l^{-1} SO_2 without added salt. However, leaf injury was negligible in the treatment of 250 nl l^{-1} SO_2 and salt, indicating a protective function for salinity against SO_2 injury. As shown in the scanning electron micrographs (Figs. 7.4, 7.5, 7.6 & 7.7), leaf cells had collapsed at both the lower and upper surfaces in the treatment of 250 nl l^{-1} SO_2 alone while

Table 7.5 Effects of SO_2, salinity and their combination on the growth of soybean shoots and roots from the 1992 experiment

Source of Variation	*d.f.*	Shoot dry weight (g)	Root dry weight (g)	Ratio of shoot : root	Root length (m)
SO_2 (A)	2	*	*	**	***
1st contrast	1	*	n.s.	*	n.s.
2nd contrast	1	n.s.	*	*	***
Salinity (B)	1	**	n.s.	**	***
A x B	2	n.s.	n.s.	n.s.	*
1st contrast	1	n.s.	n.s.	*	*
2nd contrast	2	n.s.	n.s	n.s.	*
Non SO_2, nonsaline		0.58	0.37	1.57	38.4
" saline		0.51	0.34	1.47	29.4
Low SO_2, nonsaline		0.73	0.37	1.93	34.6
" saline		0.49	0.31	1.56	24.2
High SO_2, nonsaline		0.51	0.29	1.73	24.7
" saline		0.44	0.26	1.65	22.1

n.s., not significant; * $P=0.05$; ** $P=0.01$; *** $P=0.001$.
See Table 7.3 caption for a description of the contrast terms.



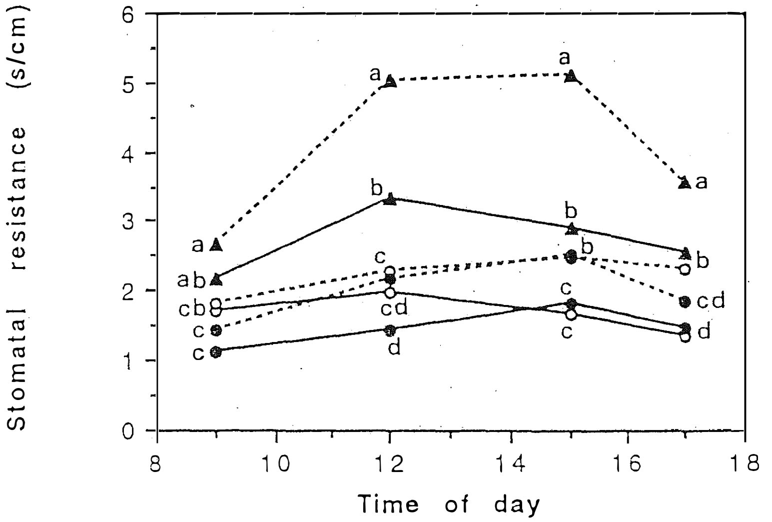


Figure 7.3 Diurnal changes of stomatal resistance in a sunny day (top) and in a cloudy day (lower) after exposure to SO_2 and salinity for 8 and 10 days, respectively, in the 1992 experiment. Within each column, means without the same letter are significantly different at the P level of 0.05 (Duncan's multiple range test).

— o —, no SO_2 + no salt; -- o --, no SO_2 + salt;

— ● —, 110 nl l^{-1} SO_2 + no salt; -- ● --, 110 nl l^{-1} SO_2 + salt.

— ▲ —, 250 nl l^{-1} SO_2 + no salt; -- ▲ --, 250 nl l^{-1} SO_2 + salt.

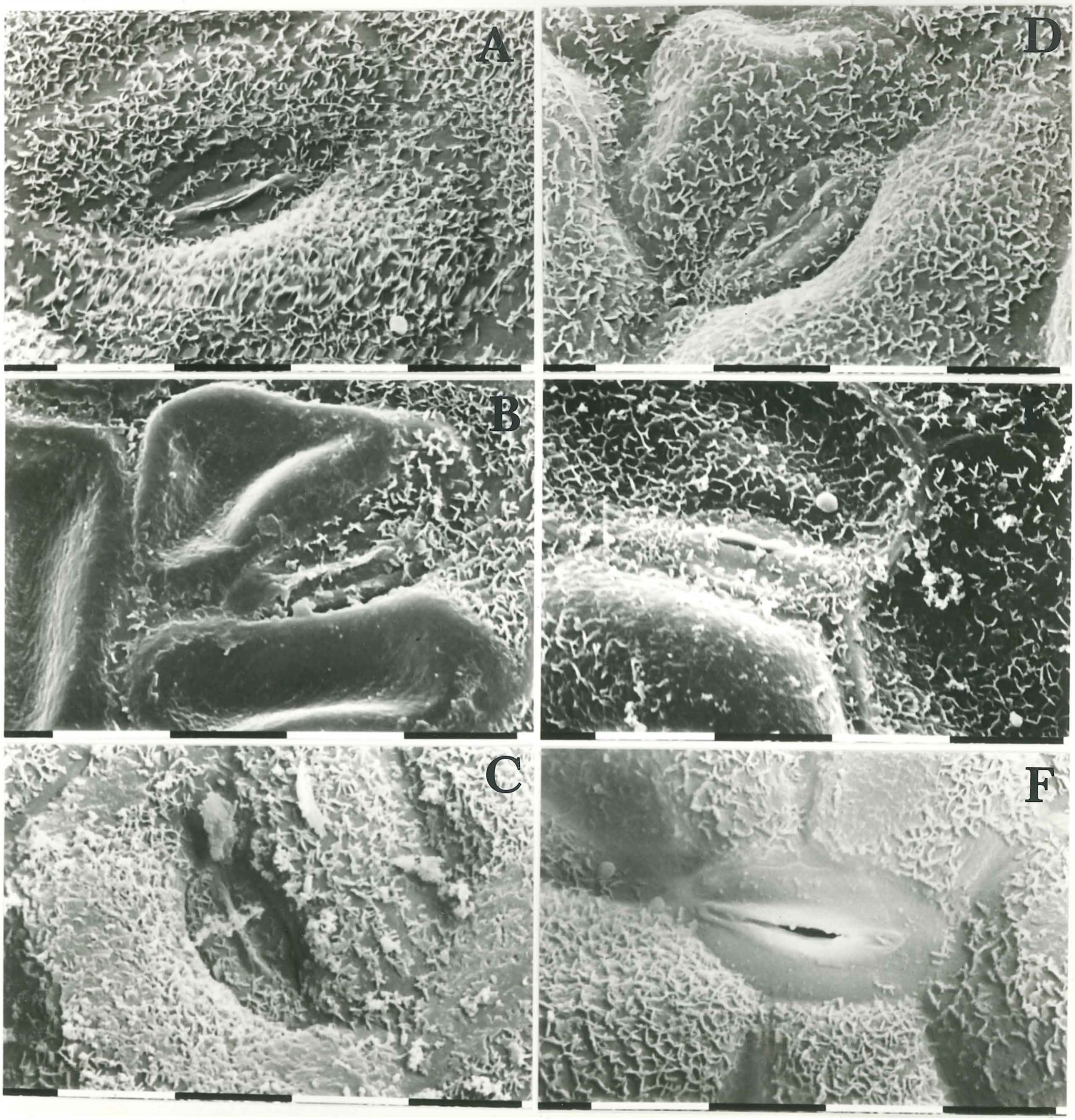


Figure 7.4 Scanning electron micrographs (X2650) are showing the interactive effects of SO_2 and salinity on foliar injury of old soybean leaves. A, B, C are for the lower surfaces and D, E, F are for the upper surfaces. Control SO_2 + nonsaline (A & D); 250 nl l^{-1} SO_2 (B & E); 250 nl l^{-1} SO_2 + salinity (C & F).

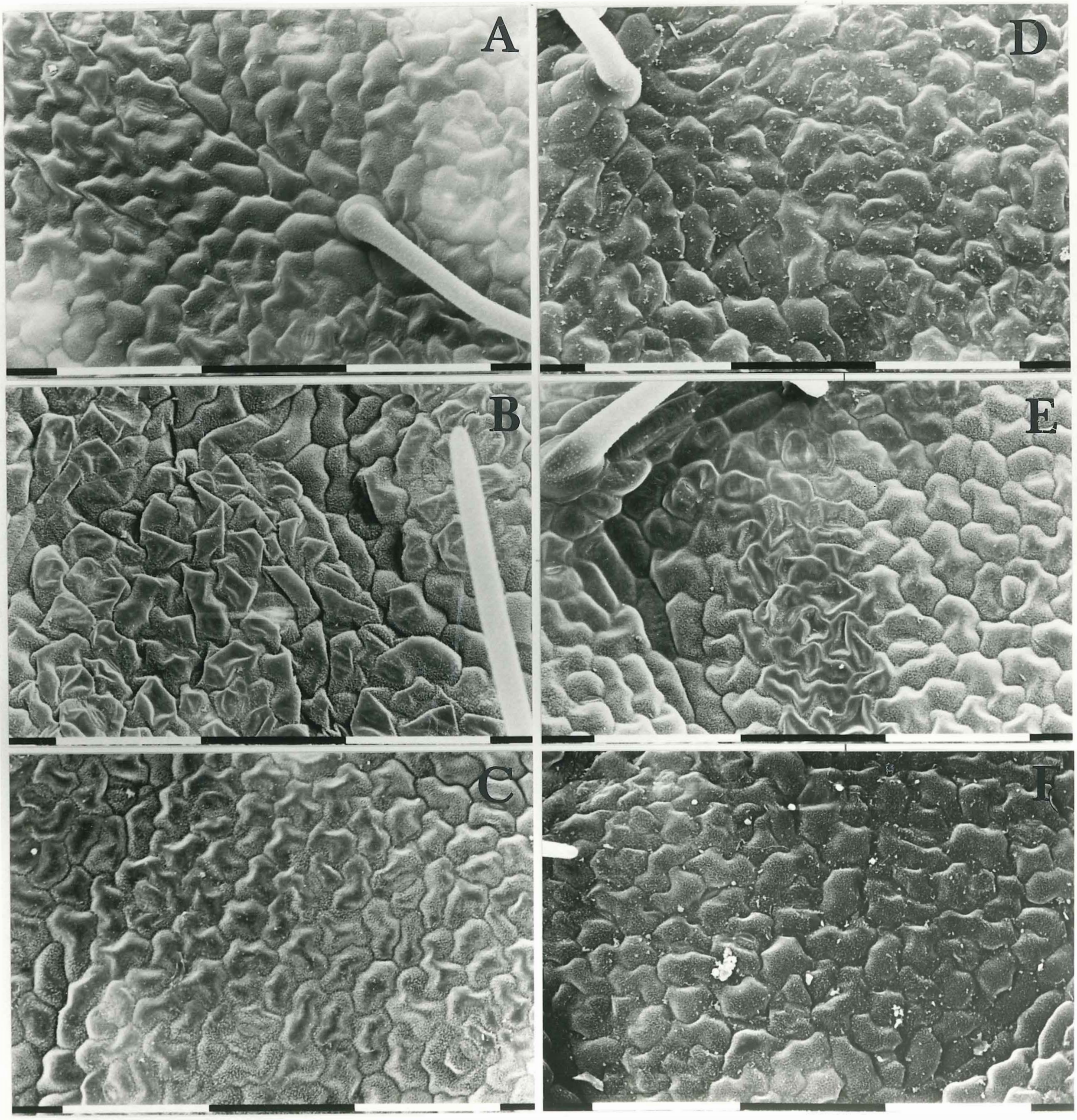


Figure 7.5 Scanning electron micrographs (X325) are showing the interactive effects of SO_2 and salinity on foliar injury of old soybean leaves. A, B, C are for the lower surfaces and D, E, F are for the upper surfaces. Control SO_2 + nonsaline (A & D); 250 nl l^{-1} SO_2 (B & E); 250 nl l^{-1} SO_2 + salinity (C & F).

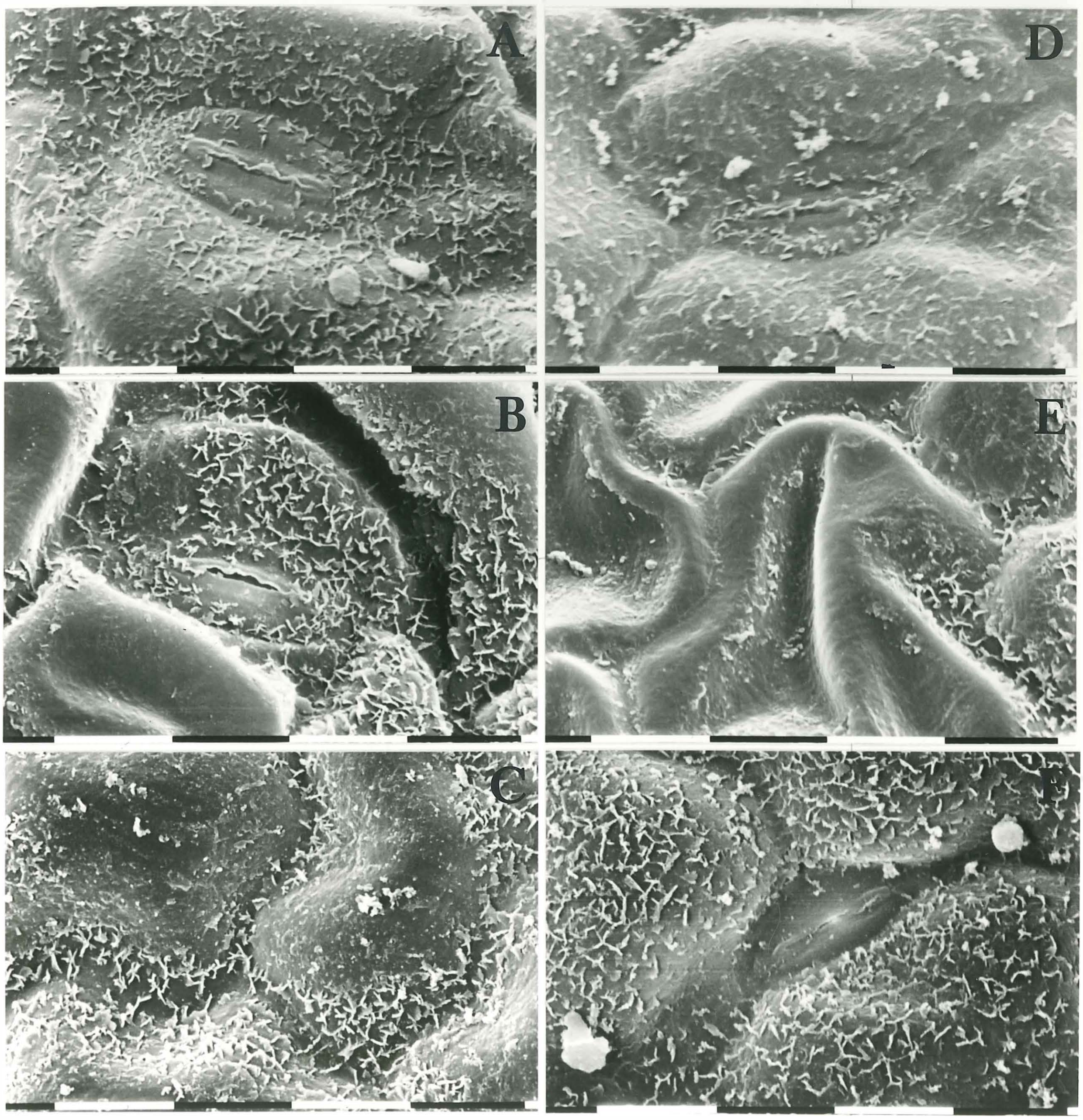


Figure 7.6 Scanning electron micrographs (X2650) are showing the interactive effects of SO_2 and salinity on foliar injury of young soybean leaves. A, B, C are for the lower surfaces and D, E, F are for the upper surfaces. Control SO_2 + nonsaline (A & D); 250 nl l^{-1} SO_2 (B & E); 250 nl l^{-1} SO_2 + salinity (C & F).

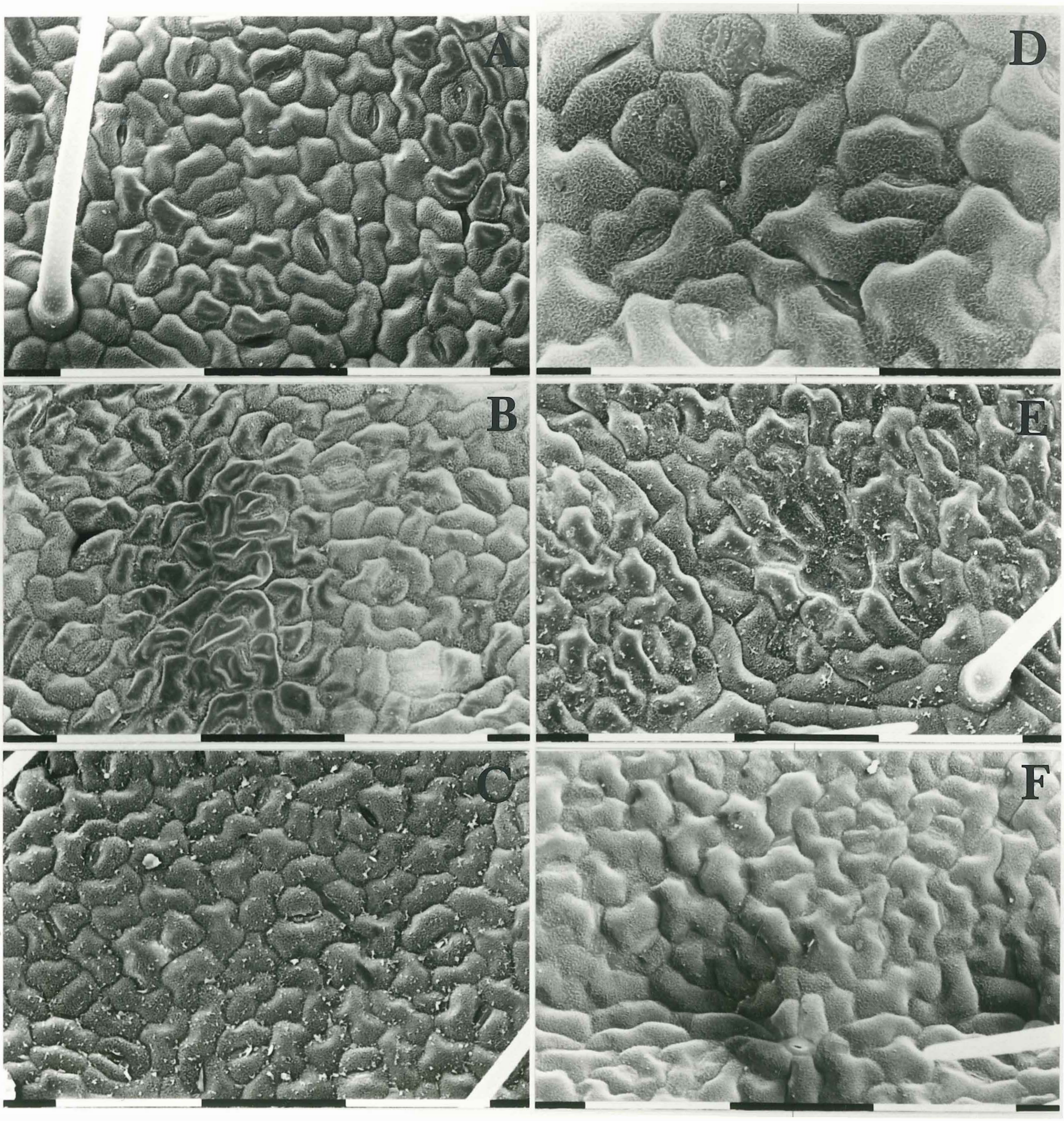


Figure 7.7 Scanning electron micrographs (X325) are showing the interactive effects of SO_2 and salinity on foliar injury of young soybean leaves. A, B, C are for the lower surfaces and D, E, F are for the upper surfaces. Control SO_2 + nonsaline (A & D); 250 nl l^{-1} SO_2 (B & E); 250 nl l^{-1} SO_2 + salinity (C & F).

leaf cells in the treatment of 250 nl l^{-1} SO_2 with added salt were showing a normal morphology. Neither 110 nl l^{-1} SO_2 nor soil salinity caused any foliar injury.

7.4 DISCUSSION

The salinity level used in both study seasons was quite moderate. It mainly aimed to induce an osmotic stress in plants which may cause stomatal closure to test if SO_2 injury would be ameliorated through decreased uptake of the pollutant. Evidence in support of this hypothesis was provided by measurement of stomatal resistance and scanning electron microscopy. Soil salinity significantly increased stomatal resistance of the leaves. Twelve days after exposure to SO_2, leaf cells had collapsed at both the lower and upper surfaces in the treatment of 250 nl l^{-1} SO_2 alone, but leaf cells in the treatments of 250 nl l^{-1} SO_2 plus salt were well protected. Stomatal closure induced by 250 nl l^{-1} SO_2 appeared to be attributable to the collapse of guard cells. This finding agrees with the results discussed in chapter five, that SO_2-induced leaf injury was more severe in the nonsaline plants than in the saline plants, with leaf sulphur concentrations significantly decreased by salinity stress.

The antagonistic effects of salinity against 250 nl l^{-1} SO_2 was also observed in root length, nodule number, specific nodule activity and plant nitrogen concentrations. However, soil salinity and SO_2 did not significantly interact on the shoot and root dry weight. This is probably because biomass production is usually the last parameter to respond to the environmental stresses following physiological and biochemical processes. Under field conditions this interaction may become even more complicated due to interactions with other environmental factors. Olszyk *et al.* (1988) also found no overall interaction between ozone and salinity on alfalfa growth and yield in a field study.

In the 1991 season, salt stress decreased the number of nodules per plant but not nodule fresh weight by increasing average nodule size. This was probably because fewer nodules were competing for photosynthates. Similar findings were reported in several previous studies on chickpea and field bean (Balasubramanian and Sinha, 1976; Yousef and Sprent, 1983; Elsheikh and Wood, 1990). Increased size of nodules in salt-stressed plants may partially compensate for reduced specific nitrogenase activity (Yousef and Sprent, 1983). However, such compensation was not observed in this experiment, which showed that total and specific nitrogenase activities were significantly suppressed by the salinity treatment over the sampling intervals. This may indicate that the salt stress had reduced the number of N-fixation-active nodules. Plant nitrogen measurements on DAP 37 showed that both shoot and root nitrogen concentrations were decreased by salinity.

Air pollutants can alter the pattern of assimilate allocation favouring shoot growth at the expense of root growth (Whitmore *et al.*, 1982; McLaughlin and McConathy, 1983; Darrall, 1989). As a result, ratios of shoot to root biomass can be increased (Freer-Smith, 1985; Murray, 1985*a*; Qifu and Murray, 1991*a*). Similarly, in the presence of 40 mmol NaCl, the degree of carbon partitioning towards the root system was inhibited by 39% (Jeschke *et al.*, 1992). Therefore, a combination of SO_2 and salt stress can be harmful to root growth. However, this study did not show significant interactions of SO_2 and salinity on root biomass. A stress interaction on the shoot to root ratios was possibly due to the stimulatory effect of 110 nl l^{-1} SO_2 and the inhibitive effect of 27 mmol NaCl on shoot growth. Although 110 nl l^{-1} SO_2 did not reduce root length, 250 nl l^{-1} SO_2 had a substantial effect. Decrease in root length may have more significant impacts than root biomass on the uptake of water and nutrients by plants, and consequently polluted plants may impair their resistance to other environmental stresses such as drought, salt and nutrient deficiency.

The SO_2-induced increases in the shoot to root ratios could partially account for SO_2-induced decreases of root nodules and nodule activities in the 250 nl l^{-1} SO_2 chambers. Blum and Tingey (1977) demonstrated that when plant tops were exposed to ozone, root growth and nodule number were reduced, but when ozone was excluded from the plant foliage, no effect on the roots and nodules were observed. It is likely that air pollution stress could influence root nodulation and N_2 fixation via alterations in metabolic processes in the shoots, and one of the major possibilities is less photosynthate being available for translocation to roots and nodules in the polluted plants (Jones *et al.*, 1985).

The effects of SO_2 on root nodulation may, in turn, affect the shoot growth by impairing the supply of nitrogen compounds from the roots. In this study, plants were not nitrogen-fertilized and nitrogen supply would totally depend upon N_2 fixing activity in the nodules after the cotyledon reserves were used up. The results showed that exposure to 250 nl l^{-1} SO_2 substantially reduced both shoot and root nitrogen concentrations. Although 250 nl l^{-1} SO_2

caused growth reduction of roots, it did not significantly suppress shoot growth. This might imply that SO_2-induced nitrogen reduction took longer to affect shoot growth than root growth.

CHAPTER EIGHT

General Discussion

Like other environmental stresses (*e.g.* drought, salt, chill, *etc.*), SO_2 may induce a direct strain beyond the elastic limit (or homeostatic safety margin) of the plant and therefore a direct plastic strain which produces an acute injury. Such injury is due to the rapid absorption of SO_2 and usually occurs when the plant is exposed to high SO_2 concentrations for short periods (Kozlowski and Mudd, 1975; Ayazloo and Bell, 1981; also see Chapter Seven). SO_2 may also induce an elastic strain which is reversible and, therefore, not injurious of itself. If maintained for a long enough time, however, this strain may give rise to an indirect plastic (irreversible) strain which results in reductions in growth, yield and quality of the plant. This usually occurs when the plant is exposed to low SO_2 concentrations. For example, the inhibition of net photosynthesis in response to short-term fumigation with SO_2 has often been found to be reversible and not accompanied by visible injury (Muller *et al.*, 1979; Hallgren, 1984; Darrall, 1986, 1989). The response of stomata to SO_2 is also reversible in the case of short exposure (Majernik and Mansfield, 1970; Unsworth *et al.*, 1972; Black and Unsworth, 1980).

SO_2 may also injure a plant, not by the strain it produces, but by predisposing the plant to a second stress. SO_2-induced assimilate allocation favouring shoot growth at the expense of root growth (Jones and Mansfield, 1982; McLaughlin and McConathy, 1983; Taylor *et al.*, 1986), may have negative consequences in the event of severe shortage of soil moisture and nutrients since the transpirational surface is increased while the absorptive surface of the roots may become limiting. A number of studies have shown that prior exposure to SO_2 may not be injurious, but it amplifies the effect of drought stress on photosynthetic capacity, protein concentration, enzyme activity and even plant survival in *Picea abies* plants, (Cornic, 1987; Pierre and Queiroz, 1988; Macrez and Hubac, 1988; Dotzler and Schutt, 1990).

Obviously, SO_2 exposure conditions (concentration, duration and pattern) play an important role in determining the characteristics of SO_2-induced injury. However, the expression of effects of SO_2 on plants also depends to a large extent upon other environmental factors such as light intensity and air temperature (Jones and Mansfield, 1982), relative humidity (McLaughlin and Taylor, 1981), soil moisture and wind speed (Black, 1985; Roberts, 1989). When plants encounter both SO_2 and other environmental stresses, stress interactions may induce a change in the threshold doses of SO_2 probably through avoidance mechanisms by modulating stomatal aperture and thus SO_2 uptake. Therefore, it is of vital importance to consider the potential interactions of gaseous air pollutants and other environmental stresses when establishing air quality criteria.

This thesis aims to examine the effects of soil moisture and salt (NaCl) stresses on crop responses to SO_2 exposure, as water stress and soil salinity are the most common and important environmental stresses in the arid and semi-arid regions. The major findings of my research are that SO_2-induced foliar injury was more severe in the well-watered plants than in the water-stressed plants of potatoes (Figs. 4.1a and 4.1c). Similarly, Na salinity also ameliorated the toxic effects of SO_2 showing more severe SO_2 injury in the nonsaline plants than in the saline plants of soybeans (Figs. 7.5, 7.6, 7.7 and

7.8). This was probably because both water stress and saline conditions induced stomatal closure which caused less SO_2 uptake. Measurements of foliar total sulphur showed that the water-stressed potato plants or salt-treated soybean plants had much lower leaf sulphur accumulation than did the well-watered or nonsaline plants at the same SO_2 fumigation levels. Previous studies also found that plant injury by SO_2 was highly correlated with percentage of soil moisture, and both the injury and soil moisture were highly correlated with stomatal conductance and plant water potential in pinto bean and poinsettia (Davids *et al.*, 1981; Krizek *et al.*, 1986). Rapid stomatal closure upon exposure to O_3 under water-stressed conditions protected sensitive plant material from injury (Rich and Turner, 1972). Studies on salinity-pollutant interactions on plants also showed that soil salinity decreased the injurious effects of O_3 in alfalfa, pinto bean and garden beet grown under controlled conditions (Maas *et al.*, 1973; Ogata and Maas, 1973; Hoffman *et al.*, 1975).

Figure 8.1 summarizes plant responses to simultaneous exposure to SO_2 and soil salinity (or water stress), or to sequential exposure with first salinity and then SO_2 under field conditions. As in the case of simultaneous exposure to salt and SO_2, pretreatment with low salinity (27 mM NaCl) also ameliorated the adverse effect of SO_2 on plants. However, high salinity (48 mM NaCl) treated plants were severely injured by subsequent SO_2 exposure especially high SO_2 (300 nl l^{-1}). The different predisposition characteristics between the low and high salinities was probably related to the nature of the salt-induced strains. The low salinity may produce a moderate strain which is quite easily reversible, but salt-induced osmotic

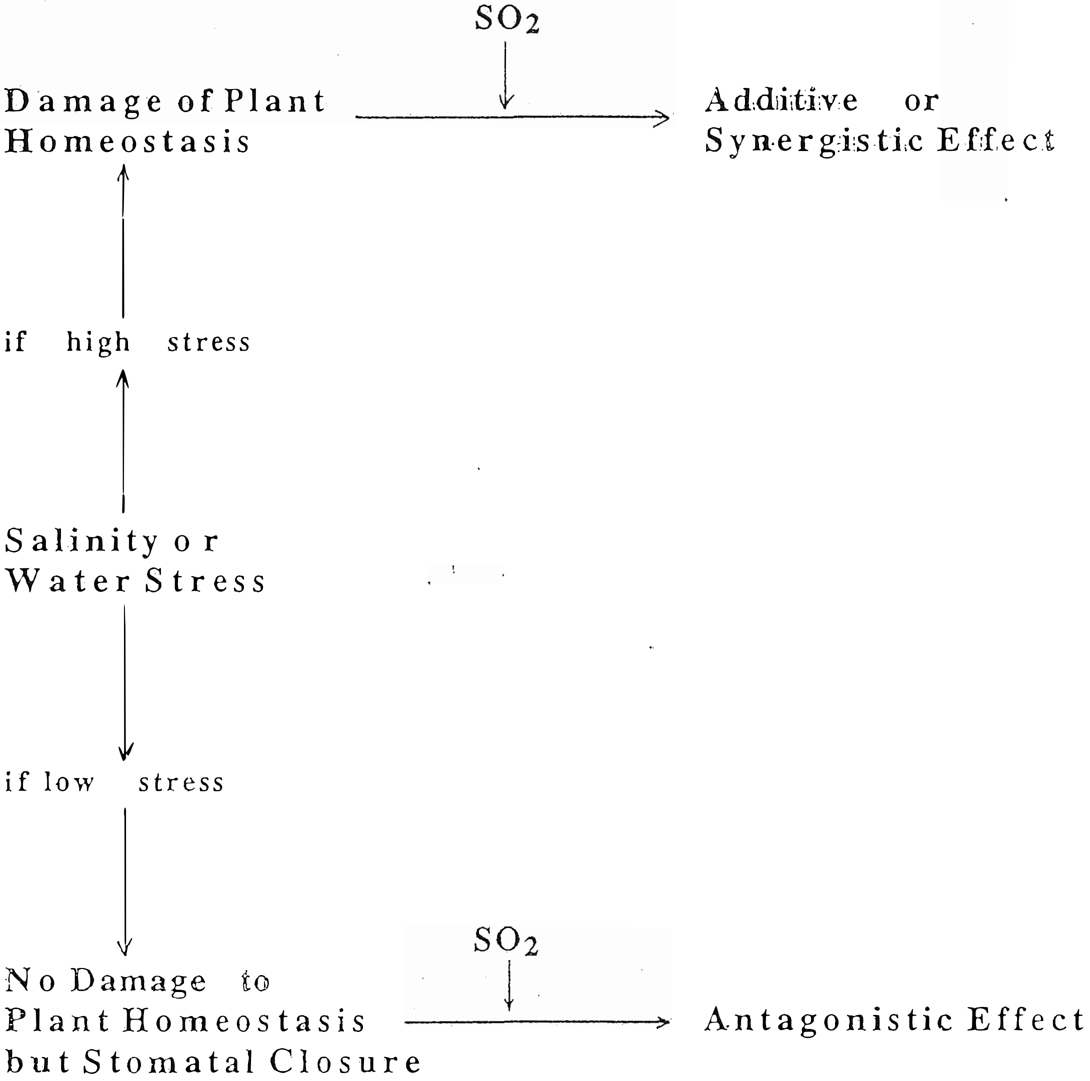


Fig. 8.1 Conceptual model of plant responses to simultaneous or sequential exposure to SO_2 and soil salinity or water stress

stress may contribute to the protective function against SO_2 through stomatal mechanisms as stomatal closure of salt-treated plants was apparent during the episode of SO_2 exposure. By comparison, pretreatment with high salinity, despite also inducing high stomatal resistance, may produce a severe strain which was beyond the elastic limit of the plant and eventually destroyed plant homeostatic balance (the ability of repairing and compensating for SO_2 perturbation) due to direct injury of high concentrations of ions. Consequently, the high salt-treated plants became very susceptible to SO_2 injury even in the case of fractional SO_2 uptake through stomata and cuticles. In short, the compensatory mechanisms and predisposition characteristics of salinity and SO_2 may depend upon the stress levels used. The interactive effects are usually less than additive if the stress levels are moderate, but usually greater than additive if the stress levels are high.

Plants pretreated with SO_2 were more severely injured by high salt stress than the controls. This is probably because SO_2 can weaken plants both physiologically and biochemically in the absence of visible injury (Hallgren, 1984; Heath, 1984; Darrall, 1989). SO_2-induced increase in the shoot : root ratios may cause physiological imbalance between the aboveground and belowground components of the plant (Freer-Smith, 1985; Murray, 1985; Qifu and Murray, 1991*a;* also see Chapter Six). A SO_2-induced decrease in root length (see Chapter Seven) may have significant effects on plant uptake of water and nutrients. Interestingly, Wright *et al.* (1986) found that reduced root mass in the pollutant plus water stress treatment was better able to extract water from drying soil. This feature may be associated with an increased rate of water loss from the leaves following fumigation. Mansfield *et al.* (1988) reported that there are two major ways in which plant water relations can be influenced by pollutants: a) alterations to root growth and function; b) effects on leaf water loss via the stomata or cuticle. Pollutant-induced damage to the cells in the epidermal layer, leading to malfuctioning of stomata, is mainly responsible for the reduced efficiency in water use (Neighbour *et al.*, 1988; Lucas, 1990).

Exposures to SO_2 often inhibit net photosynthesis and increase dark respiration (Reviewed by Darrall, 1989), resulting in losses of carbohydrates which are utilized in plant growth, maintenance and repair. SO_2 is also known to affect membrane permeability (Lendzian, 1984; Mudd *et al.*, 1984), cause ultrastructural injury such as chloroplast damage (Huttunen and Soikkeli, 1984), induce changes in amino acid pools and decline of free fatty acids (Heath, 1984), and modify cellular buffering capacity (Nieboer *et al.*, 1984). The SO_2-induced changes in plant physiology and metabolisms may decrease the homeostatic safety margin and consequently reduce the ability of plants to tolerate the detrimental effects of other environmental stresses such as drought, salinity and chill. During recent years, several hypotheses have been put forward to explain the rapidly increasing forest dieback observed in industrialized regions in Europe and North America. Among the potential causes involved, air pollution has received particular attention. It is widely believed that air pollutants play a significant role in forest decline (McLaughlin, 1985; Schutt and Cowling, 1985; Krause *et al.*, 1986; Dotzler and Schutt, 1990).

SO_2 exposure increased the shoot to root ratios in both potato and soybean crops, whereas soil moisture stress had the opposite effect (see Chapter Four). Increases in the root sink strength relative to the shoot induced by water stress could effectively counteract the impact of SO_2 on the partitioning of assimilates among plant organs which favours shoots, and helps maintain physiological balance between the aboveground and belowground components of the plant. Exposure of potato plants to 300 nl l^{-1} SO_2 under well-watered conditions induced an increase in the shoot to root (including tuber) ratios early in the growing season. In contrast, water stress decreased the ratios in the control and 110 nl l^{-1} SO_2 treatments, but not at 300 nl l^{-1} SO_2 indicating that the high SO_2 had disrupted this acclimatory response to soil moisture stress. Mansfield *et al.* (1986) fumigated *Poa pratensis* with SO_2 and NO_2, and found that changes in the shoot to root ratio induced by the pollutants were dependent upon the time of year that the treatment was applied. During the winter months of January and February, a significantly greater proportion of the plant weight was found in the shoots of fumigated material than in the controls. However, this was not apparent later in the year during the same fumigation.

Changes in carbon allocation among plant organs may also be an important response to other environmental stresses. As in the case of soil water stress, plants grown in an environment of suboptimal nutrient supply may alter the internal distribution of carbon, often favouring roots at the expense of shoots (Chapin *et al.*, 1987). Nutrient stress commonly results in greater allocation to fine root production in crop and tree species (Clarkson and Hanson, 1980; Cannell, 1986). Increases in root growth relative to shoot growth induced by nutrient deficiency could preclude an increased carbon allocation to shoots often observed in response to air pollution stress (Tjoelker and Luxmoore, 1991). Salt stress is another important edaphic factor inducing a physiological condition with similarities to water stress. A number of studies have found that under saline conditions root growth is almost always less affected than shoot growth, and at low salinity, root growth may not decrease at all while shoot growth declines (Delane *et al.*, 1982), or it may even increase (Ackerson and Youngner, 1975; Dudeck *et al.*, 1983; Weimberg *et al.*, 1984). The present studies showed that salt stress impaired both shoot and root growth, and in some experiments (*e.g.* Chapter Six) shoot growth was more sensitive to salt stress than roots, but did not induce any significant changes in the biomass ratios. These data may indicate that soil salinity cannot effectively counteract SO_2-induced effects on carbon allocation among plant organs.

Exposure to 250 nl l^{-1} SO_2 decreased the number and weight of root nodules, suppressed nodule nitrogenase activity. Consequently, both shoot and root nitrogen concentrations were reduced. The SO_2-induced effects on root nodulation may be due to the SO_2-induced increase in shoot to root ratios probably as a result of the inhibitory effects of SO_2 on leaf photosynthesis and thus less assimilate being available for translocation to root tissues. This reduction in translocation of photosynthate would decrease the energy available for growth, nodule formation, and the carbon skeletons needed for N fixation. In combination with low salinity, however, the adverse effects of SO_2 on nodule number, specific nodule activity and plant nitrogen concentrations were alleviated relative to that of SO_2 alone.

This stress interaction can be partly explained by the fact that soil salinity induced stomatal closure and thus it decreased SO_2 uptake.

The effects of SO_2 on root nodulation may consequently affect plant growth by impairing nitrogen resources. In addition, N-deficiency together with S-enrichment due to SO_2 uptake may unbalance the N:S ratio and affect amino acid metabolism and protein synthesis. As discussed in Chapter Seven, N-deficiency induced by 250 nl l^{-1} SO_2 took longer to influence shoot growth than root growth. But this was a short experiment, and if the growing season had been prolonged, the decrease in shoot growth was likely to have become significant. Moreover, SO_2-induced reduction of root growth especially root length may be another important factor affecting nutrient uptake by plants because the absorptive surface is limited. Apart from the effects of SO_2 on plant nutrition by either reducing N-fixation activity or absorptive surface, SO_2 is also known to affect plant acclimation to nutrient deficiency. For example, SO_2 exposure caused significant changes in the nitrogen balance of radish plants, and inhibited their ability to respond to decreasing nitrate availability, even though the nitrate concentrations used did not profoundly limit radish growth (Coleman *et al.*, 1989; Koch *et al.*, 1989).

The interactive effects of SO_2 and salinity or SO_2 and water stress on foliar injury and some physiological and biochemical processes may result in similar stress interactions on plant growth. SO_2 dose response relationships (nl l^{-1} h) were established for both drought and saline conditions (Figs. 8.2, 8.3, 8.4). As shown in Figure 8.2, percentage changes in the dry weight of potato plants had a sharp inverse linear relationship with SO_2 dose under the well-watered

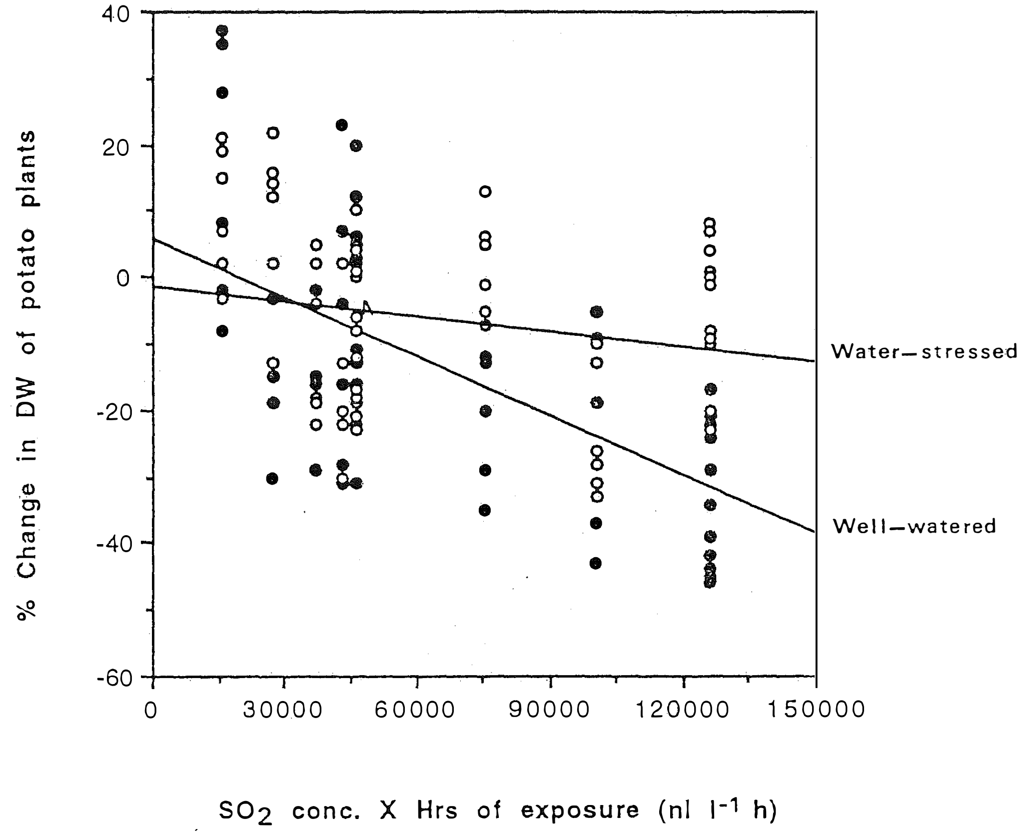


Figure 8.2 The effects of SO_2 concentration, taking into account the total hours of exposure, on the dry weight of potato plants. Solid circles denote well-watered plants, and open circles denote water-stressed plants.

For well-watered plants, $Y = 5.92 - 2.99e^{-4}X$, $r^2 = 0.38$, $P = 0.001$;

For water-stressed plants, $Y = -1.56 - 7.34e^{-5}X$, $r^2 = 0.04$, $P = 0.09$.

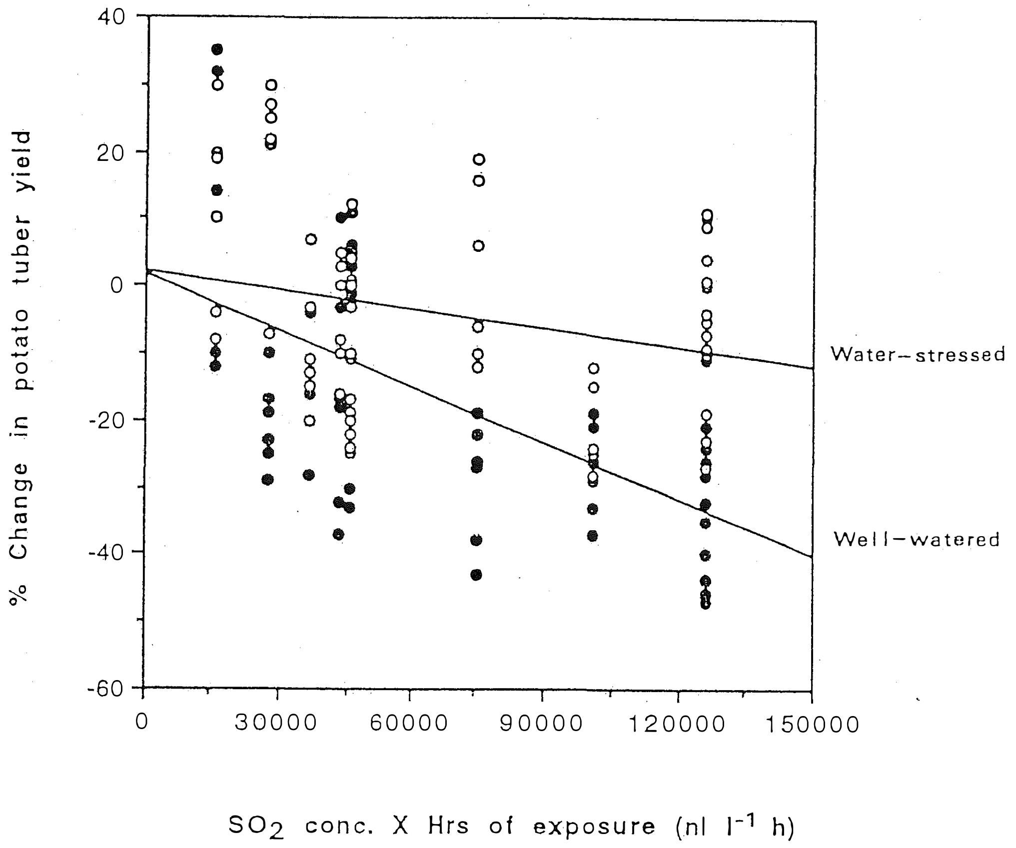


Figure 8.3 The effects of SO_2 concentration, taking into account the total hours of exposure, on potato tuber yield. Solid circles denote well-watered plants, and open circles denote water-stressed plants.
For well-watered plants, Y= 1.66 -2.81e^{-4}X, r^2= 0.36, P= 0.01
For water-stressed plants, Y= 2.26 -9.52e^{-5}X, r^2= 0.06, P= 0.10

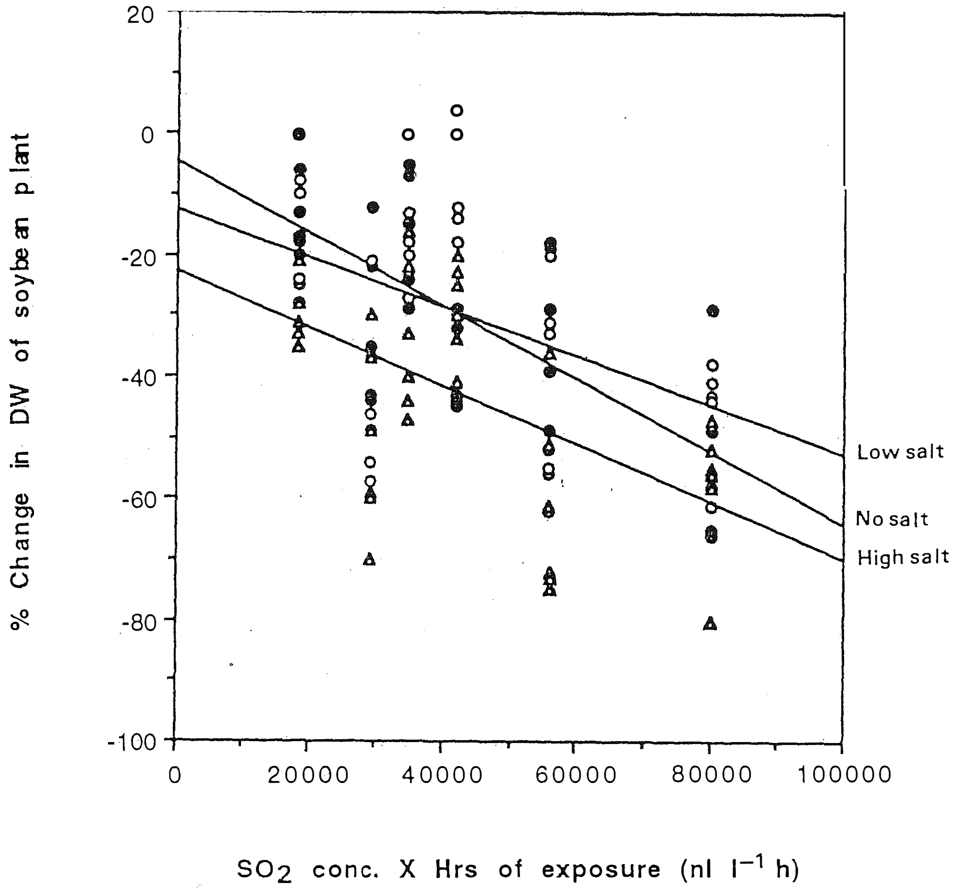


Figure 8.4 The effects of SO_2 concentration, taking into account the total hours of exposure, on the dry weight of soybean plants.
• - no salt; o - low salt; Δ - high salt.
For nonsaline plants, $Y = -4.68 - 5.97e^{-4}X$, $r^2 = 0.49$, $P = 0.001$;
For low salt treated plants, $Y = -12.85 - 3.96e^{-4}X$, $r^2 = 0.20$, $P = 0.01$;
For high salt treated plants, $Y = -22.91 - 4.74e^{-4}X$, $r^2 = 0.29$, $P = 0.01$.

conditions. By comparison, this inverse relation was largely modified under the water-stressed conditions, *i.e.* the adverse effects of SO_2 on plant growth were alleviated. Similar results were observed for potato tuber yield (Fig. 8.3). It also appeared that SO_2-salinity interactions affected the growth of soybean plants in terms of SO_2 dose response relationships (Fig. 8.4), showing some protective function from soil salinity. However, this protection was influenced by the levels of salinity used, *i.e.* low salinity provided more protection than did high salinity. Nevertheless, the R^2 values of the regressions of percentage growth change against SO_2 dose under both drought and saline conditions were usually low, due to the variations of plant response.

One of two previous studies on O_3 X water interactions on cotton under field conditions showed that during the typically hot, dry growing season in the Central Valley of California in 1981, O_3 significantly reduced cotton yield of the irrigated plants but not that of the water-stressed plants. In 1982, the weather was cooler and cloudier than normal, and cotton yields at both levels of soil moisture treatments responded similarly to O_3 (Temple *et al.*, 1985*b*). In contrast, another study conducted in Raleigh in 1985 found that there were no O_3 X water interactions on the growth variables measured (Miller *et al.*, 1988). A field study also showed no overall interaction between O_3 and salinity on alfalfa growth and yield (Olszyk *et al.*, 1988). The general lack of significant stress interactions on growth in the previous and present studies may reflect that biomass production is usually the last parameter and therefore probably less sensitive to respond to the environmental stresses following physiological and biochemical processes. In the field, climatic factors such as light, temperature and humidity may also interfere with the response of plants to SO_2, soil salinity or water stress singly and in combination. Therefore, the interactive effects of an air pollutant and other stresses on plant growth are probably less conclusive than the metabolic changes.

Since gaseous air pollutants enter the leaf mesophyll mainly by diffusing through stomata, it is widely considered and also experimentally verified that environmental factors that can modulate stomatal aperture can also modulate the effect of a gaseous pollutant (Khatamian *et al.*, 1973; Davids *et al.*, 1981; McLaughlin and Taylor, 1981; Jones and Mansfield, 1982; Tingey and Hogsett, 1985; Krizek *et al.*, 1986). This is basically true in my experiments. However, whether the stomatal mechanisms are the only explanation is still a question. Some previous studies have reported that salinity and water stress increase sugar contents in certain plants (Bernstein and Hayward, 1958; Levitt, 1980), and that high sugar levels are associated with increased resistance to O_3 injury (Dugger and Ting, 1970). Also as discussed above, high salinity pretreatment, despite inducing stomatal closure, caused the plants to be very vulnerable to SO_2 injury. These data suggest that other biochemical and physiological mechanisms may also be involved in the interactive effects of air pollutants and other environmental stresses. For instance, although both water stress and salinity can modulate stomatal aperture and thus pollutant uptake, there must be some different metabolic processes involved. Plant acclimation to salinity stress involves a greater cost in terms of both energy and organic solutes than does drought-induced osmotic adjustment (McCree, 1986). This will be no doubt more significant if plants are introduced to a high salt environment. Therefore, not only stomatal but metabolic mechanisms deserve further study on the interactive effects of gaseous air pollutants and other environmental stresses.

The weather in Western Australia is wet in the winter season when the potato experiment was conducted, but it is hot and dry in the summer season when the soybean experiments were conducted. The climate characteristics inside the fumigation chambers were similar to those outside the chambers in terms of light, temperature and relative humidity (Appendix 3). This may suggest that the results of SO_2 x water or SO_2 x salinity interactions from this project can be extrapolated to field conditions and make contributions to establish regional air quality criteria, *e.g.* in the Mediterranean regions which have similar climates to Western Australia. Moreover, it was found that growing seasons modified plant response to abiotic stresses. For instance, the effects of salinity on root nodulation and nodule activity were amplified in a mid-summer experiment compared with a late summer experiment (see Chapter Seven). The effects of SO_2 and SO_2-salinity interactions on soybean growth were also greater in mid-summer than in late summer (see Chapter Six), probably due to the influence of air temperature in regulating stomatal movements and thus SO_2 uptake. This project mainly aimed to study the effects of SO_2 and edaphic stresses on plant growth, development, yield and visual injury, and put less emphasis on physiological and biochemical changes. It is important that future research should be based on an integrated approach in which measurements of plant yield, growth and development and metabolic changes are complemented by studies of interactions between abiotic factors that impose stress on plants. When studying the effects of low concentrations of air pollutants on plants over prolonged periods, the most sensitive and informative methods should be used. Among these methods, electron microscopy, biochemical investigations of lipid and enzymatic metabolism are the most useful. Therefore, we can know how sensitive plant metabolism responds to abiotic stresses and whether any metabolic changes cause reductions of plant growth and yield production.

In conclusion of the findings of this project (referring to those hypotheses discussed in Chapter Two), **1**) soil moisture stress reduced the adverse effect of SO_2 on foliar injury, but this interaction on plant growth and yield usually showed variations over the growing season; **2**) greater proportional root growth in response to soil moisture stress precluded an increase in carbon allocation favouring shoot growth in response to 110 nl l^{-1} SO_2, however, 300 nl l^{-1} SO_2 disrupted the acclimatory response to water stress; **3**) similar to water stress, soil salinity provided a protective function against SO_2 injury by inducing stomatal closure and thus decreased SO_2 uptake; **4**) in sequential exposure, pretreatment with low salinity also alleviated the injurious effects of subsequent SO_2 exposure, in contrast, pretreatment with high salinity or SO_2 usually caused the plants to become more susceptible to subsequent stresses; **5**) low salinity modified the effects of SO_2 on root growth, nodule formation, specific nodule activity and plant nitrogen concentrations. The SO_2-induced nitrogen reduction took longer to affect shoot growth than root growth.

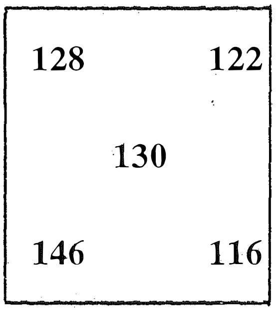
Appendix 1

Tests of SO_2 Distribution Within Fumigation Chambers (Average SO_2 concentrations, nl l^{-1}, from 1200-1600h)

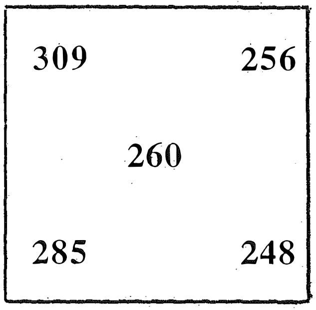
Test Date

21/7/89

Chamber 1

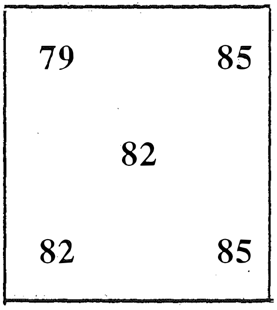


Chamber 2



24/7/89

Chamber 3



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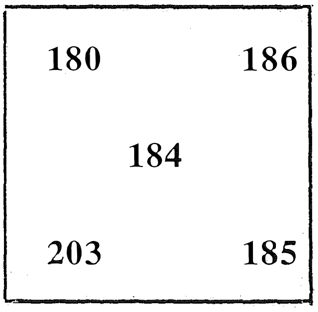
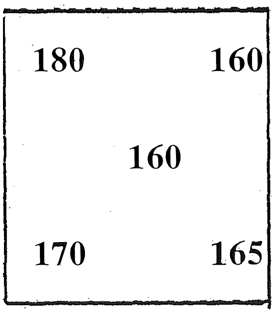
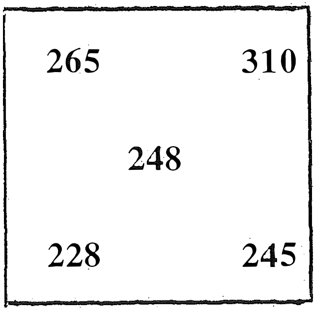


10/8/89

Chamber 5



Chamber 6



Appendix 2

Maximum 3 hour Average SO_2 Concentrations ($nl\,l^{-1}$) in Kwinana and Kalgoorlie Regions

	Kwinana		Kalgoorlie	
Month	1987	1988	1987	1988
January	184	67	521	327
February	19	97	442	269
March	80	87	419	339
April	58	129	356	173
May	121	94	393	274
June	34	76	272	149
July	52	16	284	415
August	93	70	266	222
September	150	31	257	398
October	53	100	318	281
November	63	112	349	310
December	-	148	333	-

(Adapted from Environmental Protection Authority, Perth, Western Australia; Technical Series No. 29, June, 1989)

Appendix 3

Climate Characteristics of the Fumigation Chambers (light, temperature and relative humidity)

Test Date	Parameter	Inside Chamber			Outside Chamber		
21/7/89	Light intensity	33	34	36	37	38	39
(1430h)	(mW/cm^2)	34	35	33	38	37	37
	Temp. / R.H.						
21/7/89	(1400h)	17.5 °C / 65%			15.0 °C / 55%		
	(1520h)	18.0 °C / 63%			15.0 °C / 58%		
24/7/89	(1400h)	17.0 °C / 89%			15.0 °C / 84%		
	(1600h)	16.5 °C / 87%			15.0 °C / 84%		
25/7/89	(1400h)	22.0 °C / 60%			18.5 °C / 64%		
26/7/89	(1400h)	23.0 °C / 58%			21.0 °C / 54%		
02/8/89	(1400h)	15.2 °C / 82%			13.5 °C / 81%		
11/8/89	(1200h)	24.0 °C / 40%			23.0 °C / 32%		

REFERENCES

Ackerson, R. C. & Youngner, V. B. (1975). Responses of bermudagrass to salinity. *Agronomy Journal* 67, 678-681.

Adaros, G., Weigel, H. J. & Jager, H. J. (1988). Effects of sulphur dioxide and acid rain alone or in combination on growth and yield of broad bean plants. *New Phytologist* 108, 67-74.

Amundson, R. G., Raba, R. M., Schoettle, A. W. & Reich, P. B. (1986). Response of soybean to low concentrations of ozone: II. Effects on growth, biomass allocation and flowering. *Journal of Environmental Quality* 15: 161-167.

Arad, S. & Richmond, A. E. (1976). Leaf cell water and enzyme activity. *Plant Physiology* 57, 656-658.

Ashenden, T. W. (1978). Growth reductions in cocksfoot (*Dactylis Glomerata* L.) as a result of SO_2 pollution. *Environmental Pollution* 15, 161-166.

Ashenden, T. W. & Mansfield, T. A. (1977). Influence of windspeed on the sensitivity of ryegrass to SO_2. *Journal of Experimental Botany* 28, 729-735.

Ashenden, T. W. & Williams, I. A. D. (1980). Growth reductions in *Lolium multiflorum* Lam. and *Phleum pratense* L. as a result of SO_2 and NO_2 pollution. *Environmental Pollution* 21, 131-139.

Aspinall, D. (1986). Metabolic effects of water and salinity stress in relation to expansion of the leaf surface. *Australian Journal of Plant Physiology* 13, 59-73.

Atkinson, D. E. (1977). *Cellular Energy Metabolism and its Regulation.* pp. 13-16. Academic Press, New York.

Atkinson, C. J. and Winner, W. E. (1987). Gas exchange characteristics of *Heteromeles arbutifolia* during fumigation with sulphur dioxide. *New Phytologist* 106, 423-436.

Atkinson, C. J., Winner, W. E. and Mooney, H. A. (1988). Gas exchange and SO_2 fumigation studies with irrigated and unirrigated field grown *Diplacus aurantiacus* and *Heteromeles arbutifolia. Oecologia* 75, 386-393.

Atkinson, C. J. and Winner, W. E. (1989). Modification of stomatal conductance by sulphur dioxide. *Journal of Experimental Botany* 213, 461-467.

Awang, M. B. (1979). The effects of sulphur dioxide pollution on plant growth with special reference to *Trifolium repens*. PhD Thesis, University of Sheffield, Sheffield, UK.

Ayazloo, M., Bell, J. N. B. & Daesed, S. G. (1980). Modification of chronic sulfur dioxide injury to *Lolium perenne* L. by different sulfur and nitrogen nutrient treatments. *Environmental Pollution* 22, 295-307.

Ayazloo, M. & Bell, J. N. B. (1981). Studies on the tolerance to sulphur dioxide of grass populations in polluted areas. I. Identification of tolerant populations. *New Phytologist* 88, 203-222.

Baddeley, M. S., Ferry, B. W. & Finegan, E. J. (1971). A new method for measuring lichen respiration: Response of selected species to temperature, pH and sulphur dioxide. *Lichenologist* 5, 18-25.

Baddeley, M. S., Ferry, B. W. & Finegan, E. J. (1972). The effects of sulphur dioxide on lichen respiration. *Lichenologist* 5, 283-291.

Baddeley, M. S., Ferry, B. W. & Finegan, E. J. (1973). Sulphur dioxide and respiration in lichens. In: *Air Pollution and Lichens* (Ed. by B. W. Ferry & M. S. Baddeley), pp. 299-313. Athlone Press, London.

Baker, C. K., Unsworth, M. H. & Greenwood, P. (1982). Leaf injury on wheat plants exposed in the field in winter to SO_2. *Nature* 29, 149-151.

Baker, C. K., Colls, J. J., Fullwood, A. E. & Seaton, G. G. R. (1986). Depression of growth and yield in winter barley exposed to sulphur dioxide in the field. *New Phytologist* 104, 233-241.

Balasubramanian, V. & Sinha, S. (1976). Nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.) under salt stress. *Journal of Agricultural Science* 87, 465-466.

Barlow, E. W. R., Boersma, L. & Young, J. L. (1976). Root temperature and soil water potential effects on growth and soluble carbohydrate concentration of corn seedlings. *Crop Science* 16, 59-62.

Beckerson, D. W. & Hofstra, G. (1979*a*). Stomatal responses of white bean to O_3 and SO_2 singly and in combination. *Atmospheric Environment* 13, 533-535.

Beckerson, D. W. & Hofstra, G. (1979*b*). Response of leaf diffusive resistance of radish, cucumber and soybean to O_3 and SO_2 singly or in combination. *Atmospheric Environment* 13, 533-535.

Bell, J. N. B. (1982). Sulphur dioxide and the growth of grasses. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 225-246. Butterworths, London.

Bell, J. N. B. & Clough, W. A. (1973). Depression of yield in ryegrass exposed to sulphur dioxide. *Nature* 241, 47-49.

Bell, J. N. B., Rutter, A. J. & Relton, J. (1979). Studies on the effects of low levels of sulphur dioxide on the growth of *Lolium perenne* L.. *New Phytologist* 83, 627-643.

Bernstein, L. & Hayward, H. E. (1958). Physiology of salt tolerance. *Annual Review of Plant Physiology* 9, 25-46.

Biggs, A. R. & Davis, D. D. (1980). Stomatal response of three birch species exposed to varying acute doses of SO_2. *Journal of the American Society for Horticultural Science* 105, 514-516.

Black, V. J. (1982). Effects of sulphur dioxide on physiological processes in plants. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 67-91. Butterworths, London.

Black, V. J. (1984). The effects of air pollutants on apparent respiration. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 231-248. Butterworths, London.

Black, V. J. (1985). SO_2 effects on stomatal behaviour. In: *Sulphur Dioxide and Vegetation* (Ed. by W. E. Winner, H. A. Mooney & R. A. Goldstein), pp. 96-117. California, Stanford University Press.

Black, V. J. & Unsworth, M. H. (1979*a*). Effects of low concentrations of SO_2 on net photosynthesis and dark respiration of *Vicia faba* L. *Journal of Experimental Botany* 30, 473-483.

Black, V. J. & Unsworth, M. H. (1979*b*). Resistance analysis of SO_2 fluxes to *Vicia faba*. *Nature* 282, 68-69.

Black, V. J. & Unsworth, M. H. (1980). Stomatal responses to sulfur dioxide and vapour pressure deficit. *Journal of Experimental Botany* 31, 667-677.

Bleasdale, J. K. A. (1973). Effects of coal-smoke pollution on the growth of ryegrass (*Lolium perenne* L.). *Environmental Pollution* 5, 275-285.

Bloom, A. J., Chapin, F. S. III & Mooney, H. A. (1985). Resource limitation in plants - an economic analogy. *Annual Review of Ecological System* 16, 363-392.

Blum, U. & Tingey, D. T. (1977). A study of the potential ways in which ozone could reduce root growth and nodulation of soybean. *Atmospheric Environment* 11, 737-739.

Bonte, J. (1982). Effects of air pollutants on flowering and fruiting. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 207-223. Butterworths, London.

Bressan, R. A., Wilson, L. G. & Filner, P. (1978). Mechanisms of resistance to sulfur dioxide in the *Cucurbitaceae*. *Plant Physiology* 61, 761-767.

Brun, W. A. (1976). The relation of nitrogen fixation to photosynthesis. In: *World Soybean Research* (Ed. by L. D. Hill), pp. 135-143. The Interstate Printers and Publishers, Inc., Danville, IL.

Buckenham, A. H., Parry, M. A. J. & Whittingham, C. P. (1982). The effect of aerial pollutants on the growth and yield of spring barley. *Annals of Applied Biology* 100, 179-187.

Bull, J. N. & Mansfield, T. A. (1974). Photosynthesis in leaves exposed to SO_2 and NO_2. *Nature* 250, 443-444.

Bytnerowicz, A. & Taylor, O. C. (1983). Influence of ozone, sulphur dioxide, and salinity on leaf injury, stomatal resistance, growth, and chemical composition of bean plants. *Journal of Environmental Quality* 12, 397-405.

Bytnerowicz, A., Olszyk, D. M., Kats, G., Dawson, P. J., Wolf, J. & Thompson, C. R. (1987). Effects of SO_2 on physiology, elemental content and injury development of winter wheat. *Agricultural Ecosystem and Environment* 20, 37-47.

Cannell, M. G. R. (1986). Dry matter partitioning in tree crops. In: *Attributes of Trees as Crop Plants* (Ed. by M. G. R. Cannell & J. E. Jackson), pp. 160-193.

Caput, C., Belot, Y., Auclair, D. & Decourt, N. (1978). Absorption of sulphur dioxide by pine seedlings leading to acute injury. *Environmental Pollution* 16, 3-15

Carr, W. W. & Ballard, T. M. (1979). Effects of fertilizer salt concentration on viability of seed and *Rhizobium* used for hydroseeding. *Canadian Journal of Botany* 57, 701-704.

Chapin, F. S. III (1989). Effects of multiple environmental stresses upon nutrient availability. In: *Integrated Responses of Plants to Environmental Stress* (Ed. by H. A. Mooney, W. E. Winner & E. J. Pell), Academic Press, New York.

Chapin, F. S. III, Bloom, A. J., Field, C. B. & Waring, R. H. (1987). Plant responses to multiple environmental factors. *Bioscience* 37, 49-57.

Clarke, K. & Murray, F. (1990). Stimulatory effects of SO_2 on growth of *Eucalyptus rudis* Endl.. *New Phytologist* 115, 633-637.

Clarkson, D. T. & Hanson, J. B. (1980). The mineral nutrition of higher plants. *Annual Review of Plant Physiology* 31, 239-298.

Coleman, J. S., Mooney, H. A. & Gorham, J. N. (1989). Effects of multiple stresses on radish growth and resource allocation. I. Responses of wild radish plants to a combination of SO_2 exposure and decreasing nitrate availability. *Oecologia* 81, 124-131.

Cooley, D. R. & Manning, W. J. (1987). The impact of ozone on assimilate partitioning in plants: A review. *Environmental Pollution* 47, 95-113.

Cornic, G. (1987). Interaction between sublethal pollution by sulphur dioxide and drought stress. The effect on photosynthetic capacity. *Physiologia Plantarum* 71, 115-119.

Cowling, D. W. & Koziol, M. J. (1982). Mineral nutrition and plant response to air pollutants. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 349-375. Butterworths, London.

Cowling, D. W. & Lockyer, D. R. (1978). The effect of SO_2 on *Lolium perenne* L. grown at different levels of sulphur and nitrogen solution. *Journal of Experimental Bonaty* 29, 257-265.

Crittenden, P. D. & Read, D. J. (1978). The effects of air pollution on plant growth with special reference to sulphur dioxide. II.

Growth studies with *Lolium perenne* L.. *New Phytologist* 80, 49-62.

Darrall, N. M. (1986). The sensitivity of net photosynthesis in several plant species to short term fumigation with sulphur dioxide. *Journal of Experimental Botany* 37, 1313-1322.

Darrall, N. M. (1989). The effect of air pollutants on physiological processes in plants. *Plant, Cell and Environment* 12, 1-30.

Davids, J. A., Davis, D. D. & Pennypacker, S. P. (1981). The influence of soil moisture on macroscopic sulfur dioxide injury to pinto bean foliage. *Phytopathology* 71, 1208-1212.

Davies, T. (1980). Grasses more sensitive to SO_2 pollution in conditions of low irradiance and short days. *Nature* 284, 483-485.

Davison, A. (1982). The effects of fluorides on plant growth and forage quality. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 267-291. Butterworths, London.

Davison, A. W. & Bailey, I. F. (1982). SO_2 pollution reduces the freezing resistance of ryegrass. *Nature* 27, 400-402.

Dean, C. E. & Davis, D. R. (1967). Ozone and soil moisture in relation to the occurrence of weather fleck on florida cigar-wrapper tobacco in 1966. *Plant Disease Report* 51: 72-75.

Deane-Drummond, C. E. & Glass, A. D. M. (1982). Studies of nitrate influx into barley roots by the use of $^{36}ClO^{3-}$ as a tracer for nitrate. I. Interactions with chloride and other ions. *Canadian Journal of Botany* 60, 2147-2153.

Delane, R., Greenway, H., Munns, R. & Gibbs, J. (1982). Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. I. Relationship between solute concentration and growth. *Journal of Experimental Botany* 33, 557-573.

Department of Conservation and Environment (1982). *The Kwinana Air Modelling Study.* Report 10, W. A. Department of Conservation and Environment, Perth.

Dotzler, M. & Schutt, P. (1990). Long lasting effects of O_3 and SO_2 on the sensitivity to drought in two clones of *Picea abies* (L.) Karst.. *European Journal of Forest Pathology* 20, 59-63.

Dudeck, A. E., Singh, S., Giordano, C. E., Nell, T. A. & McConnell, D. B. (1983). Effects of sodium chloride on *Cynodon* turf grasses. *Agronomy Journal* 75, 927-930.

Dugger, W. M. & Ting, I. P. (1970). Air pollution oxidants - Their effects on metabolic processes in plants. *Annual Review of Plant Physiology* 21, 215-234.

Elsheikh, E. A. E. & Wood, M. (1990). Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). *Journal of Experimental Botany* 231, 1263-1269.

EMEP/MSC-W Report (1991). Calculated budgets for Airborne Acidifying components in Europe. *Acid News* 5, 10.

Environmental Protection Authority (1989). Air Quality Measurements 1987 and 1988 Perth, Kwinana, Kalgoorlie and Port Hedland. Environmental Protection Authority, Perth, Western Australia, Technical Series No. 29, June 1989.

Esterbauer, H. & Grill, D. (1978). Seasonal variations of glutathione and glutathione reductase in needles of *Picea abies*. *Plant Physiology* 61, 119-121.

Farrar, J. F., Relton, D. & Rutter, A. J. (1977). Sulfur dioxide and growth of *Pinus sylvestris*. *Journal of Applied Ecology* 14, 861-875.

Finn, J. D. (1974). *A General Model for Multivariate Analysis*. Holt, Reinhart & Winston, New York.

Flagler, R. B. (1986). Effects of ozone and water deficit on growth, yield, and nitrogen metabolism of soybeans. Ph.D. dissertation.

Foster, K. W., Timm, H., Labanauskas, C. K. & Oshima, R. J. (1983). Effects of ozone and sulphur dioxide on tuber yield and quality of potatoes. *Journal of Environmental Quality* 1, 75-80.

Fowler, D. (1978). Wet and dry deposition of sulfur and nitrogen compounds from the atmosphere. In: *Effects of Acid Precipitation on Terrestrial Ecosystems* (Ed. by T. C. Hutchinson & M. Havas), pp. 9-27. Plenum, New York.

Fowler, D. and Cape, J. N. (1982). Air pollutants in agriculture and horticulture. In: *Effects of Gaseous Air Pollution in Agriculture And Horticulture* (Ed. by M. H. Unsworth and D. P. Ormrod) pp. 3-26. Butterworths.

Fowler, D., Cape, J. N., Leith, I. D., Paterson, I. S., Kinnaird, J. W. & Nicholson, I. A. (1988). Effects of air filtration at small SO_2 and

NO_2 concentrations on the yield of barley. *Environmental Pollution* 53, 135-149.

Freer-Smith, P. H. (1985). The influence of SO_2 and NO_2 on the growth development and gas exchange of *Betula pendula* Roth. *New Phytologist* 99, 417-430.

Gale, J. (1975). Physiological basis for the reduction of plant growth under conditions of irrigation with brackish water and possible methods of amelioration. In: *Brackish water as a factor in development* (Ed. by A. Issar), pp. 97-102. Ben-Gurion Univ. Beer-Sheeva, Israel.

Garsed, S. G. (1984). Uptake and distribution of pollutants in the plant and residence time of active species. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 83-103. Butterworths, London.

Garsed, S. G. & Read, D. J. (1974). The uptake and translocation of $^{35}SO_2$ in soybean *Glycine max* var. Biloxi. *New Phytologist* 73, 299-307.

Garsed, S. G. & Read, D. J. (1977*a*). The uptake and metabolism of $^{35}SO_2$ in plants of differing sensitivity to sulphur dioxide. *Environmental Pollution* 13, 173-186.

Garsed, S. G. & Read, D. J. (1977*b*). Sulphur dioxide metabolism in *Glycine max* var. Biloxi. II. Biochemical distribution of $^{35}SO_2$ products. *New Phytologist* 78, 583-592.

Gerini, O., Guidi, L., Lorenzini, G. & Soldatini, G. F. (1990). Leaf gas exchange of maize plants fumigated with sulfur dioxide. *Journal of Environmental Quality* 19, 154-156.

Giorgi, M. C. Di., Fichera, P. & Tropea, M. (1967). New considerations on the use of saline water: III. The influence of the potassium-sodium relationship on a saline-sensitive culture (*Citrus aurantium*). *Agrochimica* 11, 166-175.

Glinka, Z. (1971). The effect of epidermal cell water potential on stomatal response to illumination of leaf discs of *Vicia faba*. *Physiologia Plantarum* 24, 476-479.

Godzik, S. & Krupa, S. V. (1982). Effects of sulphur dioxide on the growth and yield of agricultural and horticultural crops. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 247-265. Butterworths, London.

Greenway, H. (1973). Salinity, plant growth and metabolism. *The Journal of the Australian Institute of Agricultural Science*, March, pp. 24-34.

Greenway, H., Hughes, P. G. & Klepper, B. (1969). Effects of water deficit on phosphorus nutrition of tomato plants. *Physiologia Plantarum* 22, 199-207.

Greenway, H. & Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* 31, 149-190.

Guderian, R. (1970). Untersuchungen über quantitative Beziehungen zwischen dem Schwefelgehalt von Pflanzen und dem Schwefeldioxid gehalt der Luft. I. *Zeitschrift für Pflanzenkrankenheit* 77, 200-220.

Guderian, R. (1977). *Air Pollution.* Springer Verlag, Berlin.

Halbwachs, G. (1984). Organismal responses of higher plants to atmospheric pollutants: sulphur dioxide and fluoride. In: *Air Pollution and Plant Life* (Ed. by M. Treshow), pp. 175-214. John Wiley & Sons, England.

Hale, M. G. & Orcutt, D. M. (1987). *The Physiology of Plants Under Stress.* John Wiley & Sons, New York.

Hallgren, J. E. (1978). Physiological and biochemical effects of SO_2 on plants. In: *Sulfur in the Environment: Part II Ecological Impacts* (Ed. by J. O. Nriagu), pp. 163-209. John Wiley and Sons, New York.

Hallgren, J. E. (1984). Photosynthetic gas exchange in leaves affected by air pollutants. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 147-159. Butterworths, London.

Heagle, A. S., Miller, J. E., Heck, W. W. & Patterson, R. P. (1988). Injury and yield response of cotton to chronic doses of ozone and soil moisture deficit. *Journal of Environmental Quality* 17, 627-635.

Heath, R. L. (1980). Initial events in injury to plants by air pollutants. *Annual Review of Plant Physiology* 31, 395-431.

Heath, R. L. (1984). Air pollutant effects on biochemicals derived from metabolism: organic, fatty and amino acid. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 275-290. Butterworths, London.

Heck, W. W. & Dunning, J. A. (1978). Responses of oats to sulphur dioxide: Interactions of growth exposure with exposure temperature and humidity. *Journal of the Air Pollution Control Association* 28: 241-246.

Hetherington, A. M. & Quatrano, R. S. (1991). Mechanisms of action of abscisic acid at the cellular level. *New Phytologist* 119, 9-32.

Hobbs, P. V., Tvell, J. P., Radke, L. F., Hegg, D. A. & Eltgroth, M. W. (1982). Particles and gases in the essissions from the 1980-1981 volcanic eruptions of Mt. St. Helens. *Journal of Geophysical Research* 87, 11062-11086.

Hoffman, G. J., Maas, E. V. & Rawlins, S. L. (1973). Salinity-ozone interactive effects on yield and water relations of pinto bean. *Journal of Environmental Quality* 2, 148-152.

Hoffman, G. J., Maas, E. V. & Rawlins, S. L. (1975). Salinity-ozone interactive effects on alfalfa yield and water relations. *Journal of Environmental Quality* 4, 326-331.

Houston, D. B. & Dochinger, L. S. (1977). Effects of ambient air pollution on cone, seed, and pollen characteristics of eastern white and red pines. *Environmental Pollution* 12, 1-5.

Hsiao, T. C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology* 24, 519-570.

Hunt, R. (1978). *Plant Growth Analysis.* Edward Arnold, London.

Huttunen, S. & Soikkeli, S. (1984). Effects of various gaseous pollutants on plant cell ultrastructure. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 117-127. Butterworths, London.

Ivanova, A. S. (1974). Effect of soil salinization on peach growth and development. *Fiziol. Rast.* 21, 995-999.

Jacobson, J. S. (1982). Ozone and the growth and productivity of agricultural crops. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 293-304. Butterworths, London.

Jeschke, W. D., Wolf, O. & Hartung, W. (1992). Effect of NaCl salinity on flows and partitioning of C, N, and mineral ions in whole plants of white lupin, *Lupinus albus* L. *Journal of Experimental Botany* 251, 777-788.

Johnson, C. M. & Ulrich, A. (1959). Analytical methods for use in plant analysis. *California Agricultural Experiment Station Bulletin* 766, 57-58.

Johnson, A. H. & Siccama, T. G. (1983). Acid deposition and forest decline. *Environmental Science and Technology* 17, 294-305.

Jones, T. & Mansfield, T. A. (1982). The effect of SO_2 on growth and development of seedlings of *Phleum pratense* under different light and temperature environments. *Environmental Pollution* (Series A) 27, 57-71.

Jones, A. W., Mulchi, C. L. & Kenworthy, W. J. (1985). Nodule activity in soybean cultivars exposed to ozone and sulfur dioxide. *Journal of Environmental Quality* 14, 60-65.

Kato, N. & Akimoto, H. (1992). Anthropogenic emissions of SO_2, and NOx in Asia: emission inventories. *Atmospheric Environment* 26(A), 2997-3017.

Katz, M. (1949). SO_2 in the atmosphere and its relation to plant life. *Indian Engineering Chemistry* 41, 2450-2465.

Keller, T. (1981). Folgen einer winterlichen SO_2-belastung für die Fichte. *Gartenbauwissenschaft* 46, 170-178.

Kermode, A. R. (1990). Regulatory mechanisms in the transition from seed development to germination. *Critical Reviews in Plant Science* 9, 155-195.

Khatamian, H., Adedipe, N. O. & Ormrod, D. P. (1973). Soil-plant-water aspects of ozone phytotoxicity in tomato plants. *Plant and Soil* 38, 531-541.

Klee, R. (1970). Die wirking von gas - und stabformigen immissionen auf respiration und inhaltstoffe von *Parmelia Physodes*. *Angewandte Botanik* 44, 253-261.

Klein, H., Jager, H-J., Domes, W. & Wong, C. H. (1978). Mechanisms contributing to differential sensitivities of plants to SO_2. *Oecologia* 33, 203-208.

Kobriger, J. M. & Tibbitts, T. W. (1985). Effect of relative humidity prior to and during exposure on response of peas to ozone and sulphur dioxide. *Journal of American Society in Horticultural Science* 110, 21-24.

Koch, G. W., Schulze, E-D., Percival, F. & Mooney, H. A. (1989). The nitrogen balance of *Raphanus sativus x raphanistrum*. III.

Growth, nitrogen redistribution, and photosynthesis under nitrate deprivation. *Plant, Cell, Environment* 11: 755-767.

Kondo, N. & Sugahara, K. (1978). Changes in transpiration rate of SO_2-resistant and sensitive plants with SO_2 fumigation and the participation of abscisic acid. *Plant and Cell Physiology* 19, 365-373.

Kondo, N., Maruta, I. & Sugahara, K. (1980). Abscisic acid-dependent changes in transpiration rate with SO_2 fumigation and the effects of sulfite and pH and stomatal aperture. Research Report from the *National Institute for Environmental Studies, Japan* 11, 127-136.

Kozlowski, T. T. & Mudd, J. B. (1975). Introduction. In: *Responses of Plants to Air Pollution* (Ed by J. B. Mudd & T. T. Kozlowski), pp. 2-6. Academic Press, New York.

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

Krizek, D. T., Mirecki, R. M. & Semeniuk, P. (1986). Influence of soil moisture stress and abscisic acid pretreatment in modifying SO_2 sensitivity in poinsettia. *Journal of the American Society for Horticultural Science* 111, 446-450.

Lacasse, N. L. & Treshow, M. (Eds) (1978). Fluorides. In: *Diagnosing Vegetation Injury Caused by Air Pollution*, 5.1-5.25. North Carolina Air Pollution Training Institute, U. S. Environmental Protection Agency.

Lakshmi-Kumari, M., Singh, C. S. & Subbarao, N. S. (1974). Root hair infection and nodulation in lucerne (*Nedicago sativa* L.) as influenced by salinity and alkalinity. *Plant and Soil* 40, 261-268.

Laurence, J. A. (1979). Response of maize and wheat to sulphur dioxide. *Plant Disease Reporter* 63, 468-471.

Lauter, D. J., Munns, D. N. & Clarkin, K. L. (1981). Salt response of chickpea as influenced by N supply. *Agronomy Journal* 73, 961-966.

Law, R. M. & Mansfield, T. A. (1982). Oxides of nitrogen and the greenhouse atmosphere. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 93-112. Butterworths, London.

Lendzian, K. J. (1984). Permeability of plant cuticles to gaseous air pollutants. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 77-81. Butterworths, London.

Lewis, O. A. M., Leidi, E. O. & Lips, S. H. (1989). Effect of nitrogen source on growth response to salinity stress in maize and wheat. *New Phytologist* 111, 155-160.

Levitt, L. (1980). *Responses of Plants to Environmental Stresses.* II. *Water, Radiation, Salt, and Other Stresses.* Academic Press, New York.

Linzon, S. N. (1978). Effects of airborne sulfur pollutants on plants. In: *Sulfur in the Environment: Part II Ecological Impacts* (Ed. by J. O. Nriagu), pp. 109-162. John Wiley and Sons, New York.

Lorenc-Plucinska, G. (1984). The uptake and translocation of ^{14}C in Scots pine seedlings of differing susceptibility to sulphur dioxide. *European Journal of Forest Pathology* 14, 310-318.

Lucas, P. W. (1990). The effects of prior exposure to sulphur dioxide and nitrogen dioxide on the water relations of Timothy grass (*Phleum pratense*) under drought conditions. *Environmental Pollution* 66, 117-138.

Ma, T. H. & Khan, S. H. (1976). Pollen-tube mitosis and pollen-tube growth inhibition by SO_2 in cultured pollen tubes of *Tradescantia. Environmental Research* 12, 144-149.

Maas, E. V., Hoffman, G. J., Rawlins, S. L. & Ogata, G. (1973). Salinity-ozone interactions on pinto bean: integrated response to ozone concentration and duration. *Journal of Environmental Quality* 2, 400-404.

MacLean, D. C. & Schneider, R. E. (1973). Fluoride accumulation by forage; continuous vs intermittent exposures to hydrogen fluoride. *Journal of Environmental Quality* 72, 501-503.

Macrez, V. & Hubac, C. (1988). Exposure to SO_2 and water stress in *Picea Abies* (L.) Karsten. *Water, Air and Soil Pollution* 40, 251-259.

Majernik, O. & Mansfield, T. A. (1970). Direct effect of SO_2 pollution on the degree of opening of stomata. *Nature* 227, 377-378.

Malhotra, S. S. & Khan, A. A. (1984). Biochemical and physiological impact of major air pollutants. In: *Air Pollution and Plant Life*

(Ed. by M. Treshow), pp. 153-157. John Wiley and Sons, New York.

Mansfield, T. A. (1976). Chemical control of stomatal movements. *Philosophical Transactions of the Royal Society of London, Series B* 273, 541-550.

Mansfield, T. A. & Davies, W. J. (1981). Stomata and stomatal mechanisms. In: *The Physiology and Biochemistry of Drought Resistance in Plants* (Ed. by L. G. Pale and D. Aspinall), pp. 315-346. Academic Press, Sydney.

Mansfield, T. A., Davies, W. J. & Whitmore, M. E. (1986). Interactions between the responses of plants to pollution and other environmental factors such as drought, light and temperature. In: *Effects of Air Pollution on Terrestrial and Aquatic Ecosystems*, Proceedings of a CEC and NAEP Workshop, 23-25 March 1986, Riso, Denmark.

Mansfield, T. A. & Freer-Smith, P. H. (1984). The role of stomata in resistance mechanisms. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 131-146. Butterworths, London.

Mansfield, T. A. & Majernik, O. (1970). Can stomata play a part in protecting plants against air pollutants? *Environmental Pollution* 1, 149-154.

Mansfield, T. A., Wright, E. A., Lucas, P. W. & Cottam, D. A. (1988). Interactions between air pollutants and water stress. In: *Air Pollution and Plant Metabolism* (Ed. by S. Schulte-Hostede, N. M. Darrall, L. W. Blank & A. R. Wellburn), pp. 288-306. Elsevier, Amsterdam.

McCree, K. J. (1986). Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. In: Plant Growth, Drought and Salinity' (Ed. by N. C. Turner & J. B. Passioura), pp. 33-43. CSIRO, Melbourne.

McKenzie, H. A. & Wallace, H. S. (1954). The Kjehdahl determination of nitrogen: a critical study of digestion conditions - temperature, catalyst, and oxidising agent. *Australian Journal of Chemistry* 7, 55-70.

McLaughlin, S. B. (1985). Effects of air pollution on forests: A critical review. *Journal of the Air Pollution Control Association* 35, 512-534.

McLaughlin, S. B. & McConathy, R. K. (1983). Effects of SO_2 and O_3 on allocation of ^{14}C-labelled photosynthate in *Phaseolus vulgaris*. *Plant Physiology* 73, 630-635.

McLaughlin, S. B. & Taylor, G. E. (1981). Relative humidity - important modifier of pollutant uptake by plants. *Science* 211, 167-169.

McLaughlin, S. B. & Taylor, G. E. (1985). SO_2 effects on dicot crops: some issues, mechanisms, and indicators. In: *Sulphur dioxide and Vegetation. Physiology, Ecology, and Policy Issues* (Ed. by W. E. Winner, H. A. Mooney & R. A. Goldstein), pp. 227-249. Stanford University Press, Stanford, California.

McWilliam, J. R. (1986). The national and international importance of drought and salinity effects on agricultural production. In: *Plant Growth, Drought and Salinity* (Ed. by N. C. Turner & J. B. Passioura), pp. 1-13. CSIRO, Australia.

Meidner, H. & Bannister, P. (1979). Pressure and solute potentials in stomatal cells of *Tradescantia virginiana*. *Journal of Experimental Botany* 30, 255-265.

Mejstrik, V. (1980). The influence of low SO_2 concentrations on growth reduction of *Nicotiana tabacum* L. cv. Samsun and *Cucumis sativus* L. cv. Unikat. *Environmental Pollution (Series A)* 21, 73-76.

Mercado, B. T., Malabayabas, C. A. & Gumasing, S. R. (1974). Growth of some rice varieties in NaCl-salinated soils as affected by the season. *Phillipp Agriculture* 58, 40-49.

Milchunas, D. G., Lauenroth, W. K., Dodd, J. L. & McNary, T. J. (1981). Effects of SO_2 exposure with nitrogen and sulphur fertilization on the growth of *Agropyron smithii*. *Journal of Applied Ecology* 18, 81-91.

Miller, J. E., Patterson, R. P., Heagle, A. S., Pursley, W. A. & Heck, W. W. (1988). Growth of cotton under chronic ozone stress at two levels of soil moisture. *Journal of Environmental Quality* 17, 635-643.

Moran, R. & Porath, D. (1980). Chlorophyll determination in intact tissues using N, N-dimethylformamide. *Plant Physiology* 65, 478-479.

Mudd, J. B. & Kozlowski, T. T. (Ed.) (1975). *Responses of Plants to Air Pollution*. Academic Press, New York.

Mudd, J. B., Banerjee, S. K., Dooley, M. M. & Knight, K. L. (1984). Pollutants and plant cells: effects on membranes. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 105-116. Butterworths, London.

Muller, R. N., Miller, J. E. & Sprugel, D. G. (1979). Photosynthetic response of field-grown soybeans to fumigation with sulphur dioxide. *Journal of Applied Ecology* 16, 567-576.

Munns, R. & Termaat, A. (1986). Whole-plant responses to salinity. In: *Plant Growth, Drought and Salinity* (Ed. by N. C. Turner & J. B. Passioura), pp. 143-160. CSIRO, Australia.

Murray, F. (1984*a*). Effects of sulphur dioxide on three *Eucalyptus* species. *Australian Journal of Botany* 32, 139-145.

Murray, F. (1984*b*). Responses of subterranean clover and ryegrass to sulphur dioxide under field conditions. *Environmental Pollution* 36, 239-249.

Murray, F. (1985*a*). Changes in growth and quality characteristics of lucerne (*Medicago sativa* L.) in response to sulphur dioxide exposure under field conditions. *Journal of Experimental Botany* 164, 449-457.

Murray, F. (1985*b*). Some responses of ladino clover (*Trifolium repens* L. cv. Regal) to low concentrations of sulphur dioxide. *New Phytologist* 100, 57-62.

Murray, F. (1986). Under what conditions can sulfur dioxide be beneficial to plants? In: *Proceedings of the 7th World Clean Air Congress* Vol. III. Ed. by H. F. Hartmann. Clear Air Society of Australia and New Zealand, Melbourne, pp. 126-132.

Murray, F. & Wilson, S. (1988*a*). Joint action of sulphur dioxide and hydrogen fluoride on growth of *Eucalyptus tereticornis*. *Environmental and Experimental Botany* 28, 343-349.

Murray, F. & Wilson, S. (1988*b*). The joint action of sulphur dioxide and hydrogen fluoride on the yield and quality of wheat and barley. *Environmental Pollution* 55, 239-249.

Murray, F. & Wilson, S. (1988*c*). Effects of sulphur dioxide, hydrogen fluoride and their combination on three *Eucalyptus* Species. *Environmental Pollution* 52, 265-279.

Murray, F. (1989). Acid rain and acid gases in Australia. *Arch. Environ. Contam. Toxicol.* 18, 131-136.

Murray, F. & Wilson, S. (1989*a*). The relationship between sulphur dioxide concentrations and yield of five crops in Australia. *Clean Air* 23: 51-55.

Murray, F. & Wilson, S. (1989*b*). Sulfur dioxide-induced growth changes in Eucalyptus calophylla. *European Journal of Forest Pathology* 19, 193-199.

Murray, F. & Wilson, S. (1990*a*). Growth responses of barley exposed to SO_2. *New Phytologist* 114, 537-541.

Murray, F. & Wilson, S. (1990*b*). Yield responses of soybean, maize, peanut and navy bean exposed to SO_2, HF and their combination. *Environmental and Experimental Botany* 30, 215-223.

Neely, G. E., Tingey, D. T. & Wilhour, R. G. (1977). Effects of ozone and sulfur dioxide singly and in combination on yield, quality, and nitrogen fixation in alfalfa. *National Technical Information Service* 264233, pp. 633-673. National Technical Information Service, Springfield, VA.

Neighbour, E. A., Cottam, D. A. & Mansfield, T. A. (1988). Effects of sulphur dioxide and nitrogen dioxide on the control of water loss by birch (*Betula spp.*). *New Phytologist* 108, 149-157.

Nieboer, E., MacFarlane, J. D. & Richardson, D. H. S. (1984). Modification of plant cell buffering capacities by gaseous air pollutants. In: *Gaseous Air Pollutants and Plant Metabolism* (Ed. by M. J. Koziol & F. R. Whatley), pp. 313-330. Butterworths, London.

Norby, R. J. & Kozlowski, T. T. (1981). Response of SO_2-fumigated *Pinus resinosa* seedlings to post-fumigation temperature. *Canadian Journal of Botany* 59, 470-475.

Norby, R. J. & Kozlowski, T. T. (1983). Flooding and SO_2 stress interaction in *Betula papyrifera* and *B. nigra* seedlings. *Forest Science* 29, 739-750.

Noyes, R. D. (1980). The comparative effects of sulphur dioxide on photosynthesis and translocation in bean. *Physiological Plant Pathology* 16, 73-79.

Oertli, J. J. (1959). Effect of salinity on susceptibility of sunflower plants to smog. *Soil Science* 87, 249-251.

Ogata, G. & Maas, E. V. (1973). Interactive effects of salinity and ozone on growth and yield of garden beet. *Journal of Environmental Quality* 2, 518-520.

Olszyk, D. M. & Tingey, D. T. (1984). Phytotoxicity of air pollutants. *Plant Physiology* 74, 999-1005.

Olszyk, D. M., Maas, E. V., Kats, G. & Francois, L. E. (1988). Soil sanility and ambient ozone: lack of stress interaction for field-grown alfalfa. *Journal of Environmental Quality* 17, 299-304.

Ormrod, D. P. (1982). Air pollutant interactions in mixtures. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 307-331. Butterworths, London.

Osmond, C. B. (1968). Ion absorption in Atriplex leaf tissue. I. Absorption by mesophyll cells. *Australian Journal of Biological Science* 21, 1119-1130.

Otto, H. W. & Daines, R. H. (1969). Plant injury by air pollutants: Influence of humidity on stomatal apertures and plant responses to ozone. *Science* 163, 1209-1210.

Palfi, G., Bito, M. & Palfi, Z. (1973). Free proline and water deficit in plant tissues. *Fiziol. Rast.* 20, 233-238.

Pande, P. C. (1985). An examination of the sensitivity of five barley cultivars to SO_2 pollution. *Environmental Pollution* (Series A) 37, 27-41.

Pande, P. C. & Mansfield, T. A. (1985). Responses of spring barley to SO_2 and NO_2 pollution. *Environmental Pollution* (Series A) 38, 87-97.

Pell, E. J., Pearson, N. S. & Vinten-Johansen, C. (1988). Qualitative and quantitative effects of ozone and/or sulphur dioxide on field-grown potato plants. *Environmental Pollution* 53, 171-186.

Petitte, J. M. & Ormrod. D. P. (1988). Effects of sulphur dioxide and nitrogen dioxide on shoot and root growth of Kennebec and Russet Burbank potato plants. *American Potato Journal* 65, 517-527.

Pierre, M. & Queiroz, O. (1982). Modulation by leaf age and SO_2 concentration of the enzymic response to subnecrotic SO_2 pollution. *Environmental Pollution* (Serial A) 28, 209-217.

Pierre, M. & Queiroz, O. (1988). Air pollution by SO_2 amplifies the effects of water stress on enzymes and total soluble proteins of spruce needles. *Physiologia Plantarum* 73, 412-417.

Pierre, M. & Savoure, A. (1990). Effects of water-stress and SO_2 pollution on spruce endoproteases. *Plant Physiology and Biochemistry* 28, 95-104.

Piggott, T. J. (1986). Vegetable crops. In: *Plant Analysis: An Interpretation Manual* (Ed. by D. J. Reuter & J. B. Robinson), pp. 148-187. Inkata Press, Sydney.

Poorter, H. & Lewis, C. (1986). Testing differences in relative growth rate: a method avoiding curve fitting and pairing. *Physiologia Plantarum* 67, 223-226.

Qifu, M. & Murray, F. (1991*a*). Responses of potato plants to sulphur dioxide, water stress and their combination. *New Phytologist* 118, 101-109.

Qifu, M. & Murray, F. (1991*b*). Soil salinity modifies SO_2 sensitivity in soybean. *New Phytologist* 119, 269-274.

Quatrano, R. S. (1987). The role of hormones during seed development. In: *Plant Hormones and their Role in Plant Growth and Development* (Ed. by P. J. Davies), pp. 494-514. Dordrecht/Kluwer Academic Publishing.

Quebedeaux, B. Jr. & Ozbun, J. L. (1973). Effects of ammonium nutrition on water stress, water uptake, and root pressure in *Lycopersicon esculentum* Mill. *Plant Physiology* 52, 677-679.

Queiroz, O. (1983). Interactions between external and internal factors affecting the operation of PEP carboxylase. *Physiol. Veg.* 21: 963-975.

Rai, R. & Prasad, V. (1983). Salinity tolerance of *Rhizobium* mutants: growth and relative efficiency of symbiotic nitrogen fixation. *Soil Biology and Biochemistry* 15, 217-219.

Reich, P. B., Schoettle, A. W. & Amundson, R. G. (1985). Effects of low concentrations of O_3, leaf age and water stress on leaf diffusive conductance and water use efficiency in soybean. *Physiologia Plantarum* 63, 58-64.

Reinert, R. A. & Gray, T. N. (1981). The response of radish to nitrogen dioxide, sulphur dioxide and ozone alone and in combination. *Journal of Environmental Quality* 10, 240-243.

Rich, S. & Turner, N. C. (1972). Importance of moisture on stomatal behaviour of plants subjected to ozone. *Journal of the Air Pollution Control Association* 22, 718-722.

Rist, D. L. & Davis, D. D. (1979). The influence of exposure temperature and relative humidity on the response of pinto-bean foliage to sulfur dioxide. *Phytopathology* 69. 231-235.

Roberts, T. M. (1984*a*). Effects of air pollutants on agriculture and forestry. *Atmospheric Environment* 18, 629-652.

Roberts, T. M. (1984*b*). Long-term effects of sulphur dioxide on crops: an analysis of dose-response relations. *Phil. Trans. R. Soc. Lond.* B. 305, 299-316.

Roberts, T. M., Darrall, N. M. & Lane, P. (1984). Effects of gaseous air pollutants on agriculture and forestry in the UK. *Advances in Applied Biology* 9, 1-142.

Roberts, B. R. (1989). Some physiological considerations on sulphur dioxide sensitivity in drought-stressed seedlings of sweetgum (*Liquidambar styraciflua* L.). *Scientia Horticulturae* 41, 141-149.

Sachs, R. M., Kretchun, T. & Mock, T. (1975). Minimum irrigation requirements for landscape plants. *Journal of American Society for Horticultural Science* 100, 499-502.

Satyanarayana, N. V., Prasad, P. & Madhava Rao, K. V. (1985). Effects of sulphur dioxide on growth and nodulation of pigeonpea. *Proceeding of Indian Academic Science (Plant Science)* 95, 199-202.

Saxe, H. (1983). Long-term effects of low levels of SO_2 on bean plants (*Phaseolus vulgaris*). II. Immission-response effects on biomass production: Quantity and quality. *Physiologia Plantarum* 57, 108-113.

Schutt, P. & Cowling, E. B. (1985). Waldsterben, a general decline of forests in central Europe. Symptoms development and possible causes. *Plant Disease* 69, 544-558.

Sekiya, J., Wilson, L. G. & Filner, P. (1982). Resistance to injury by sulfur dioxide. Correlation with its reduction to, and emission of hydrogen sulfide in *Cucurbitaceae*. *Plant Physiology* 70, 437-441.

Shanklin, J. & Kozlowski, T. T. (1985). Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO_2. *Environmental Pollution (Series A)* 38, 199-212.

Sij, J. W. & Swanson, C. A. (1974). Short-term kinetics on the inhibition of photosynthesis by sulfur dioxide. *Journal of Environmental Quality* 3, 103-107.

Singleton, P. W. & Bohlool, B. B. (1984). Effect of salinity on nodule formation by soybean. *Plant Physiology* 74, 72-76.

Singleton, P. W., El-Swaify, S. A. & Bohlool, B. B. (1982). Effect of salinity on *Rhizobium* growth and survival. *Applied Environmental Micrology* 44: 884-890.

Taniyama, T. (1972). Studies on the development of symptoms and the mechanism of injury caused by sulphur dioxide in crop plants. *The Bulletin of the Faculty of Agriculture*, No. 44 Mie University, Tsu, Japan.

Taylor, O. C. & Eaton, F. M. (1966). Suppression of plant growth by nitrogen dioxide. *Plant Physiology* 41, 132-135.

Taylor, G. E. & Tingey, D. T. (1983). Sulfur dioxide influx into leaves of *Geranium carolinianum*. Evidence of a non-stomatal or residual resistance. *Plant Physiology* 72, 237-244.

Taylor, G. E.; Tingey, D. T. & Gunderson, C. A. (1986). Photosynthesis, carbon allocation and growth of sulphur dioxide ecotypes of *Geranium carolinianum* L. *Oecologia* (Berlin) 68, 350-357.

Temple, P. J., Fa, C. H. & Taylor, O. C. (1985*a*). Effects of sulphur dioxide on stomatal conductance and growth of *Phaseolus vulgaris*. *Environmental Pollution (Series A)* 37, 267-279.

Temple, P. J., Taylor, O. C. & Benoit, L. F. (1985*b*). Cotton yield responses to ozone as mediated by soil moisture and evapotranspiration. *Journal of Environmental Quality* 14, 55-60.

Tibbitts, T. W. & Kobriger, J. M. (1983). Mode of action of air pollutants in injuring horticultural crops. *Horticultural Science* 18, 675-680.

Tingey, D. T. & Blum, U. (1973). Effects of ozone on soybean nodules. *Journal of Environmental Quality* 2, 341-342.

Tingey, D. T. & Hogsett, W. E. (1985). Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiology* 77, 944-947.

Tingey, D. T., Thutt, G. L. Gumpertz, M. L. & Hogsett, W. E. (1982). Plant water stress influences ozone sensitivity of bean plants. *Agricultural Environment* 7, 243-254.

Tingey, D. T. & Reinert, R. A. (1975). Effect of ozone and sulphur dioxide singly or in combination on plant growth. *Environmental Pollution* 9: 117-125.

Tjoelker, M. G. & Luxmoore, R. J. (1991). Soil nitrogen and chronic ozone stress influence physiology, growth and nutrient status of *Pinus taeda* L. and *Liriodendron tulipifera* L. seedlings. *New Phytologist* 119, 69-81.

Tu, J. C. (1981). Effect of salinity on *Rhizobium*-root hair interaction, nodulation and growth of soybean. *Canadian Journal of Plant Science* 61, 231-239.

United States Environmental Protection Agency (1978). *Diagnosing Vegetation Injury Caused by Air Pollution* (Ed. by N. L. Lacosse & M. Treshow), pp. 4.1-4.2.3. Air Pollution Training Institute.

Unsworth, M. H., Biscoe, P. V. & Pinckney, H. R. (1972). Stomatal responses to sulfur dioxide. *Nature* 239, 458-459.

Unsworth, M. H. & Black, V. J. (1981). Stomatal responses to pollutants. In: *Stomatal Physiology* (Ed. by P. E. Jarvis & T. A. Mansfield), pp. 187-203.

Unsworth, M. H., Crawford, D. V., Gregson, S. K. & Rowlatt, S. M. (1985). Pathway for sulfur from the atmosphere to plants and soil. In: *Sulfur Dioxide and Vegetation: Physiology, Ecology and Policy Issues* (Ed. by W. E. Winner, H. A. Mooney & R. A. Goldstein), pp. 375-388. Stanford University Press, California.

Unsworth, M. H. & Ormrod, D. P. (1982). Appendix 2 Units, physical properties and conversion factors. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 513-515. Butterworths, London.

Varshney, S. R. K. & Varshney, C. K. (1981). Effects of sulphur dioxide on pollen germination and pollen tube growth. *Environmental Pollution* 24, 87-92.

Weimberg, R., Lerner, H. R. & Poljakoff-Mayber, A. (1984). Changes in growth and water-soluble solute concentrations in *Sorghum bicolor* stressed with sodium and potassium salts. *Physiologia Plantarum* 62, 472-480.

Weinstein, L. H. (1977). Fluoride and plant life. *Journal of Occupation Medicine* 19, 49-78.

Weinstein, L. H. & Alscher-Herman, R. (1982). Physiological responses of plants to fluorine. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 139-167. Butterworths, London.

Wellburn, A. R. (1982). Effects of SO_2 and NO_2 on metabolic function. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 169-187. Butterworths, London.

Wesselius, J. C. & Brouwer, R. (1972). Influence of water stress on photosynthesis, respiration and leaf growth of *Zea mays* L. *Meded. Landbovwhogesh. Wageningen* 72, 1-15.

Whitmore, M. E. (1985). Relationship between dose of SO_2 and NO_2 mixtures and growth of *Poa pratensis. New Phytologist* 99, 545-553.

Whitmore, M. E., Freer-Smith, P. H. & Davies, T. (1982). Some effects of low concentrations of SO_2 and/or NO_2 on the growth of grasses and poplar. In: *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (Ed. by M. H. Unsworth & D. P. Ormrod), pp. 483-485. Butterworths, London.

Whitmore, M. E. & Mansfield, T. A. (1983). Effects of long-term exposure to SO_2 and NO_2 on *Poa pratensis* and other grasses. *Environmental Pollution* (Serial A) 31, 217-235.

Wilson, S. & Murray, F. (1990). SO_2-induced growth reductions and sulphur accumulation in wheat. *Environmental Pollution* 66, 179-191.

Winner, W. E. and Mooney, H. A. (1980*a*). Ecology of SO_2 resistance. I. Effects of fumigations on gas exchange of deciduous and evergreen shrubs. *Oecologia* 44, 290-295.

Winner, W. E. and Mooney, H. A. (1980*b*). Ecology of SO_2 resistance. III. Metabolic changes of C_3 and C_4 *Atriplex* species due to SO_2 fumigations. *Oecologia* 46, 49-54.

World Health Organisation (1987). *Air Quality Guidelines for Europe: WHO Regional Publications: European Series* No 23. WHO, Copenhagen.

Wright, E. A., Lucas, P. W., Cottam, D. A. & Mansfield, T. A. (1986). Physiological responses of plants to SO_2, NO_2, and O_3.

Implications for drought resistance. In: *Direct Effects of Dry and Wet Deposition on Forest Ecosystems - In Particular Canopy Interactions*. EEC Cost Workshop, Lokeberg, Sweden.

Yousef, A. N. & Sprent, J. I. (1983). Effects of NaCl on growth, nitrogen incorporation and chemical composition of inoculated and NH_4NO_3 fertilized *Vicia faba* (L.) plants. *Journal of Experimental Botany* 145, 941-950.

Zhukovskaya, N. V. (1973). Phosphate uptake and accumulation by the plants under conditions of soil salinization. *Fiziol. Rast.* 20, 71-78.

Ziegler, I. (1972). The effect of SO^{2-} on the activity of ribulose1,6-diphosphate carboxylase in isolated spinach chloroplasts. *Planta* 103, 155-163.

Ziegler, I. (1973). Effect of sulfite on phosphoenolpyruvate carboxylase and malate formation in extracts of *Zea mays*. *Phytochemistry* 12, 1026-1030.