Quantification of SO_2 effects on physiological processes, plant growth and crop production

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Proefschrift

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Stellingen

1. Lange-termijn effecten van SO_2 op planten worden voor een belangrijk deel bepaald door de samenstelling en verwerking van de door de planten opgenomen voedingsstoffen.

   H.-J. Jäger & H. Klein (1976). Eur. J. For. Path. 6: 347-354.
   Dit proefschrift

2. In de door Laisk *et al.* ontwikkelde modellen voor de opname van SO_2 door bladeren en de invloed daarvan op de pH van subcellulaire oplossingen wordt de rol van metabolische pH-regulatiemechanismen ten onrechte verwaarloosd.

   A. Laisk *et al.* (1988). Planta 173: 230-240; 241-252.
   Dit proefschrift

3. Het vaststellen van de effecten van luchtverontreiniging op de translocatiesnelheid van assimilaten, geeft alleen dan inzicht in het belang van deze effecten voor de groei en productie van gewassen, als de effecten op het dagelijkse verloop van de translocatiesnelheid worden geanalyseerd.

4. Het werkelijke voordeel van gemengde teelt kan niet worden gekarakteriseerd met behulp van de LER (Land Equivalent Ratio), die is afgeleid uit de resultaten van additieve experimenten, waarin de dichtheden van de afzonderlijke soorten niet zijn gevarieerd.

   C.J.T. Spitters (1983). Neth. J. Agric. Sci. 31: 1-11; 143-155.
   W.C.H. van Hoof (1987). Proefschrift Landbouwuniversiteit Wageningen.

5. Het aantal onkruiden per soort en per eenheid van oppervlak is geen goede indikator voor het verlies in gewasopbrengst dat deze onkruiden kunnen veroorzaken.

6. De algemeen heersende opvatting, dat onkruidbestrijdingsmaatregelen slechts effectief zijn als de onkruiden volledig afsterven, staat de ontwikkeling van milieuvriendelijker alternatieven in de weg.

7. Het is slechts zinvol om software voor Computer Ondersteund Onderwijs te ontwikkelen voor het hoger agrarisch onderwijs, als daarmee onderwijsdoelstellingen kunnen worden gerealiseerd, die met de bestaande hulpmiddelen niet of nauwelijks te realiseren zijn.

8. De productie van bio-ethanol uit tarwe in de EG is pas concurrerend met vergelijkbare producten uit fossiele brandstoffen, als de tarweprijs in de EG met een factor acht zou dalen en zal vanwege de geringe netto energieopbrengst ook bij stijgende energieprijzen geen oplossing zijn voor de problemen rond de graanoverschotten in de EG.

   R. Rabbinge (1982). Landbouwkundig tijdschrift 94: 25-30.
   Landbouwschap (1988). Interne notitie, 23 September.
   G. van der Bijl (1989). Spil 79-80: 21-27.

9. Milieu-onderzoekers maken zich ongeloofwaardig, wanneer zij denken aan de behoefte van beleidsmakers tegemoet te komen door eenduidige antwoorden te geven zonder de onzekerheden helder te presenteren.

10. Zowel vanuit landbouwkundig als milieukundig oogpunt is het noodzakelijk, dat het aardappelras Bintje haar - zichzelf instandhoudende - monopoliepositie verliest.

11. Gezien het rijgedrag van automobilisten in Nederland, is het bij opbod verkopen van de bevoegdheid om automobilisten te bekeuren bij overschrijding van de maximum snelheid een effectieve en winstgevende milieumaatregel.

Stellingen behorende bij het proefschrift van Martin Kropff:
Quantification of SO_2 effects on physiological processes, plant growth and crop production.

Wageningen, 29 september 1989.

Abstract

SO_2 may cause damage on crops and vegetation. This thesis aimes to explain the impact of SO_2 on plant growth and crop production on basis of a quantitative analysis of SO_2 effects on physiological processes. Photosynthesis of leaves was found to be depressed at high radiation levels, by competition between SO_2 and CO_2 for the carboxylating enzyme. A model for the flux of SO_2 into the leaf and effects of its metabolites on photosynthesis was developed and used to estimate values for model parameters at the biochemical level from data on the effect of SO_2 on photosynthesis. Differences in sensitivity between species and cultivars appeared to be largely based on variation in the rate of sulphite oxidation. The model was used to analyse the mechanism behind temperature effects on photosynthetic sensitivity to SO_2. The submodel for SO_2 effects at the leaf level was coupled to a model for photosynthesis for leaf canopies. The effects of SO_2 on canopy photosynthesis were simulated accurately.

SO_2 effects on growth and production of broad bean (*Vicia faba* L.) crops were studied using an open-air exposure system. Yield was depressed by 9-17% at SO_2 concentrations ranging from 74-165 μg SO_2 m^{-3}. Chronic injury, leaf damage in the older leaves after long exposures, caused substantial reductions in leaf area at the end of the growing period.

The mechanism behind the observed depression in crop yield was analysed by mechanistic simulation models for crop growth, extended with the submodels for SO_2 effects on leaf photosynthesis. Direct effects of SO_2 on photosynthesis explained about 10% of the observed yield reduction. An increased rate of maintenance respiration, observed in field exposed leaves, explained an extra 10% of the observed yield reduction. The major part was explained by chronic leaf injury at the end of the growing period.

Because chronic injury may be related to a disturbance of intracellular pH regulation, a conceptual model was proposed for regulation of intracellular pH in relation to uptake and assimilation of nutrients and uptake of N and S containing air pollutants by the shoots of plants.

The results of this study are discussed in view of the development of mechanistic models for estimation of the impact of air pollutants on crops, forests and (semi-) natural vegetation.

additional index words: photosynthesis, respiration, stomata, sulphite, model, chronic injury, *Vicia faba* L., intracellular pH, yield reduction, open-air exposure.

Aan Nynke, Wietske en Femke

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Het in dit proefschrift beschreven onderzoek is uitgevoerd in het kader van een samenwerkingsverband tussen het Instituut voor Plantenziektenkundig Onderzoek (IPO) en de vakgroepen Luchthygiëne- en verontreiniging (LUVO) en Theoretische Produktie-ecologie (TPE) van de Landbouwuniversiteit. De konkrete projecten binnen deze samenwerking werden deels gefinancierd door het Nationaal Programma Verzuringsonderzoek. Ik hoop dat deze vruchtbare samenwerking in de komende jaren gecontinueerd zal worden.

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Contents

ACCOUNT

Parts of this thesis have been included, in part or in whole, in the following publications:

Chapter 2 — Kropff, M.J., 1987. Physiological effects of sulphur dioxide. 1. The effect of SO_2 on photosynthesis and stomatal regulation of *Vicia faba* L. Plant, Cell and Environment 10: 753-760.

Chapter 3 — Kropff, M.J., 1989. Modelling short-term effects of sulphur dioxide. 1. A model for the flux of SO_2 into leaves and effects on leaf photosynthesis. Netherlands Journal of Plant Pathology, in press.

Chapter 4 — Kropff, M.J., 1989. Modelling short-term effects of sulphur dioxide. 2. Quantification of biochemical characteristics determining the effect of SO_2 on photosynthesis of leaves. Netherlands Journal of Plant Pathology, in press.

Chapter 5 — Kropff, M.J., Smeets, W., Meijer, E., van der Zalm, A.J.A. & Bakx, E.J., 1989. Effects of sulphur dioxide on photosynthesis: The role of temperature and humidity, submitted.

Chapter 6 — Kropff, M.J. & Goudriaan, J., 1989. Modelling short term effects of sulphur dioxide. 3. The effect of SO_2 on photosynthesis of leaf canopies. Netherlands Journal of Plant Pathology, in press.

Chapter 7 — Kropff, M.J., Mooi, J., Goudriaan, J., Smeets W., Leemans, A., Kliffen, C. & van der Zalm, A.J.A., 1989. The effects of long-term open-air fumigation with SO_2 on a field crop of broad bean (*Vicia faba* L.). I. Depression of growth and yield. New Phytologist, in press.

Chapter 8 — Kropff, M.J., Mooi, J., Goudriaan, J., Smeets, W., Leemans, A., Kliffen, C., 1989. The effects of long-term open-air fumigation with SO_2 on a field crop of broad bean (*Vicia faba* L.) II. Effects on growth components, leaf area development and elemental composition. New Phytologist, in press.

Chapter 9 — Kropff, M.J., 1989. Effects of long-term open-air fumigation with SO_2 on a field crop of broad bean (*Vicia faba* L.). III. Quantitative analysis of damage components, submitted.

Chapter 10 — Kropff, M.J., 1989. Long term effects of sulphur dioxide on plants, SO_2 metabolism and regulation of intracellular pH, submitted.

Chapters 7-9 — Kropff, M.J., Smeets,W., Mooi, J., Goudriaan, J., & Leemans, A., 1989. Growth and production of faba bean crops exposed to SO_2 in the field: Experimental data analysed with a simulation model. Proceedings of the 8th World Clean Air Congress, The Hague, Holland, 1989, in press.

Abbreviations

RBP = Ribulose 1,5-bisphosphate
S(IV) = $[SO_2]_{aq} + [HSO_3^-] + [SO_3^{2-}]$
1 ppm equals about 2700 μg SO_2 m^{-3}

VPD	Vapour Pressure Deficit	(mbar)
PPFD	Photosynthetic Photon Flux Density	(μE m^{-2} s^{-1})
LAI	Leaf Area Index	(m^2 m^{-2})
SLA	Specific Leaf Area	(m^2 g^{-1})

Chapter 1

General introduction

Effects of air pollutants were already recognized in the nineteenth century, when plants growing near emission sources showed severe injurious symptoms. The devastation of landscapes in the vicinity of these sources intrigued scientists who questioned the ecological impact of air-pollutants and economical implications for agricultural crops. Since the end of the nineteenth century many studies have been undertaken to analyse the effects of air pollutants on plants.

Sulphur dioxide, which is a common byproduct of fossil fuel consumption, is one of the major air pollutants that can damage vegetation. The effects of SO_2 on plants range from severe macroscopic damage to leaf foliage as a result of (short) exposures to high SO_2 concentrations, to growth reductions without visible damage at low concentrations (see reviews by Unsworth & Ormrod, 1982; Winner, Mooney & Goldstein, 1985). Besides these harmful effects, SO_2 stimulates growth of plants growing on soils with insufficient sulphur to meet the sulphur requirements for plant growth (Thomas *et al.*, 1943; Thomas, Hendricks & Hill, 1944; Olsen, 1957; Faller, Herwig & Kuhn, 1970).

Three categories of SO_2 injury on plants may be distinguished: acute, chronic and subtle injury (Linzon, 1978). Acute injury results from short exposures (less than one day) to high SO_2 concentrations, generally in the ppm range[1)] and appears as irreversible visible damage in leaf foliage (necrosis). Initially, the young fully unfolded leaves show grayish-green spots between the veins. Acute injury is assumed to be caused by the rapid accumulation of bisulphite and sulphite. Chronic injury (chlorotic or necrotic damage) is the result of long term exposure (days to years) to variable concentrations of SO_2. Chronic effects are assumed to be related to the accumulation of excess sulphur in the plant. Generally, the older leaves are damaged, followed by early abscission. Subtle effects are defined as changes in physiological and biochemical processes, causing growth reductions (or stimulation) without visible injury. Because no relationship was found between the sensitivity of plants to the different types of injury, the biochemical backgrounds are assumed to be different for these types of injury (Bell, 1985; Garsed, 1985).

Most early studies concentrated on visible symptoms produced by high SO_2 concentrations, associated with emissions from point sources that affected surrounding ve-

[1)] 1 ppm equals about 2700 μg SO_2 m^{-3}

getation (Posthumus, 1981). As a result of emission standards and limits, high pollutant concentrations causing acute injury have been strongly diminished in Western Europe and North America during the past decades. The increase of tall stacks on power plants and smelters built to reduce the concentrations in the immediate surrounding of the sources, resulted in long distance transport causing elevated SO_2 concentrations in extended areas in industrialized countries. Such elevated concentrations may cause chronic and subtle injury which may go unrecognized in the field, because there is no reference to plants growing in clean air. In the 1970's, sophisticated experimental equipment was developed in which plants could be fumigated artificially (*e.g.* open top chambers, Heagle, Body & Neely, 1974). This led to experiments which showed that air pollutants cause changes in plant growth and crop production without causing visible injury. Widespread concern that agricultural crops and vegetation was affected by SO_2 at sublethal concentrations was based upon evidence that plant growth may be reduced by SO_2 concentrations as low as 40 $\mu g\ m^{-3}$, without causing visible injury (Bell, Rutter & Relton, 1979). This resulted in a gradual transition of research efforts from studies on acute injury to studies on SO_2 effects on plant growth and the biochemical and physiological backgrounds of these reductions. Research of SO_2 effects on physiological processes like photosynthesis and respiration started prior to 1950 by Thomas and co-workers (Thomas, 1951) and was intensified in the past two decades (Winner *et al.*, 1985). It is well recognized now that severe damage on agricultural crops and vegetation may occur during long-term exposures to the low concentrations of SO_2 and has been observed in large areas of Western Europe and North America without causing acute injury symptoms.

Atmospheric SO_2 levels and SO_2 deposition

Emission of SO_2 into the atmosphere may result from natural and man-made sources. Background levels of SO_2 in the atmosphere, caused by natural emissions (microbial activity, volcanoes, sulphur springs, sea-spray and weathering processes), are about 3 $\mu g\ m^{-3}$ (Kellog *et al.*, 1972). The contribution of man-made sulphur to the total sulphur inputs into the atmosphere is considerable. Natural worldwide emissions amount to about 128 Mt of sulphur per year, whereas in the mid-1970's (when sulphur emissions were highest) man-made emissions were about 77 Mt of sulphur per year (Wellburn, 1988). More than 90% of these man-made emissions arise from the urban and industrialized areas in Europe, North America, India and the Far East (Wellburn, 1988). The burning of coal is by far the largest source of man-made emissions.

In heavily industrialized areas in Western Europe like the Ruhr area in the Federal Republic of Germany and the industrial Midlands and North of England, the annual mean SO_2 concentration may exceed 100 $\mu g\ m^{-3}$ (Fowler & Cape, 1982). They estimated that 1% of the total area in Western Europe was exposed to mean SO_2 concentrations exceeding 100 $\mu g\ m^{-3}$ and 5% to concentrations between 50-100 $\mu g\ m^{-3}$. Yearly mean SO_2

concentrations in 1986 in the Netherlands ranged from 8-43 $\mu g\ m^{-3}$ (Anon., 1988). Although SO_2 emissions have been reduced in the past decade, yearly mean SO_2 concentrations in Western Europe may locally range from 5 to over 100 $\mu g\ m^{-3}$, with even higher concentrations during short episodes. The EC limit is 120 $\mu g\ m^{-3}$ for annual median daily concentrations and the EC guide value for annual mean SO_2 concentrations is 40-60 $\mu g\ m^{-3}$ (Saunders, 1985).

The behaviour of smoke plumes from stacks depends upon the prevailing weather conditions, depositing them nearby within a few days or carrying them away for hundreds of kilometers. The rate of dry deposition depends on properties of the receiving surfaces. The flux of SO_2 into the plant through the stomata may be a considerable part of the total dry deposition of SO_2 onto vegetation (Unsworth, Biscoe & Black, 1976). Cowling & Koziol (1982) estimated an annual sulphur deposition onto crops (deposition to both the leaf canopy and the soil) of 38 kg S $ha^{-1}\ yr^{-1}$, at a concentration of 30 $\mu g\ SO_2\ m^{-3}$, whereas the sulphur demands of grass and wheat amount to 12-23 kg S $ha^{-1}\ yr^{-1}$. The sulphur demand of non-fertilized, slowly growing vegetation will be much lower, because sulphur metabolism is strictly coupled to nitrogen metabolism (Dijkshoorn & van Wijk, 1967). The annual deposition of sulphur in the Netherlands was estimated at 22 kg $ha^{-1}\ yr^{-1}$ in 1986 (Anon., 1988). These data indicate that crops and vegetation are exposed to excess sulphur in large parts of Western Europe. However, excess sulphur can only be related to effects on plant growth, when deposition to soil and plant surfaces are separated from the flux of SO_2 into the leaf interior.

SO_2 effects on plant growth

Effects of SO_2 at all levels of biological organization have been reported (see reviews by Unsworth & Ormrod, 1982; Koziol & Whatley, 1984; Winner *et al.*, 1985; Schulte-Hostede *et al.*, 1988). Studies of SO_2 effects on crop production were mainly directed towards the quantification of yield losses, by description of data from fumigation experiments with dose-response relationships. An example is the relationship between the logarithm of dose (SO_2 concentration x exposure time) and yield loss. However, responses strongly vary between species, cultivars and exposure systems (Roberts, 1984; Bell, 1985; McLaughlin & Taylor, 1985). In most experiments in which the effects of air pollution on plant growth are studied, some form of enclosure is used in controlling the pollutant, ranging from indoor growth cabinets to open-top chambers in the field. The extrapolation of the effects observed in these studies to the field situation may lead to over or underestimation of the effects, because the environmental conditions differ from the field situation (Olszyk *et al.*, 1986) and because the growth of isolated plants, or poorly bordered plots strongly differs from the growth of crops in closed canopies (de Wit, 1968).

Open-air studies, in the absence of any form of plant enclosure have also been performed for many years. The exposure methodology in these studies ranged from

placing plants at different distances from point sources (like smelters), to gas-dispersing tubes, constructed in experimental field plots (McLeod, Fackrell & Alexander, 1985). Recently, the technique for open-air exposure has been strongly improved by the development of circular or square line sources in which a field crop of up to 100 m^2 can be exposed to reasonably uniform SO_2 concentrations by means of computer-controlled gas release. Although fluctuations occur within short time spans due to changes in windspeed, high concentration peaks are avoided. (Greenwood *et al.*, 1982; McLeod *et al.*, 1985; Mooi & van der Zalm, 1986).

Studies at the physiological (*e.g.* photosynthesis, transpiration and respiration) and biochemical level (*e.g.* membrane functioning, photosynthetic electron transport, carboxylation) have been mainly directed towards the explanation of subtle injury. The rate of photosynthesis at light saturation appears to be negatively correlated with the rate of uptake of SO_2 by the leaf (Black, 1982; Carlson, 1983; Winner & Mooney, 1980a, b). Quantum yield is not influenced by SO_2 (Black, 1982; Hållgren, 1984). Because the stomata are the primary sites where SO_2 enters the leaves, much research has concerned the effect of SO_2 on stomatal resistance. Both opening and closure of stomata has been observed at low concentrations of SO_2. At high SO_2 concentrations only stomatal closure has been observed (Black, 1982). Both stimulation and inhibition of dark respiration have been observed at low SO_2 concentrations (Black, 1984). In most studies the effect of SO_2 on a single process is analysed, making it difficult to determine the relative importance of the reported effects with respect to growth reductions.

The magnitude of SO_2 induced effects on plants is strongly influenced by plant and environmental factors, like plant species and cultivar, plant age, nutritional status, pollutant concentration, duration of the exposure and microclimatic factors as light intensity, relative humidity and temperature. In the field the situation becomes even more complex, because environmental factors are variable throughout the growing period and the effect of SO_2 may also be influenced by stress factors like drought or other air pollutants (see reviews in Unsworth & Ormrod, 1982; Koziol & Whatley, 1984; Winner *et al.*, 1985; Schulte-Hostede *et al.*, 1988).

Mechanistic understanding of SO_2 effects on plant growth

In spite of much research effort at various levels of integration (ranging from the biochemical level to the system level) in the past decades, an integrated understanding of plant responses to SO_2 has not been achieved yet. Quantitative explanation of the links between effects observed at the different levels of integration received minor attention. Mechanistic models may provide a useful tool to bridge the gaps in knowledge and may help to explain air pollutant effects at the system level, on the basis of phenomena observed at the biochemical and process level. Especially for prediction of long-term air pollutant effects on forests and vegetation this approach may be helpful to integrate effects

which are observed at the process level to estimate the impact at the system level, because experimentation at the system level is not feasible. These models may be used to estimate environmental benefits from proposed measures to reduce emission of air pollutants.

In the past decades, models for growth and development of crops and forests have been developed on current insight in physiological processes (de Wit *et al.*, 1978; Penning de Vries & van Laar, 1982; Mohren, 1987). Such models can be extended with submodels for SO_2 uptake by the canopy, the sulphur balance in the plant and the effects of sulphur metabolites on plant functioning. The number of processes included in the model depends on the production situation. For a crop growing at a potential production situation (optimal supply of water and nutrients and free of pests, diseases and weeds), effects of SO_2 metabolites on photosynthesis, respiration, dry matter partitioning and morphological development have to be quantified in submodels. However, when the approach is used to evaluate air-pollutant effects on systems growing at suboptimal conditions, processes related to the water and/or nutrient balance of the soil-plant system have to be included in the model. The interference of SO_2 with growth processes becomes far more complex, and such models require much more insight in SO_2 effects at the process level. Crop growth models for different production situations are available, although the models for the more complex production situations still have a preliminary status (Penning de Vries & van Laar, 1982; Rabbinge, Ward & van Laar, 1989).

The relatively few models that have been developed for analysis or prediction of SO_2 effects on plant growth and crop production have not been evaluated with independent experimental data sets, nor were they used for a detailed analysis of the mechanisms behind effects observed at the system level (Kercher & King, 1985; Krupa & Kickert, 1987; Luxmoore, 1988).

Objectives and approach

The central objective of this study was to quantitatively explain the effects of SO_2 on growth and production of crops based on insight in foliar SO_2 uptake, SO_2 metabolism in the plant, and the impact of SO_2 metabolites on physiological processes. This led to a combination of experimental and theoretical research at different levels of integration. To permit the quantitative analysis of the relations between phenomena observed at the different levels of integration, all experiments were conducted with broad beans (*Vicia faba* L., cv. Minica) cultivated at potential growing conditions. This species was found to be relatively sensitive for SO_2 with respect to subtle injury (Black & Unsworth, 1979b).

Because direct effects of SO_2 on physiological processes like photosynthesis are assumed to be responsible for subtle SO_2 effects on crop growth, a large part of this study was directed towards the development, parameterization and evaluation of mechanistic submodels for foliar SO_2 uptake, SO_2 metabolism in the plant, and the effects of SO_2 metabolites on physiological processes. The second part of the study deals with effects of SO_2 on growth and production of broad bean crops in newly developed equipment for the

exposure of field crops. The backgrounds of these effects were analysed with mechanistic crop growth models, extended with submodels developed in the first part of the study. A conceptual model was developed for the mechanistic understanding of chronic SO_2 effects in relation to regulation of intracellular pH and nutrient supply and uptake. This conceptual model may be used as a framework for the development of models for more complex production situations. The work presented in this thesis may provide a basis for the development of models for the effects of air pollutants on crops, forests and vegetation growing at more complex production situations.

Outline of the thesis

The structure of this thesis is illustrated in Fig. 1.1. Experimental and theoretical work on effects of SO_2 on physiological processes is presented in Chapters 2 - 6. The short-term effects of SO_2 on photosynthesis, stomatal behaviour and respiration are discussed in Chapter 2. Data from gas exchange measurements are interpreted in biochemical terms. On the basis of the findings presented in Chapter 2 and analysis of literature data from studies at the biochemical and physiological level, a simulation model for the uptake and short-term effects of SO_2 on leaf photosynthesis was developed. This model, described in Chapter 3, explains effects of SO_2 on photosynthesis on the basis of processes at the underlying biochemical level. Because parameterization of the model was hampered by the lack of suitable literature data, the model was used to determine parameter values for biochemical characteristics from data on photosynthetic responses to SO_2 in Chapter 4. In Chapter 5, the model was used to determine the role of temperature and humidity in photosynthetic reductions during SO_2 exposure. Data from gas exchange measurements, in which temperature and humidity were varied, were interpreted in biochemical terms by the model. The results were evaluated by experimental evaluation at the (bio)chemical level. The submodel for SO_2 effects on leaves was included in a model for canopy photosynthesis in order to determine SO_2 effects on photosynthesis of whole canopies in Chapter 6. Model performance was evaluated with experimental data from fumigation experiments with enclosed leaf canopies.

The experimental analysis of SO_2 effects at the system level is described in Chapters 7 and 8. In a newly developed open-air exposure system, broad bean crops were exposed to elevated SO_2 concentrations in three years. SO_2 effects were studied by analysis of growth, morphological development and elemental composition. The analysis of the mechanisms behind effects of SO_2 on crop growth and production using mechanistic simulation models is described in Chapter 9. A quantitative analysis of various damage components was performed with a mechanistic simulation model for crop growth, extended with the physiological models developed in first part of the study. Simulation of SO_2 uptake of by the plants as a part of the dry deposition to the crop, was evaluated using experimental data on sulphur uptake by the crops.

Because chronic effects played a major role in long-term effects of SO_2 on crop growth, a conceptual model for mechanisms of chronic effects from effects of SO_2 on intracellular pH regulation is presented in Chapter 10.

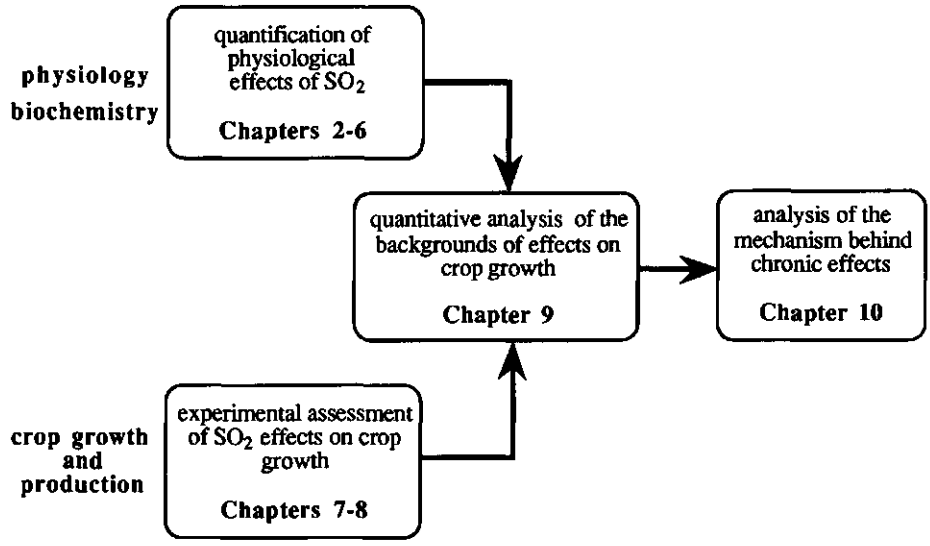


Fig 1.1 Outline of the thesis.

Chapter 2

SO_2 effects on photosynthesis and stomatal behaviour of *Vicia faba* L.

Abstract The effect of short-term SO_2 fumigation on photosynthesis and transpiration of *Vicia faba* L. was measured at different irradiances and SO_2 concentrations. At high irradiances photosynthetic rates were reduced when leaves were exposed to SO_2, and the magnitude of the reduction was linearly related to the rate of SO_2 uptake through the stomata. Photosynthetic rates stabilized within two hours after the start of fumigation.

The effect of SO_2 on photosynthesis was measured at different CO_2 concentrations to analyse the contribution of stomatal and non-stomatal factors to photosynthetic inhibition. Mesophyll resistance to CO_2 diffusion increased as a result of SO_2 exposure and caused a rapid reduction in photosynthesis after the start of fumigation. Stomatal resistance was not affected directly by SO_2 fumigation, but indirectly as a result of a feedback loop between net photosynthesis and internal CO_2 concentration.

Analysis of gas-exchange measurements in biochemical terms indicated that photosynthetic inhibition during SO_2 exposure can be explained by a stronger reduction in the affinity of RBP carboxylase/oxygenase for CO_2 than for O_2.

2.1 Introduction

Sulphur dioxide is one of the major gaseous air pollutants that cause damage to agricultural crops and natural vegetation. Exposure of plants to high concentrations of SO_2 can cause chlorosis and necrosis of leaf tissue which lead to reductions in growth. Reduced plant growth in the absence of visible injury has also been observed at relatively low ambient SO_2 concentrations (Lockyer, Cowling & Jones, 1976; Ashenden & Mansfield, 1977; Sprügel *et al.*, 1980). The magnitude of SO_2-induced effects on plant growth depends not only on pollutant concentration but also on plant status (physiological status dependent on plant age, growing conditions like nutrient availability, water supply, irradiance and temperature) and microclimatic factors (Black, 1982).

The rate of photosynthesis at light saturation appears to be negatively correlated with the rate of uptake of SO_2 through the stomata (Black, 1982; Black & Unsworth, 1979b;

Carlson, 1983; Winner & Mooney, 1980a, b). Photosynthetic light use efficiency is not influenced by SO_2 (Black & Unsworth, 1979b; Hållgren & Gezelius, 1982). Because the stomata are the primary sites where SO_2 enters the leaf tissue, much research has concerned the effect of SO_2 on stomatal resistance. Both stomatal opening and closure has been observed at low concentrations of SO_2. At high SO_2 concentrations only stomatal closure has been observed (Black, 1982).

Environmental factors such as windspeed, humidity and light intensity have a strong effect on stomatal responses to SO_2 (Black & Unsworth, 1979a). In recent studies attempts have been made to separate SO_2-induced effects on photosynthesis into stomatal and non-stomatal components. Non-stomatal factors (*e.g.* an increase in mesophyll resistance) appear to be primarily responsible for the reduction in photosynthesis (Barton, McLaughlin & McConathy, 1980; Winner & Mooney, 1980b). No consistent effects of SO_2 on dark respiration rates have been found. Both stimulation and inhibition of dark respiration have been observed at low SO_2 concentrations (Black, 1984). Ziegler (1975) concluded on the basis of *in vitro* studies that the biochemical mechanism of inhibition of net photosynthesis by SO_2 is competition between SO_2 and CO_2 for binding sites on the carboxylating enzyme RBP carboxylase/oxygenase. Gezelius & Hållgren (1980), however, suggested a non-competitive or a mixed effect from *in vitro* measurements with pine chloroplasts.

In the present study the short-term effects of SO_2 on photosynthetic characteristics of *Vicia faba* leaves are analysed quantitatively. The effect of SO_2 on stomatal regulation was analysed by making a time-dependent distinction between stomatal and non-stomatal components of photosynthetic changes. The results of the CO_2 gas exchange measurements are also interpreted in biochemical terms in an effort to relate these results to published results of *in vitro* measurements.

2.2 Materials and methods

Plant material and experimental system

Plants of *Vicia faba* L.(cv. Minica) were grown in 11 cm diameter plastic pots filled with a commercial potting mixture in a greenhouse at an average temperature of 16 °C and about 50% relative humidity. Supplementary illumination provided a photoperiod of 16 hours. The soil-moisture level was maintained at field capacity. CO_2 assimilation measurements were started when the plants were flowering and had about 14 leaves. The sulphur content of the leaves was 7.7 ± 0.6 mg S g^{-1} dry matter.

Rates of CO_2 assimilation, respiration and transpiration of the youngest fully unfolded leaf were measured with equipment for routine measurements of photosynthesis comparable to the type described by Louwerse & van Oorschot (1969). SO_2 was supplied from a cylinder (1000 ppm SO_2 in N_2) through a flowmeter and was injected into the air supply of the leaf chamber. Gas samples from the air lines leaving the chambers were

drawn continuously through teflon tubing and analysed with a Philips SO_2 gas analyser (type PW 9700). Relative humidity in the leaf chamber was about 40-50% and air temperature was 23 °C. The incoming SO_2 flow was continuously adjusted to prevent large changes in SO_2 concentration in the leaf chamber.

Calculations and experimental procedure

Data on differences in CO_2 concentration and water vapour content of the air stream entering and leaving the leaf chambers, temperature, irradiance and air humidity were recorded every 5 minutes by a microcomputer. SO_2 concentration was monitored as well. Measurements were performed during a prefumigation period of 2 hours to obtain stable rates, during a subsequent SO_2 fumigation period of 2 hours, and finally during a dark period of 1 hour. Rates of net photosynthesis and transpiration, stomatal resistance and internal CO_2 concentration were calculated following the procedure of Goudriaan & van Laar (1978), in which stomatal resistance to CO_2 is calculated from the transpiration rate, corrected for differences in diffusion coefficients between CO_2 and H_2O. Internal CO_2 concentration (C_i) is computed with the resistance model for CO_2 diffusion through the stomata from the rate of photosynthesis (P), external CO_2 concentration (C_a), stomatal resistance (r_s) and the experimentally determined boundary layer resistance (r_b): $C_i = C_a - P\,(r_s + r_b)$. The flux of SO_2 into the leaf interior was calculated by dividing the SO_2 concentration in the leaf chamber by the sum of the calculated stomatal resistance and an experimentally determined boundary layer resistance (about 7 s m^{-1}) for SO_2. The SO_2 concentration at internal leaf surfaces was assumed to be zero. This is a reasonable assumption because the resistance for SO_2 going into solution at the wet surface of the stomatal cavity is very low during short exposures (Unsworth *et al.*, 1976; Black & Unsworth, 1979a; Carlson, 1983). Since the cuticular resistance for SO_2 is extremely high compared to stomatal resistance, the flux of SO_2 through the cuticle is negligible (Unsworth *et al.*, 1976).

Three series of measurements were performed. In series 1 the effect of fumigation with 400 μg SO_2 m^{-3} on photosynthesis was measured at irradiances (visible 400-700 nm) ranging from 0-300 J m^{-2} s^{-1} at a constant ambient CO_2 concentration of 340 ppm to analyse the effect of SO_2 on the photosynthesis-light-response characteristics of leaves. The CO_2 assimilation-light-response curve for individual leaves can be described by a negative exponential function (Goudriaan, 1982):

$$P = (P_{max} + R_d)\left\{1 - \exp\left(\frac{-\varepsilon I}{P_{max} + R_d}\right)\right\} - R_d \qquad (2.1)$$

with:

P	= net CO_2 assimilation rate	(μg CO_2 m^{-2} s^{-1})
P_{max}	= net CO_2 assimilation rate at light saturation	(μg CO_2 m^{-2} s^{-1})

R_d	= dark respiration rate	(μg CO_2 m^{-2} s^{-1})
ε	= initial light use efficiency	(μg CO_2 J^{-1})
I	= absorbed radiation	(J m^{-2} s^{-1})

The parameters P_{max}, R_d and ε were determined by using an optimization program. In the second series of measurements the effect of SO_2 concentrations ranging from 0-800 μg SO_2 m^{-3} on photosynthesis was measured at light saturation (300 J m^{-2} s^{-1}) and a CO_2 concentration of 340 ppm. In the third series of measurements the effect of a single concentration of SO_2 (800 μg m^{-3}) on photosynthesis was measured at light saturation (300 J m^{-2} s^{-1}) and CO_2 concentrations ranging from 30-850 ppm CO_2. The confounding effect of differences in stomatal resistance was eliminated by relating the CO_2 assimilation rate to internal CO_2 concentration. This relationship can be described mathematically by (J. Goudriaan, pers. comm.):

$$P = P_{max,c}\left\{1 - \exp\left(\frac{-g_m (C_i - \Gamma)}{P_{max,c}}\right)\right\} \qquad (2.2)$$

with

P	= net CO_2 assimilation rate	(μg CO_2 m^{-2} s^{-1})
$P_{max,c}$	= net CO_2 assimilation rate at CO_2 saturation	(μg CO_2 m^{-2} s^{-1})
C_i	= internal CO_2 concentration	(μg CO_2 m^{-3})
Γ	= CO_2 compensation point	(μg CO_2 m^{-3})
g_m	= mesophyll conductance	(m s^{-1})

The mesophyll resistance to CO_2 is the inverse of the mesophyll conductance g_m ($r_m = 1/g_m$; dimension s m^{-1}).

Separation of stomatal and non-stomatal effects

The effect of SO_2 on the mesophyll resistance to CO_2 can be analysed by fitting Equation 2.2 to data on net photosynthesis at different CO_2 concentrations. Equation 2.2 cannot be used to analyse the effect of SO_2 on stomatal resistance because it relates photosynthesis to the internal CO_2 concentration. Several methods have been developed to quantify the relative importance of mesophyll and stomatal components to a change in photosynthetic rate during stress situations (Jones, 1985; Rabbinge, Jorritsma & Schans, 1985). Winner & Mooney (1980b) showed that both components contribute to a reduction in photosynthetic rates after fumigation, but did not analyse their relative contributions during the fumigation period. When both components are responsible for changes in photosynthesis their relative effects are 'path dependent' (Jones, 1985), which makes it necessary to analyse the time course of photosynthesis and C_i during fumigation. This is illustrated in Fig. 2.1. If the stomatal resistance increases, the internal CO_2 concentration

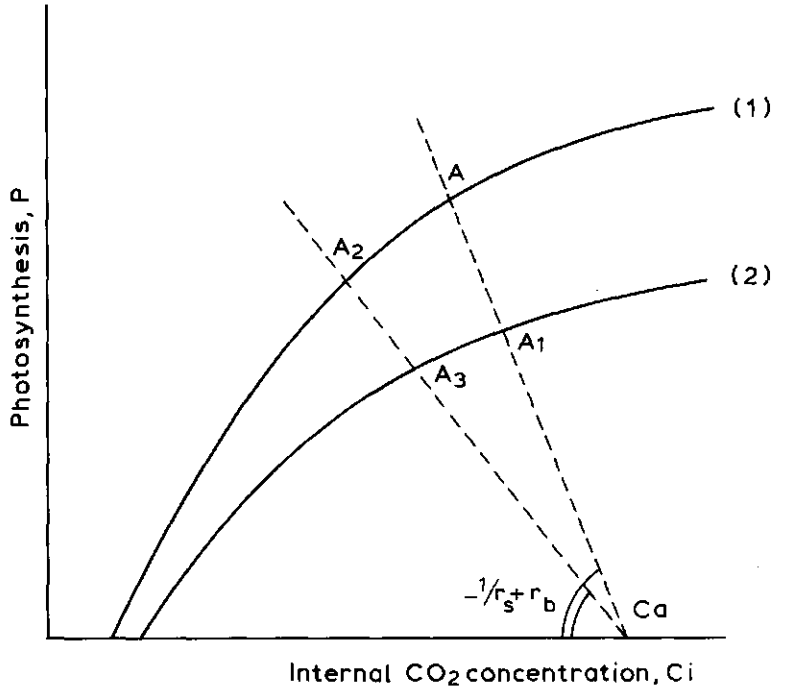


Fig. 2.1 Partitioning of stomatal and non-stomatal contributions to a change in net photosynthesis. When stomata change first, the trajectory will be A-A_2-A_3; when the mesophyll resistance changes first the trajectory will be A-A_1-A_3, The dotted lines represent the supply functions (C_i=C_a-$P(r_s+r_b)$ (where r_s and r_b are the stomatal and boundary layer resistance, resp.), with a slope of -1/(r_s+r_b). Solid lines represent the response of photosynthesis to varying internal CO_2 concentrations for control plants (1) and for stressed plants (2). After Jones (1985).

will drop, so that the photosynthetic rate will be reduced according to the photosynthesis-C_i curve (trajectory A-A_2). If the mesophyll resistance to CO_2 increases and stomatal resistance remains unchanged, C_i will increase according to the dotted line (trajectory A-A_1), representing the so-called 'supply function': a linear resistance model for CO_2 diffusion into the stomatal cavities. If both the stomatal and mesophyll resistance increase (A-A_3), the trajectory will be A-A_2-A_3 when stomata close first (relative contribution of the stomatal component is (A-A_2)/(A-A_3), and A-A_1-A_3 when the mesophyll resistance increases first (relative contribution of the stomatal component is (A_1-A_3)/(A-A_3)).

If a stress factor induces an increase in mesophyll resistance first, stomatal closure may subsequently occur as the result of the feedback loop between photosynthesis and stomatal resistance. This feedback loop results in a constant ratio between C_i and C_a (the ambient CO_2 concentration) which is about 0.7 for C_3 plants (Goudriaan & van Laar,

1978; Bell, 1982; Farquhar & Sharkey, 1982). This constant ratio can be used to describe stomatal behaviour in simulation models for crop growth. Stomatal resistance can then be calculated from the rate of photosynthesis using the resistance model for CO_2 diffusion through the stomata:

$$r_s = \frac{C_a - C_i}{P} - r_b \tag{2.3}$$

where r_b is the boundary layer resistance to CO_2, and r_s is the stomatal resistance. This procedure can be used for the calculation of canopy transpiration (Goudriaan, 1977, 1982; de Wit *et al.*, 1978) and can be used for the calculation of SO_2 uptake, when SO_2 does not alter stomatal behaviour. Any influence of SO_2 on stomatal behaviour will be reflected in the C_i/C_a ratio.

Biochemical interpretation of gas exchange measurements

The hyperbolic Michaelis-Menten equation can be used to analyse the biochemical mechanism of SO_2 inhibition of net photosynthesis with *in vivo* data on leaf photosynthesis at varying CO_2 concentrations (Edwards & Walker, 1983):

$$V = \frac{V_c (C_i - \Gamma)}{C_i + K_c \left(1 + \frac{O}{K_o}\right)} \tag{2.4}$$

where V is the net photosynthetic rate, V_c is the photosynthetic rate at high CO_2 concentrations, Γ is the CO_2 compensation point, K_c is the Michaelis-Menten constant for binding of CO_2 to RBP carboxylase/oxygenase, K_o is the inhibition constant due to O_2 competition and O is the oxygen concentration in the leaf. An expression for the mesophyll resistance (the inverse of the initial slope at the CO_2 compensation point) can be derived from this equation:

$$r_m = \frac{\Gamma + K_c}{V_c} \left(1 + \frac{O}{K_o}\right) \tag{2.5}$$

The CO_2 compensation point also can be interpreted in biochemical terms by means of the Michaelis Menten equations for carboxylation and oxygenation (Laing, Ogren & Hageman, 1974):

$$\Gamma = \frac{t V_o K_c O}{V_c K_o} \tag{2.6}$$

where t is the fraction of glycolate carbon released (0.5) and V_o the maximum rate of

oxygenation. If the mechanism of inhibition of net photosynthesis by SO_2 is competition between SO_2, CO_2 and O_2 for the binding sites of the RBP carboxylase/oxygenase, as suggested by Ziegler (1975), then the mesophyll resistance should increase as a result of SO_2 fumigation:

$$r_m = \frac{\Gamma + K_c}{V_c}\left(1 + \frac{O}{K_o} + \frac{S}{K_s}\right) \tag{2.7}$$

where S is the concentration of sulphur metabolites in the cells and K_s is the inhibition constant. The CO_2 compensation point, however, should remain unchanged.

2.3 Results and discussion

Inhibition of net photosynthesis in plants exposed to high concentrations of SO_2 has been reported by many researchers, but the effect of lower, more realistic concentrations (< 0.1 ppm) has seldom been analysed (Black, 1982).

A typical time response curve of net photosynthesis of fumigated and control plants at light saturation is shown in Fig. 2.2. A strong decrease in net photosynthesis of the fumigated plants occurred within the first 20 minutes of exposure to SO_2 and stable rates were obtained within 2 hours. This pattern is in agreement with the results of Black &

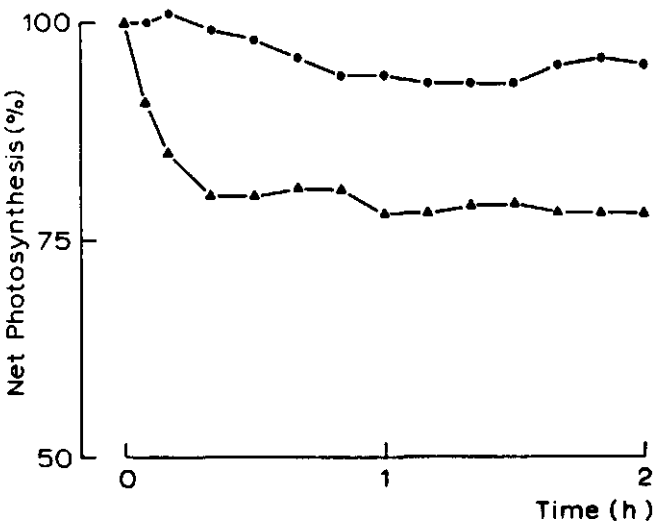


Fig. 2.2 Typical time course of net photosynthesis of *Vicia faba* leaves after the start of fumigation with 800 μg SO_2 m^{-3} for control plants (●) and fumigated plants (▲) at light saturation (mean values of 5 plants; SE did not exceed 4.9% of the mean in fumigated plants and 3.2% for control plants).

Unsworth, 1979b; Barton *et al.*, 1980; Sisson, Booth & Throneberry, 1981; Sij & Swanson, 1974; and Darrall, 1986. Because steady photosynthetic rates were obtained after a short fumigation period, it can be concluded that the concentration of toxic intermediate oxidation metabolites (sulphite, bisulphite) also reached stable values. These values depend upon the rate of uptake of SO_2 and the rates of oxidation of dissolved SO_2 to sulphate and the subsequent metabolites (Black & Unsworth, 1979b).

The CO_2 assimilation light-response curve was significantly affected by SO_2 fumigation. The fit of Equation 2.1 to the data is presented graphically in Fig. 2.3 and the estimated parameter values for photosynthetic rate at light saturation (P_{max}), dark respiration (R_d) and initial light use efficiency (ε) are given in Table 2.1. The estimated value of P_{max} decreased by 15% as a result of 2 hours of fumigation with 400 μg SO_2 m^{-3} ($P < 0.1$). Estimated dark respiration (R_d) increased as a result of fumigation with SO_2 but not significantly. The initial light use efficiency (ε) was not affected by SO_2 fumigation. The effect of SO_2 on the photosynthesis light-response curve of individual leaves (Fig. 2.3) was similar to that found for whole plants of *Vicia faba* (Black & Unsworth, 1979b). However, Black & Unsworth (1979b), found a much stronger effect of SO_2 on dark respiration rates. The difference may be explained by increased respiration in organs other than leaves. Contradictory reports on the effect of SO_2 on dark respiration in a number of studies (reviewed by Black, 1984) indicate the need for more detailed research. The absence of an effect of SO_2 on initial light use efficiency has also been observed by Hållgren & Gezelius (1982) for pine seedlings.

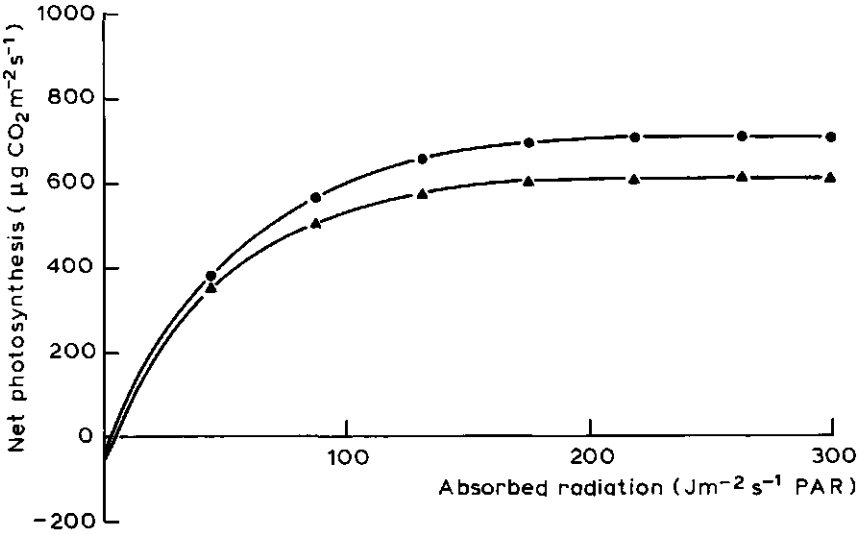


Fig. 2.3 Fitted net CO_2 assimilation light response curves for *Vicia faba* leaves before (●) and after (▲) a 2 hours fumigation period with 400 μg SO_2 m^{-3}. Markers do not indicate measured data.

Table 2.1 Estimated parameter values ± SE of CO_2 assimilation at light saturation (P_{max}), the initial light use efficiency (ε) and dark respiration (R_d) before and after a fumigation period of 2 hours with 400 μg SO_2 m^{-3} (n = 49).

		before	after
P_{max}	(μg CO_2 m^{-2} s^{-1})	724.0 ± 21.0	615.0 ± 15.0
ε	(μg CO_2 J^{-1})	13.9 ± 1.4	14.0 ± 1.3
R_d	(μg CO_2 m^{-2} s^{-1})	40.5 ± 11.8	48.8 ± 10.2

The effect of SO_2 on photosynthesis at light saturation was analysed in relation to the calculated flux of SO_2 into the leaf interior at the end of the fumigation period instead of the external SO_2 concentration. The rate of CO_2 assimilation after 2 hours of fumigation, relative to prefumigation rates, decreased linearly as the rate of SO_2 uptake increased from 0 to 3 μg m^{-2} s^{-1} (Fig. 2.4). This relation is similar to that reported by Black & Unsworth (1979c) over the range of rates employed here, but they found no further reduction in photosynthesis at rates of SO_2 uptake exceeding 1.5 μg m^{-2} s^{-1}. The reduction appeared to be reversible since prefumigation rates of photosynthesis were obtained when plants which had been fumigated with 800 μg m^{-3} SO_2 were measured after a 2 hour recovery period without fumigation.

The ratio of CO_2 assimilation to transpiration was not significantly affected by SO_2 fumigation (Table 2.2). The simultaneous reduction of CO_2 assimilation and transpiration may have been caused either directly by an increase in stomatal resistance or indirectly by an increase in mesophyll resistance.

Table 2.2 Ratio of rates of Photosynthesis and Transpiration (P/T) before and after a 2 hour fumigation period with SO_2 (n is number of replicates).

		P/T	
n	SO_2 (μg m^{-3})	before	after
4	100	7.37 ± 0.79	7.05 ± 0.70
4	200	8.97 ± 0.47	8.48 ± 0.56
14	400	9.88 ± 0.37	8.94 ± 0.27
6	800	9.02 ± 1.01	8.06 ± 1.44

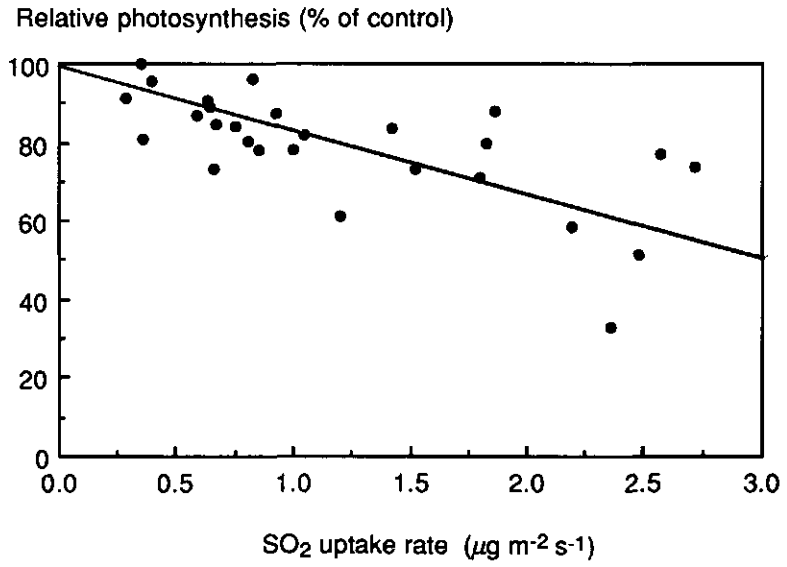


Fig. 2.4 Rates of CO_2 assimilation after a fumigation period of 2 hours relative to control rates before fumigation of *Vicia faba* leaves in relation to SO_2 uptake rates (F in μg SO_2 m^{-2} s^{-1}). P_n (% of control)=100 - 16.2 F (r^2=0.37, n=32).

The effect of fumigation with 800 μg SO_2 m^{-3} on CO_2 assimilation at light saturation and varying CO_2 concentrations is shown in Fig. 2.5. The estimated parameter values for the photosynthetic rate at high CO_2 concentrations ($P_{max,c}$), the mesophyll resistance (g_m) and the CO_2 compensation point (Γ) of plants fumigated with 800 μg SO_2 m^{-3} and of control plants are given in Table 2.3. The parameter values of the control plants did not change during the two hour period. At low concentrations of CO_2, the CO_2 assimilation rate was reduced by fumigation with SO_2, but at high CO_2 concentrations no effect of SO_2 fumigation could be detected (Fig. 2.5). Both the estimated CO_2 compensation point and the mesophyll resistance to CO_2 increased as a result of SO_2 exposure (Table 2.3). The lack of inhibition of CO_2 assimilation by SO_2 at high CO_2 concentrations was also reported by Carlson (1983) for soybean leaves. Black (1982) demonstrated that the suppression of the effects of SO_2 at high CO_2 concentrations was not caused by stomatal closure due to enhanced CO_2 concentrations by relating relative photosynthesis to the rate of SO_2 uptake.

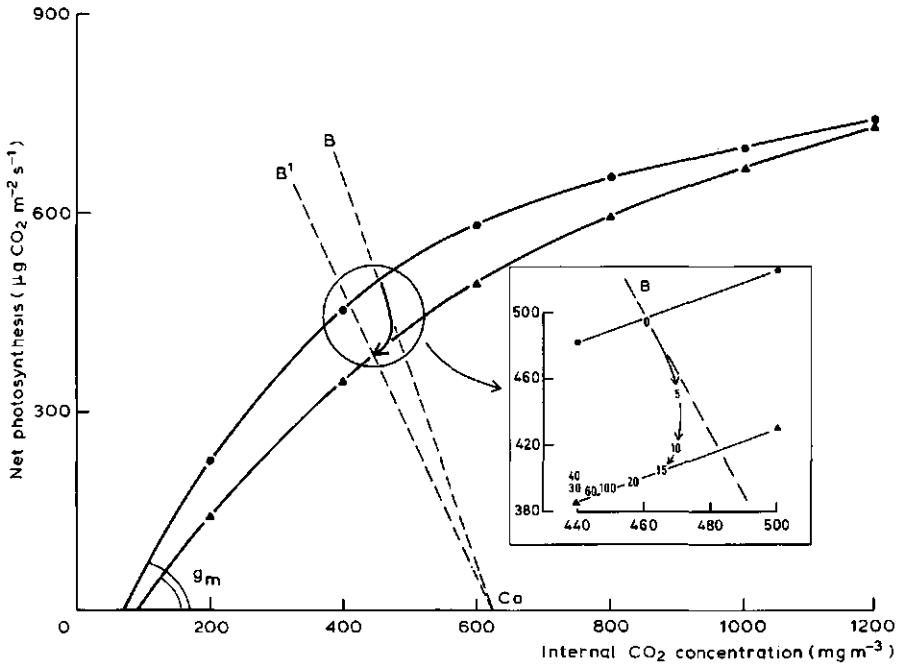


Fig. 2.5 Net CO_2 assimilation rate of *Vicia faba* leaves in relation to calculated internal CO_2 concentration (C_i) before (●), and after 2 hours (▲) fumigation with 800 μg SO_2 m^{-3} fitted with Equation 2.2. Markers do not indicate measured data. Dotted lines represent the CO_2 supply functions before (B) and after fumigation (B^1) of plants measured at an ambient CO_2 concentration of 340 ppm CO_2 (average value of five plants). The measured time course of the change in CO_2 assimilation and C_i of these plants is enlarged in the inset. The numbers give time in minutes after the start of fumigation.

Table 2.3 Values of CO_2 assimilation at high irradiance and high CO_2 concentration ($P_{max,c}$), mesophyll conductance of CO_2 (g_m) and the CO_2 compensation point (Γ) before and after a 2 hour fumigation period with 800 μg SO_2 m^{-3} (n= 22).

		$P_{max,c}$ (μg CO_2 m^{-2} s^{-1})	g_m (mm s^{-1})	Γ (mg CO_2 m^{-3})	
Fumigated	before	761 ± 55	2.1 ± 0.4	75 ± 9	(41 ± 5 ppm)
	after	830 ± 102	1.5 ± 0.3	98 ± 23	(53 ± 12 ppm)
Control	before	791 ± 34	3.3 ± 0.5	87 ± 10	(48 ± 6 ppm)
	after	766 ± 38	3.3 ± 0.6	85 ± 12	(46 ± 7 ppm)

The role of stomatal resistance in the observed reduction of the rate of CO_2 uptake was analysed by plotting the time course of leaf net photosynthesis versus C_i at an ambient CO_2 concentration of 340 ppm (Fig. 2.5 inset). A strong reduction in net photosynthesis occurred during the first 10 minutes of fumigation, with a trajectory that closely followed the CO_2 supply function (dotted lines), indicating that the reduction was entirely due to an increasing mesophyll resistance. An increase in stomatal resistance occurred later, as can be observed by following the trajectory of the photosynthesis-C_i curve in time. These results suggest that SO_2 induces an increase in mesophyll resistance which results in lowered photosynthetic rates. Stomata close later as a result of a feedback loop between net photosynthesis, internal CO_2 concentration and stomatal resistance. The constant ratio between internal CO_2 concentration and ambient CO_2 concentration both before and after fumigation (Table 2.4) support the conclusion that stomatal behaviour is not influenced by SO_2. Further analysis of Carlson's data (1983) showed that SO_2 did not affect the C_i/C_a ratio in soybeans either, supporting the conclusion that stomatal behaviour is not altered by SO_2.

Table 2.4 The ratio between internal CO_2 concentration (C_i) and external CO_2 concentration (C_a) before and after a 2 hour fumigation period at high irradiances (300 J m^{-2} s^{-1})

n	C_a (ppm)	SO_2 (μg m^{-3})	C_i/C_a	
			before	after
4	340	100	0.79 ± 0.02	0.79 ± 0.02
3	340	200	0.75 ± 0.01	0.76 ± 0.02
14	340	400	0.72 ± 0.01	0.70 ± 0.03
7	340	800	0.74 ± 0.02	0.70 ± 0.04
6	850	800	0.76 ± 0.04	0.75 ± 0.01

Several workers also found stomatal closure in *Vicia faba* plants and other species exposed to low concentrations of SO_2 at low relative humidity, but stomatal opening at high relative humidity (Majernik & Mansfield, 1971; Black & Unsworth, 1980). Black & Unsworth (1980) observed stomatal opening at both low and high relative humidity in *Phaseolus vulgaris*, while Temple, Cao Hong & Taylor (1985) observed stomatal closure in this species. Other workers reported no change or a slight reduction in stomatal conductance (Barton *et al.*, 1980) at low concentrations of SO_2 or reductions in stomatal conductance (*cf.* Müller, Miller & Sprügel, 1979; Olszyk & Tibbitts, 1981). The contradictory results of many studies were discussed by Black (1982) and Mansfield &

Freer-Smith (1984). The mechanism behind stomatal opening in response to SO_2 was analysed by Black & Black (1979) who observed damage in the epidermal cells of *Vicia faba* leaves surrounding the intact guard cells. Stomatal responses to light were unchanged. A possible explanation for the absence of such an effect in other studies could be a different physiological status of the plants used. In most studies, the effect of SO_2 on stomatal behaviour and photosynthesis are analysed separately. The method of analysis presented in this paper may help to obtain more insight into the interaction between various physiological reactions of plants during exposure to air pollutants.

From *in vitro* gas exchange measurements it appears that the effects of SO_2 are reversible and suppressed at high CO_2 concentrations (Fig. 2.5; Black, 1982; Carlson, 1983), which supports the competitive mechanism of SO_2 inhibition suggested by Ziegler (1975). From Table 2.3 it appears that both the CO_2 compensation point and the mesophyll resistance increased after SO_2 fumigation. An increase in the CO_2 compensation point was also reported by Furukawa, Natori & Totsuka (1980) and Jensen & Noble (1984). This increase in Γ indicates that the effect of SO_2 on CO_2 assimilation cannot be explained by an equal competitive effect of sulphur metabolites with respect to CO_2 and O_2. The observed increase in the CO_2 compensation point and mesophyll resistance can only be explained by a stronger effect of sulphur compounds on the affinity of the enzyme for CO_2 (K_c) than on its affinity for O_2 (K_o). These effects can be quantified in gas exchange measurements at a range of CO_2 concentrations at both normal and low oxygen concentrations to separate SO_2 effects on carboxylation and oxygenation of RBP carboxylase/oxygenase.

Symbols used in Chapter 2

C_a	ambient CO_2 concentration	(μg CO_2 m^{-3})
C_i	internal CO_2 concentration	(μg CO_2 m^{-3})
ϵ	initial light use efficiency of leaf photosynthesis	(μg CO_2 J^{-1})
g_m	mesophyll conductance	(m s^{-1})
Γ	CO_2 compensation point	(μg CO_2 m^{-3})
I	absorbed radiation	(J m^{-2} s^{-1})
K_c	Michaelis-Menten constant for binding of CO_2 to RBP carboxylase/oxygenase	(μg CO_2 m^{-3})
K_o	inhibition constant for CO_2 binding by O_2 competition	(μg O_2 m^{-3})
K_s	inhibition constant for CO_2 binding by SO_2 competition	(μg SO_2 m^{-3})
O	oxygen concentration in the leaf	(μg O_2 m^{-3})
P	net CO_2 assimilation rate	(μg CO_2 m^{-2} s^{-1})
P_{max}	net CO_2 assimilation rate at light saturation	(μg CO_2 m^{-2} s^{-1})
$P_{max,c}$	net CO_2 assimilation rate at CO_2 saturation	(μg CO_2 m^{-2} s^{-1})
R_d	dark respiration rate	(μg CO_2 m^{-2} s^{-1})
r_b	boundary layer resistance to CO_2	(s m^{-1})
r_s	stomatal resistance to CO_2	(s m^{-1})
r_m	mesophyll resistance to CO_2	(s m^{-1})
S	concentration of sulphur metabolites in the cells	(μg SO_2 m^{-3})
t	fraction of glycolate carbon released after oxygenation	(-)
V	net rate of carboxylation	(μg CO_2 m^{-2} s^{-1})
V_c	rate of carboxylation at high CO_2 concentrations	(μg CO_2 m^{-2} s^{-1})
V_o	maximum rate of oxygenation	(μg O_2 m^{-2} s^{-1})

Chapter 3

Modelling the flux of SO_2 into leaves and effects on leaf photosynthesis

Abstract A model for the flux of atmospheric SO_2 into leaves and the effects of SO_2 metabolites (S(IV) compounds) on leaf photosynthesis and stomatal resistance is presented. The S(IV) balance in the leaf is determined by the rate of SO_2 uptake and S(IV) removal by oxidation to sulphate. Toxic S(IV) compounds reduce the rate of photosynthesis and induce stomatal closure as a result of feedback control of stomatal resistance by photosynthesis. Other proposed mechanisms, like effects through a pH reduction, are not likely to play a role in short-term effects of realistic SO_2 concentrations. The model contains two key parameters which describe biochemical characteristics: a time coefficient for S(IV) oxidation and a parameter describing the sensitivity of photosynthesis for S(IV).

Simulation results demonstrate the potential of plants to avoid extremely toxic concentrations of S(IV) in the leaf by three mechanisms: (*i*) rapid oxidation of S(IV) to less toxic sulphate, (*ii*) relatively high resistance to SO_2 uptake and (*iii*) feedback control between photosynthesis and stomatal resistance. S(IV) concentrations in the leaf and SO_2 concentrations in the stomatal cavities in stable situations are less than 1% of concentrations which build up without these mechanisms. Leaf thickness appeared to be an important factor determining the susceptibility of plants to air pollutants. Thin leaves will be more sensitive than thicker leaves. It is concluded that effects of SO_2 on photosynthesis should be related to the uptake per unit of leaf volume instead of the commonly used flux per unit leaf area. The model accurately described the time course of photosynthetic reduction during a short fumigation period and subsequent recovery period.

3.1 Introduction

The effects of SO_2 on plants, studied extensively in the past decades, indicate generally depressing effects at different organization levels (cell, organ, plant, crop) (see reviews: Ziegler, 1975; Hållgren, 1978; Unsworth & Ormrod, 1982; Winner *et al.*, 1985). However, it is still impossible to predict the effects of specific SO_2 concentrations on plant

growth. This is mainly due to the modification of the effects of SO_2 on plant growth by environmental factors (like windspeed, irradiation, temperature, humidity) and by the physiological status of the plant, which strongly depends upon growth conditions (Black, 1982).

The effects of SO_2 on plants can be separated into reversible (*e.g.* effects of SO_2 on photosynthesis) and irreversible effects (*e.g.* leaf necrosis). Irreversible effects have been observed after short-term exposures to extremely high concentrations (ppm range) or during long-term exposures to more realistic concentrations (< 400 μg SO_2 m^{-3}). However, long-term exposures may result in decreased plant growth at concentrations as low as 40-400 μg SO_2 m^{-3} without causing visible injury (Bell *et al.*, 1979; Ashenden & Mansfield, 1977; Sprügel *et al.*, 1980).

The influence of various factors on the intensity of SO_2 induced effects on plants makes it difficult to interpret and generalize results of fumigation experiments, performed in indoor-fumigation chambers and open-top chambers (Black, 1982). This also holds for experimental assessment of air pollutant effects on crop growth and production and on natural vegetation under field conditions. Moreover, the few systems developed for open-air exposure of vegetation are too expensive to allow sufficient replicates to obtain reliable data on air pollutant effects in the field.

The large variation in growth responses of plants to SO_2 reflect differences in effects at a metabolic level. Prediction of effects should be based upon quantitative insight into the links between the effects of SO_2 observed at different levels of organization and their interactions with other environmental factors. Deterministic models for crop growth may provide a tool for quantitative evaluation of the complex links between SO_2 effects on plant metabolism and effects on plant growth and production (de Wit *et al.*, 1978; Penning de Vries & van Laar, 1982). Such models enable the integration of existing knowledge on ecophysiological processes that determine crop growth, including the effects of air pollutants on those processes. Since air pollutants can influence physiological processes only upon entrance in cellular solutions, submodels are needed to quantify air pollutant fluxes into the leaf, the metabolism of pollutants in cellular solutions and the effects of toxic metabolites on physiological processes. These submodels should explain the wide range of photosynthetic responses which have been reported on the basis of quantitative insight in effects at the biochemical level.

Kercher (1978) developed a very detailed submodel at the leaf level for the uptake and effects of SO_2 and H_2S. Laisk *et al.* (1988a) and Laisk, Pfanz & Heber (1988b) modelled the effect of SO_2 on intracellular pH. Both models require many parameters and have not been tested with independent data sets on the effect of SO_2 on photosynthesis Moreover, as will be shown in this chapter, it is unlikely that short-term effects of SO_2 on photosynthesis are due to changes in cellular pH.

In Chapter 2, the short-term effects of relatively low SO_2 concentrations on photosynthesis and stomatal behaviour were analysed quantitatively for broad bean leaves (*Vicia faba* L.). It was shown that exposure to SO_2 increased the mesophyll resistance for CO_2, resulting in reduced rates of photosynthesis at high radiation levels. Stomatal

behaviour was not directly influenced by SO_2. In the study presented here, the flux of SO_2 into leaves and short-term effects of SO_2 on photosynthesis are modelled in order to find a quantitative mechanistic explanation for the observed effects. Such models may also provide a tool to single out a possible difference in the mechanisms underlying short-term and long-term effects.

In this Chapter, a model for the effect of SO_2 on gas exchange of leaves is derived on the basis of reported effects at the biochemical level. The model simulates foliar SO_2 uptake, the balance of toxic SO_2 metabolites in the leaf, their direct effects on photosynthesis and their indirect effects on stomatal behaviour. The objectives of this study are to explain and predict effects at the leaf level based on as few processes as possible. In a subsequent paper (Chapter 4), species characteristics which determine SO_2 effects on photosynthesis of single leaves will be quantified from analysis of experimental data.

3.2 General structure of the model

A schematic representation of the dynamic simulation model for the SO_2 flux into the leaf and for effects of sulphur metabolites on photosynthesis is given in Fig. 3.1. The central state variable in the model is the amount of toxic S(IV) compounds in the leaf: sulphur dioxide $[SO_2]_{aq}$, bisulphite $[HSO_3^-]$ and sulphite $[SO_3^{2-}]$.

The rate of SO_2 uptake by the leaf is calculated from the difference between the ambient SO_2 concentration, the SO_2 concentration in the stomatal cavities and from the leaf resistance for diffusion of SO_2 into the leaf. Stomatal regulation is described on basis of the observation that the internal CO_2 concentration in the stomatal cavities tends to be constant at a given ambient CO_2 concentration (Chapter 2). The leaf resistance to CO_2 is calculated from the rate of photosynthesis and a preset value of the internal CO_2 concentration. From the leaf resistance to CO_2 the resistance to SO_2 can be calculated. The reaction of stomatal behaviour to humidity can be included in the model by introduction of a relation between the vapour pressure deficit (VPD) and the ratio between internal and ambient CO_2 concentration (Morison, 1987). The influence of possible direct effects on stomata (like the opening of stomata during SO_2 exposure which has been observed by several workers (*cf.* Mansfield & Freer-Smith, 1984)) can be analysed with the model, although more quantitative information should come available. The SO_2 concentration in the stomatal cavities is assumed to be in equilibrium with the SO_2 concentration in the aqueous phase in the leaf. This equilibrium is determined by the solubility of SO_2 in water. The amount of SO_2 dissolving and solution pH (which is between 7 and 8 for the cytosol where SO_2 first enters) determine the amount of protons produced and the distribution of S(IV) over the different dissociation products. Since the model is developed for short-term effects at realistic SO_2 concentrations, effects on cellular pH (Laisk *et al.*, 1988a, b) are negligible as will be shown later in this Chapter.

Toxic S(IV) compounds are oxidized to sulphate (Asada & Kiso, 1973), which is less toxic than sulphite (Marques & Anderson, 1986). Sulphate can also be reduced to S^{2-} which can be incorporated into organic compounds (Rennenberg, 1984) or emitted as H_2S (Hållgren & Frederiksson, 1982). The toxic S(IV) compounds influence the rate of photosynthesis by competition with CO_2 (and O_2) for binding sites at RBP carboxylase/oxygenase (Chapter 2). The model operates with time steps of 1 minute.

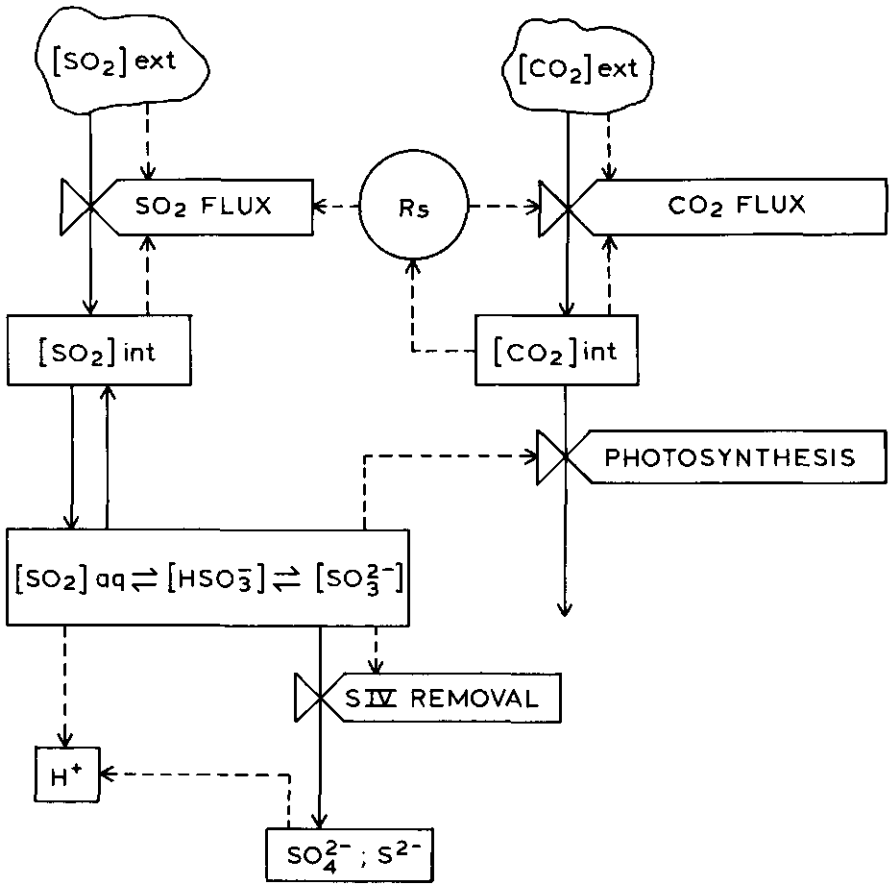


Fig. 3.1 A relational diagram of the model for fluxes of SO_2 and CO_2 into the leaf and the effects of S(IV) on photosynthesis. Boxes represent state variables (amounts); circles contain intermediate variables; valve symbols represent rate variables; solid lines indicate flows of material and broken lines flows of information. R_S represents the stomatal resistance.

3.3 Model description

SO_2 flux into the leaf

For the calculation of SO_2 effects on leaf photosynthesis, the fluxes of SO_2 and CO_2 through the stomata into the leaf interior must first be quantified. The fluxes of SO_2, CO_2 and water vapour are coupled, since they all pass the same diffusion barriers: the aerodynamic boundary layer resistance which is governed by the windspeed, and the stomatal resistance, determined by plant physiological processes. The sum of these resistances is often called the leaf resistance. The use of resistance models based upon electrical analogy for diffusion processes of air pollutants has been discussed by Unsworth *et al.* (1976). The most important pathway of air pollutants into the leaf interior are the stomata, since the cuticular resistance is at least ten times greater than stomatal resistance (Black & Unsworth, 1979a).

Stomatal regulation is modelled on the basis of a feedback loop between internal CO_2 pressure and photosynthesis, which has been observed for many plant species (Goudriaan & van Laar, 1978; Wong, Cowan & Farquhar, 1979; Louwerse, 1980; Bell, 1982; Farquhar & Sharkey, 1982). This feedback loop results in a constant ratio between internal (C_i) and ambient CO_2 concentration (C_a), which is about 0.7 for C_3 plants and 0.4 for C_4 species. It has been shown that stomatal behaviour, characterized by constant C_i/C_a ratios in broad bean leaves was not influenced by SO_2 after a 2-hour fumigation period at 0-800 μg SO_2 m^{-3} (Chapter 2). Analysis of data of Carlson (1983) did not show an SO_2 effect on C_i/C_a ratios for soybean leaves either. With data from long-term fumigation experiments, Saxe (1983) showed that the rates of photosynthesis and transpiration remained closely correlated, indicating that a direct effect of SO_2 on stomatal behaviour is unlikely also in the long run.

Several workers observed also stomatal closure at low humidity, but stomatal opening at high humidities (Majernik & Mansfield, 1971). Black & Unsworth (1980) however, observed stomatal opening during fumigation at both high and low relative humidities in beans while Temple *et al.* (1985) observed stomatal closure in the same species. Others reported no changes in stomatal resistance at low SO_2 concentrations (Barton *et al.*, 1980) or reduced stomatal resistance (*cf.* Olszyk & Tibbitts, 1981). Black & Black (1979) analysed the mechanism behind stomatal opening and observed damage in the epidermal cells surrounding the guard cells. However, stomatal responses to radiation remained relatively unchanged. The contradictory results of these and other studies has been discussed by Black (1982) and Mansfield & Freer-Smith (1984). The problem in most studies on stomatal reactions to SO_2 is that photosynthesis and stomatal reactions are not analysed simultaneously.

When it is assumed that direct effects on stomata do not occur and stomatal behaviour remains intact as observed in a previous study (Chapter 2), leaf resistance during SO_2 exposure can be computed from the photosynthetic rate and a preset value of the internal CO_2 concentration, using the linear resistance model for CO_2 diffusion into the

leaf interior (Gaastra, 1959):

$$r_s = \frac{C_a - C_i}{P} - r_b \qquad (3.1)$$

where r_s and r_b are the stomatal and boundary layer resistances to CO_2 (s m^{-1}) respectively, P is the rate of photosynthesis (μg CO_2 m^{-2} s^{-1}), and C_a and C_i are the ambient and internal CO_2 concentrations (μg CO_2 m^{-3}), respectively. The standard version of the model assumes that changes in stomatal resistance and changes in photosynthesis rates occur simultaneously. In practice however the stomatal reaction to a changed photosynthesis is delayed by about 5-10 minutes (Chapter 2). The effect of this delay is analysed with an adapted version of the model. The influence of a direct effect of SO_2 on stomata could be evaluated with a modified version of the model.

To account for differences in diffusion characteristics between CO_2 and SO_2, the boundary layer and stomatal resistance for SO_2 ($r_{b,s}$ and $r_{s,s}$) can be calculated from their molecular weights (M) (which determines their diffusion coefficient) (Monteith, 1973; Unsworth *et al.*, 1976) with :

$$\frac{r_{s,s}}{r_s} = \left(\frac{M_{CO_2}}{M_{SO_2}}\right)^{-\frac{1}{2}} \qquad \frac{r_{b,s}}{r_b} = \left(\frac{M_{CO_2}}{M_{SO_2}}\right)^{-\frac{1}{3}}$$

This results in:

$$r_{s,s} = 1{\cdot}21\, r_s \quad ; \quad r_{b,s} = 1{\cdot}13\, r_b \qquad (3.2)$$

The rate of SO_2 uptake can be calculated in analogy with CO_2 diffusion by:

$$F = \frac{S_a - S_i}{r_{s,s} + r_{b,s}} \qquad (3.3)$$

in which S_a and S_i represent the ambient and internal (in the gas phase!) concentrations of SO_2 (mmol m^{-3}), respectively, and F is the rate of SO_2 uptake (mmol m^{-2} s^{-1}). The uptake of SO_2 through the cuticle is negligible (Unsworth *et al.*, 1976).

Sulphur balance in the leaf

Inside the leaf, SO_2 dissolves in the aqueous phase at the cell wall. The internal gaseous SO_2 concentration (S_i) in the stomata just above the aqueous cell walls is assumed to be in equilibrium with the aqueous SO_2 concentration in the leaf ($[SO_2]_{aq}$) and can be

calculated with the solubility constant H ca. 33 at 20 °C for SO_2 (Cape, 1984):

$$[SO_2]_g = \frac{[SO_2]_{aq}}{H} \tag{3.4}$$

It is assumed that the protons which are released when SO_2 reacts with water, are buffered by metabolic and chemical mechanisms. This is a reasonable assumption for short-term exposures, since cellular solutions have high buffering capacities which are mainly based upon metabolic processes (biochemical reactions, such as the synthesis or breakdown of organic acids), instead of chemical buffering mechanisms based upon chemical equilibria (Raven, 1986). Many biochemical reactions in cells involve the production or consumption of protons. Smith & Raven (1979) suggest that the pre-existing buffer capacity of cellular solutions is effective in countering pH changes of 0.2-0.3 for a few minutes only. However, leaf cells have a strong capacity to maintain their internal pH. This was illustrated by Sakaki & Kondo (1985) and Smith & Raven (1979) who showed that protoplasts suspended in an acidic medium can maintain their pH. Roberts *et al.* (1981) provided evidence for regulation of cytosolic pH. They measured the rate of proton efflux (stimulated by K^+) by titration. From the buffer capacity of cells it was expected that pH would rise with 0.6 pH unit per hour. In practice however, no pH changes were detected.

When metabolic buffering mechanisms in the cells *in vivo* are neglected, no pH changes influencing photosynthesis are to be expected during short-term exposure to SO_2, as will be illustrated in the following. When leaves are exposed to high SO_2 concentrations for short periods (1350 μg SO_2 m^{-3} for 1 hour) strong effects on photosynthesis can be observed (up to 40 % reduction; Darrall, 1986). The H^+ production rate in leaves (Darrall, 1986) can then be calculated using the following assumptions: leaf resistance for H_2O equals 280 s m^{-1}, about 1.5 mol protons are produced per mol SO_2 dissolved, internal SO_2 concentration is zero and leaf thickness is 0.3 mm. The buffering capacity of the cellular solution in isolated barley protoplasts was estimated at 35 mol H^+ m^{-3} pH unit $^{-1}$ by Pfanz & Heber (1986). The proton production in this example is 0.72 mol H^+ m^{-3} leaf h^{-1}. This should lead to a pH reduction of 0.02 pH unit per hour, which is negligible. Even if the flux into the vacuole is neglected, since SO_2 is trapped mainly in the alkaline cytosol and in the chloroplasts (which cover 40% of the total buffering capacity; Pfanz & Heber, 1986), the decrease will be not more than 0.05 pH unit per hour. Thus, even when metabolic buffering processes are not considered, no effects of SO_2 on cellular pH are to be expected.

The S(IV) concentration in the leaf consists of 3 components:

$$[S(IV)] = [SO_2]_{aq} + [HSO_3^-] + [SO_3^{2-}] \tag{3.5}$$

The concentrations of these 3 components are interrelated by the following dissociation

reactions:

$$SO_2 + H_2O \Leftrightarrow HSO_3^- + H^+$$

$$HSO_3^- \Leftrightarrow SO_3^{2-} + H^+$$

In equilibrium, the concentrations will be related according to:

$$K_1 = \frac{[HSO_3^-]\ [H^+]}{[SO_2]_{aq}} \tag{3.6}$$

$$K_2 = \frac{[SO_3^{2-}]\ [H^+]}{[HSO_3^-]} \tag{3.7}$$

The equilibrium constant of the first reaction is about 0.0148 mol l^{-1} (K_1) and of the second reaction $7 \cdot 10^{-8}$ mol l^{-1} (K_2) (at 20 °C ; Cape, 1984). The concentration of the various compounds is pH dependent: below pH 7 the dominant compound is HSO_3^- and above pH 7 the SO_3^{2-} concentration becomes more important. When SO_2 influxes are low, the leaf will be able to buffer the protons produced during SO_2 uptake, and maintain a constant pH. When the SO_2 influx is extremely high or SO_2 is absorbed by solutions with a low buffer capacity, the pH will be reduced. The relative concentrations of S(IV) compounds will change, necessitating the use of iterative procedures for the calculation of equilibrium concentrations (Laisk *et al.*, 1988a). For situations in which we are interested, changes in pH may be neglected, so that the concentration of $[SO_2]_{aq}$ can be calculated directly from the total S(IV) concentration in the leaf and a preset value for the pH of the cellular solution by combining Equations 3.5, 3.6 and 3.7:

$$[SO_2]_{aq} = \frac{[S(IV)]}{1 + \frac{K_2}{[H^+]}\left(1 + \frac{K_2}{[H^+]}\right)} \tag{3.8}$$

The concentrations of the other S(IV) compounds can be similarly calculated.

For the calculation of the S(IV) balance it is necessary to include the processes by which the S(IV) is removed from cellular solutions. S(IV) may be oxidized to sulphate by atmospheric oxygen or by a photo-induced enzymatic oxidation process (Asada & Kiso, 1973; Kondo *et al.*, 1980). Sulphate may be transported into the vacuole or reduced to sulphide, which can be released as H_2S (Hällgren & Frederiksson, 1982; Rennenberg & Filner, 1982), or incorporated in organic compounds. The process of S(IV) removal is described here as a first-order reaction with respect to its concentration. In the model developed by Kercher (1978) it is assumed that the rate of S(IV) oxidation follows a Michaelis-Menten shaped curve. This resulted in the explanation of a threshold concentration of ambient SO_2, below which no effects should occur, because S(IV) is

removed quickly at low concentrations (first order kinetics), but when concentrations are above the threshold (the point where the Michaelis Menten curve bends off), the rate of S(IV) removal is constant (not proportional), resulting in strong accumulation of S(IV). However the data of Alscher, Bower & Zipfel (1987) and Miller & Xerikos (1979) show that S(IV) removal is a first order reaction, even in situations where photosynthesis is reduced by 80%. The values for the time coefficient for sulphite removal ranged from 1200-3000 seconds. Because it is generally accepted that oxidation is the main process determining the removal of S(IV), the removal will be indicated as oxidation in the rest of this thesis.

The change in S(IV) concentration (mmol S(IV) l^{-1}) can be described with the differential equation:

$$\frac{dS(IV)}{dt} = \frac{F}{d} - \frac{S(IV)}{\tau_2} \qquad (3.9)$$

where τ_2 is the time coefficient for S(IV) removal (s), and F is the rate of SO_2 uptake (in mmol m^{-2} s^{-1}) and d is the leaf thickness in mm.

Effects on photosynthesis

Based on the underlying biochemical mechanisms, the explanation of observed pollutant effects on photosynthesis is complex. A large number of effects at the biochemical level have been reported (review by Hållgren, 1978; Malhotra & Khan, 1984). In most studies, photosynthesis is related to the SO_2 concentration or rate of SO_2 uptake. Such studies, however, give no information on the underlying mechanisms. Most *in vitro* studies in which the metabolic mode of action is analysed, indicate an effect of S(IV) on the carboxylation process, which can be interpreted as a competitive inhibition of the binding of CO_2 to RBP carboxylase/oxygenase by SO_2 (Ziegler, 1975; Hållgren, 1978). However, conflicting data were published recently by Gezelius & Hållgren (1980) who reported non-competitive inhibition while Khan & Malhotra (1982) observed competitive inhibition and confirmed the conclusions of Ziegler (1975). Parry & Gutteridge (1984) observed a mixed type of inhibition and discussed the complexity of interpretation of *in vitro* experiments. A large number of factors may have contributed to the reported differences, like the use of enzymes with low activity. Black (1982), Carlson (1983) and Kropff (1987) observed that SO_2 effects decrease at higher CO_2 concentrations, which indicates a competitive or mixed inhibition. On the basis of mathematical analysis of *in vivo* measurements on leaf photosynthesis, it was demonstrated in Chapter 2 that the mechanism could be based upon differences in competitive inhibition of O_2 and CO_2 binding by SO_2.

Other *in vitro* studies show effects of SO_2 on processes coupled to the light reactions of photosynthesis. However, Alscher *et al.* (1987) showed that even at high

concentrations (800 ppb SO_2) effects on light reactions of photosynthesis are of minor importance. Recently, Pfanz *et al.* (1987) suggested another mechanism of photosynthetic inhibition by SO_2 based on changes in the cellular pH. However, they used protoplasts suspended in solutions containing very high sulphite concentrations. Earlier in this Chapter, using data from Pfanz & Heber (1986) and Darrall (1986), it was calculated that pH decreased less than 0.05 pH unit after 1 hour fumigation with 1350 μg SO_2 m^{-3}. According to data from Pfanz & Heber (1986) and Sakaki & Kondo (1985) the decrease in photosynthesis is about 40% per pH unit decrease. Thus, for the data of Darrall (1986) a photosynthetic reduction of 2% is expected when metabolic buffering is neglected, whereas 40% reductions were observed for some species. Another argument against the mechanism proposed of Pfanz *et al.* (1987) is the reduction of SO_2 induced effects at enhanced CO_2 concentrations at equal SO_2 uptake rates (Black, 1982), whereas higher CO_2 concentrations would be expected to cause an even larger reduction of cellular pH.

In a number of studies it has been shown that translocation of sugars is inhibited to a larger extent than the rate of photosynthesis (Noyes, 1980; Jones & Mansfield, 1982; McLaughlin *et al.*, 1982; Teh & Swanson, 1982; Lorenc-Plucinska, 1986). However, it is not likely that photosynthesis is affected in the short-term by a feedback effect of accumulating sugars resulting from inhibited translocation, because no relationship exists between photosynthetic reduction and inhibition of translocation (Noyes, 1980). Moreover, the recovery of translocation rate after an exposure period is very slow (Noyes, 1980; Teh & Swanson, 1982; Lorenc-Plucinska, 1986), whereas photosynthesis recovers very rapidly (Chapter 2). The observed suppression of SO_2 effects at elevated CO_2 concentrations (Black, 1982; Carlson, 1983; Chapter 2) also contradicts with such an explanation.

Sakaki & Kondo (1985) showed that reductions in photosynthesis depend upon the intracellular S(IV) concentration. They found a constant relative reduction of photosynthesis within isolated *Vicia faba* chloroplast at different pH values in the medium. In addition, the rate of photosynthesis appeared to be very sensitive to the pH of the medium in which the protoplasts were suspended. This indicates that possibly both HSO_3^- and SO_3^{2-} or $[SO_2]_{aq}$ compete with CO_2 for RBP carboxylase/oxygenase, as their relative concentrations vary with pH.

The relationship between intracellular S(IV) and photosynthesis of leaves *in vivo* has been rarely studied. The only data where both S(IV) concentrations and photosynthesis have been measured are from Alscher *et al.* (1987), who studied photosynthesis of *Pisum sativum* during SO_2 exposure *in vivo*, and from Sakaki & Kondo (1985) who used isolated *Vicia faba* protoplasts and chloroplasts in sulphite solutions. Their data were used to construct Fig. 3.2, which relates the rate of photosynthesis to the intracellular sulphite concentration. The relation between sulphite concentration and photosynthesis appeared to be similar for both varieties of *Pisum sativum* (although they strongly differed in sensitivity) and for the isolated protoplasts of

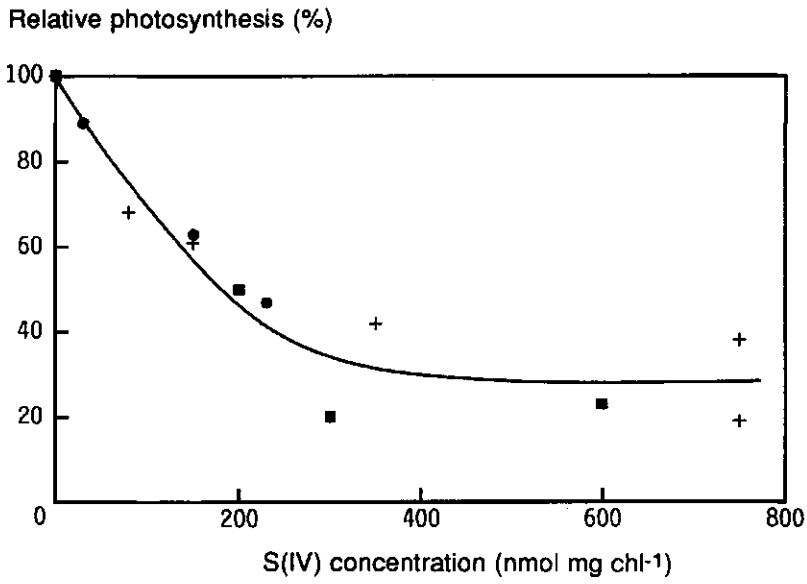


Fig. 3.2 The relation between the relative rate of photosynthesis and intracellular S(IV) concentration (nmol mg^{-1} chlorophyll). Data derived from Alscher *et al.* (1987) (*Pisum sativum* cv. Nugget (■) and cv. Progress (●)) and Sakaki & Kondo (1985) (*Vicia faba* cv. Otafuku (+)). Curve was fitted by hand.

Vicia faba (Fig. 3.2). The extreme difference in sensitivity of photosynthesis to ambient SO_2 between the two pea varieties (Alscher *et al.*, 1987) is clearly not based upon its sensitivity to the sulphite concentration. The relationship between sulphite and photosynthesis is linear up to photosynthetic reductions of 70%, which have been observed at extremely high SO_2 concentrations (2160 μg SO_2 m^{-3}) (Alscher *et al.*, 1987). For realistic situations below 200 nmol S(IV) mg^{-1} chlorophyll, a simple linear relationship can be used:

$$P = P_0 (1 - k\ S(IV)); \ S(IV) < 200 \text{ nmol mg}^{-1} \text{ chl.} \qquad (3.10)$$

where P_0 is the rate of photosynthesis before fumigation and k is a constant which describes the relative effect of S(IV) on photosynthesis. The value for k was estimated from Fig. 3.2 (0.0025 [nmol S(IV) (mg chl.)$^{-1}$]$^{-1}$). Sakaki & Kondo (1985) reported that the volume of the protoplasts they used was 0.33 ml (mg chl.)$^{-1}$, so that the k value on a volume basis was 0.825 (mmol S(IV) $l^{-1})^{-1}$ (=1 x 10^{-5} (μg S(IV) $l^{-1})^{-1}$). The value of k can also be calculated on a leaf area basis from its leaf thickness (*i.e.* for 0.4 mm thick leaves (measured on greenhouse-grown *Vicia faba* plants, by measuring both the leaf area

and the leaf volume, by putting the leaf in a measuring glass with water), k equals 2.05 (mmol S(IV) $m^{-2})^{-1}$ or 0.000025 (μg S(IV) $m^{-2})^{-1}$.

Effects at variable radiation levels

The photosynthesis-light response of individual leaves can be described by:

$$P_n = P_{max} \left\{ 1 - \exp\left(\frac{-\varepsilon I}{P_{max}} \right) \right\} \quad (3.11)$$

where P_{max} (μg CO_2 m^{-2} s^{-1}) is the rate of photosynthesis at light saturation, ε is the initial light use efficiency (μg CO_2 J^{-1}) and I is the absorbed radiation (PAR in J m^{-2} s^{-1}). It has been shown that SO_2 influences the rate of photosynthesis only at high levels of irradiation (Black, 1982; Hållgren, 1984; Chapter 2), so that the effect of S(IV) on photosynthesis can be described with an adapted version of Eqn. 3.10:

$$P_{max,s} = P_{max,0} (1 - k \ S(IV)) \quad (3.12)$$

where $P_{max,0}$ is the maximum rate of photosynthesis for control leaves and $P_{max,s}$ is the maximum rate of photosynthesis during exposure to SO_2. Since the rate of sulphite oxidation in leaves is light dependent (Rothermel & Alscher, 1985), the time coefficient for sulphite oxidation should be made light-dependent in the model when used for simulation of photosynthesis at low radiation levels, but more quantitative information should come available.

3.4 Results and discussion

Model behaviour was analysed at a concentration of 100 μg SO_2 m^{-3} during the first 1000 minutes after the onset of SO_2 exposure. Model parameters were based upon own

Table 3.1 Standard parameters used in the simulation model for the effects of SO_2 on photosynthesis of leaves.

pH of the leaf solution		7.5
P_0	(initial rate of photosynthesis)	1000 μg CO_2 m^{-2} s^{-1}
k	(sensitivity parameter)	0.825 (mmol S(IV) $l^{-1})^{-1}$
C_i/C_a	(ratio internal and ambient CO_2)	0.75
d	(leaf thickness)	0.4 mm
τ_2	(time coefficient for S(IV) oxidation)	2400 s

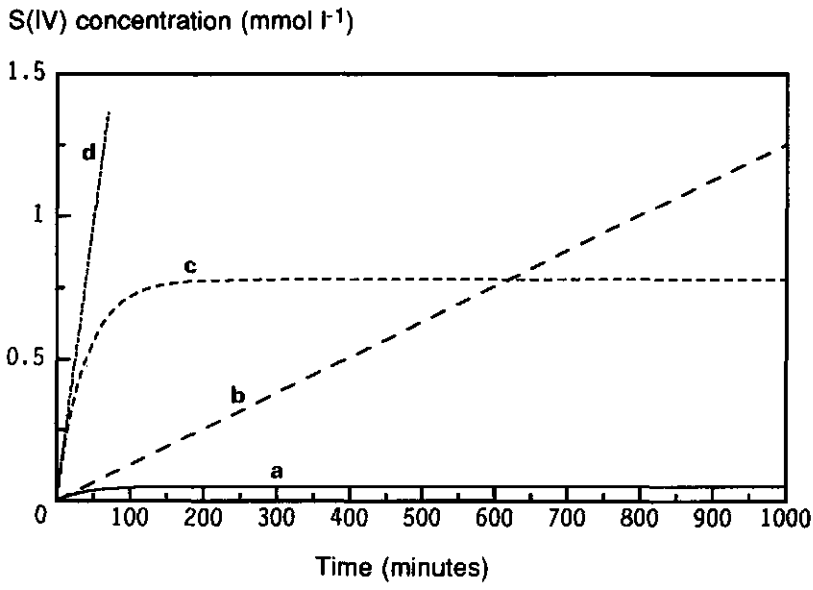


Fig. 3.3 S(IV) concentration in leaves after the start of exposure to 100 μg SO_2 m^{-3} as simulated with the model, assuming no effects on photosynthesis for a leaf with (a) and without (b) S(IV) oxidation and for a leaf with a strongly reduced leaf resistance (10 s m^{-1}) with (c) and without (d) S(IV) oxidation. Model parameters are given in Table 3.1.

Vicia faba L. data and the effect parameter was estimated from data published by Alscher *et al.* (1987) and Sakaki & Kondo (1985). Parameter values are listed in Table 3.1.

The effect of leaf resistance and time coefficient for sulphite oxidation on the time course of the S(IV) concentration were analysed with a model version in which effects on photosynthesis were neglected. From the parameters listed in Table 3.1, it can be calculated that the leaf resistance for CO_2 equals 155 s m^{-1}. Fig. 3.3 shows the change in leaf sulphite concentration after the start of fumigation. An increase of S(IV) is simulated during the first 100 minutes after the start of the exposure, after which it stabilizes at 0.05 mmol S(IV) l^{-1}, a very low concentration compared to that in equilibrium with the atmospheric SO_2 concentration (77.6 mmol l^{-1} at pH 7.5). Without oxidation the S(IV) concentration in the leaf increases constantly, but so slowly that it would take weeks to reach equilibrium concentrations with atmospheric SO_2 (Fig. 3.3).

When the leaf is considered a water layer with a boundary layer resistance of 10 s m^{-1} for CO_2, without oxidation, equilibrium concentrations are reached much faster (10 days). The equilibrium concentration at pH 7 is about 12.9 mmol l^{-1} S(IV) and is reached within about 2 days. Fig. 3.3 also shows that a low leaf resistance leads to much higher S(IV) concentrations than normal values for the leaf resistance, even when oxidation of S(IV) is included in the model. These results demonstrate that extremely toxic levels of

sulphite may accumulate in the absence of oxidation, even at very low background SO_2 concentrations.

The effect of photosynthetic feedback control of stomatal resistance on the time course of the S(IV) concentration in the leaf is demonstrated in Fig. 3.4. Without sulphite oxidation, the S(IV) concentration is strongly influenced by stomatal closure induced by depressed photosynthesis. When oxidation is considered, S(IV) concentration is hardly affected by stomatal closure, although photosynthetic depression is simulated to be about 10%. When more sulphite accumulates in the leaf (higher concentrations of SO_2, or slower oxidation rates) photosynthesis will be more strongly reduced, resulting in a stronger effect of stomatal closure.

The effect of S(IV) oxidation rate on photosynthesis, the S(IV) concentration of leaves and the internal SO_2 concentration during SO_2 exposure was subsequently analysed. Fig. 3.5 shows the influence of S(IV) oxidation on the effect of 100 μg SO_2 m^{-3} on photosynthesis, S(IV) concentration and internal SO_2 concentration. At normal time coefficients for S(IV) oxidation (1200-6000 s) a quick equilibrium in photosynthetic rate and S(IV) concentration is reached, which is the general pattern in short-term fumigation experiments (Black, 1982; Darrall, 1986; Chapter 2). The time from the start of fumigation in which stable rates of photosynthesis are reached, is strongly dependent

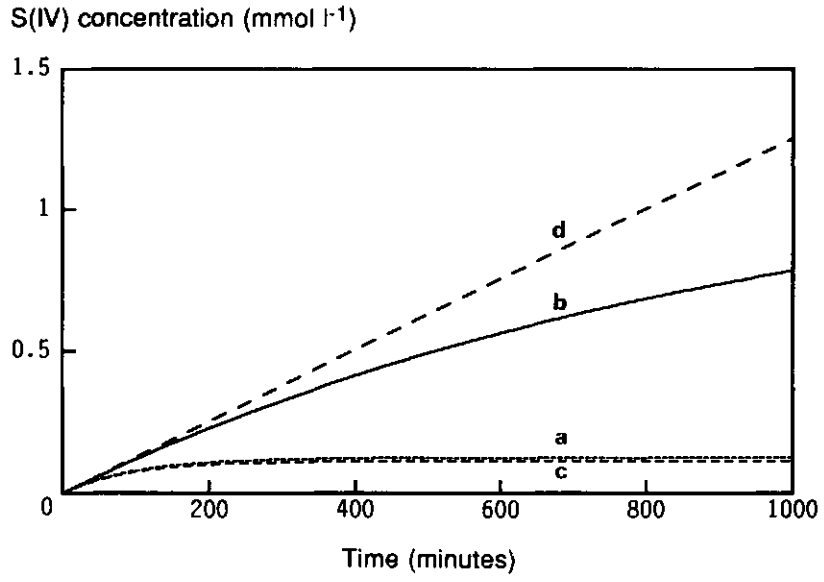


Fig. 3.4 S(IV) concentration in leaves as a function of time after the start of exposure to 100 μg SO_2 m^{-3} as simulated with the model, assuming no stomatal closure as a result of a reduced photosynthesis for a standard leaf with (a) and without (b) S(IV) oxidation and for a leaf with stomatal closure as a result of a reduced photosynthesis with (c) and without (d) S(IV) oxidation. Model parameters are given in Table 3.1. The time coefficient for S(IV) oxidation (τ_2) was 6000 seconds.

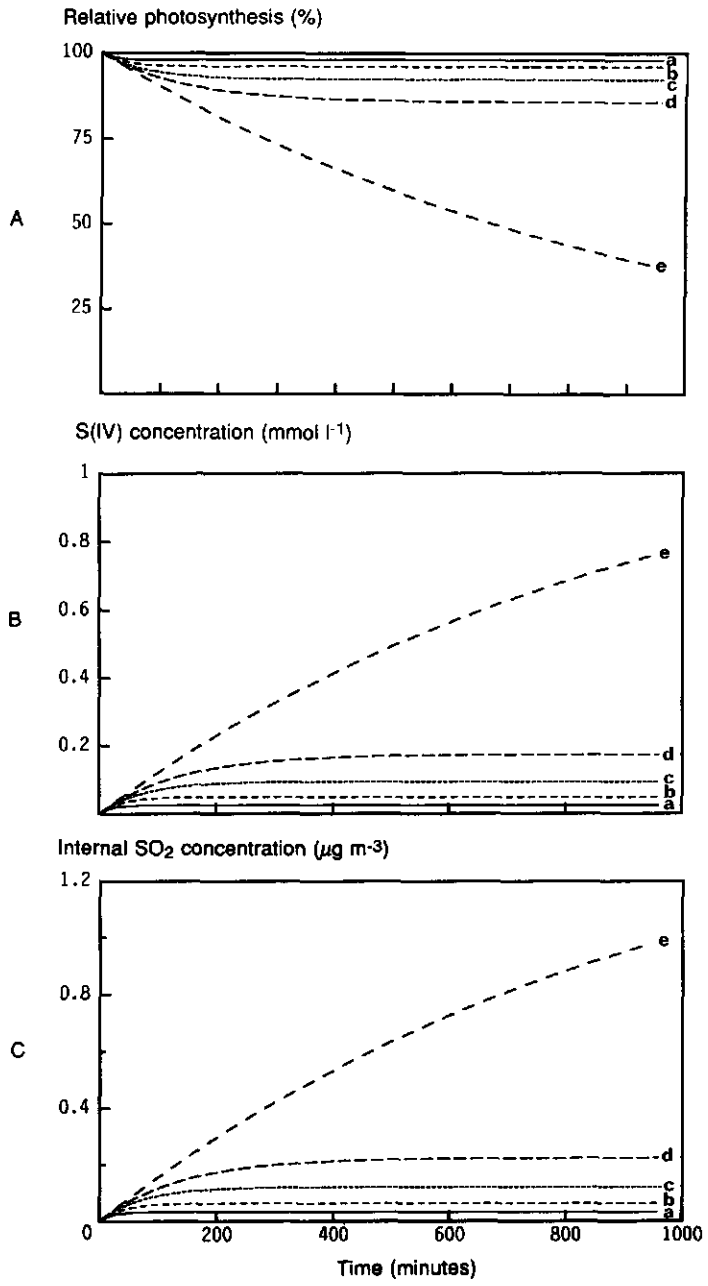


Fig. 3.5 (A). Relative photosynthesis of a leaf, (B) S(IV) concentration in the leaf and (C) internal SO_2 concentration in the stomatal cavities, as a function of time after the onset of exposure to 100 μg SO_2 m^{-3}, simulated with the model at different values of the time coefficient for S(IV) oxidation: τ_2 = 1200 (a), 2400 (b), 4800 (c), 9600 (d) or ∞ (e) seconds. Model parameters are listed in Table 3.1.

upon the rate of S(IV) oxidation (Fig. 3.5A). The same pattern is simulated for the sulphite concentration in the leaf (Fig. 3.5B). Without oxidation the rate of photosynthesis gradually decreases to very low values since sulphite accumulates in the leaf (Fig. 3.5A). Because of the feedback loop between stomatal resistance and photosynthesis, the curve is non-linear. Stomata close following the reduction of photosynthesis which leads to reduced rates of SO_2 uptake. The non-linearity is not a result of increased SO_2 concentration in the stomatal cavities reducing differences between ambient and internal concentrations (Fig. 3.5C), since the internal SO_2 concentration is less than 1% of ambient SO_2. The internal SO_2 concentration shows the same pattern as the S(IV) concentration in the leaf (Fig. 3.5C). Slow SO_2 uptake, rapid S(IV) oxidation and the feedback mechanism of stomatal resistance, results in very low internal SO_2 concentrations in the stomatal cavities (< 0.2 μg SO_2 m^{-3}), when compared to the ambient SO_2 concentration (100 μg SO_2 m^{-3}). Similar conclusions have been drawn from experimental data on gas exchange of SO_2 in leaves, where the internal SO_2 concentration was estimated to be 0 (Black & Unsworth, 1979c; Carlson, 1983).

These results demonstrate the potential of plants to avoid high equilibrium S(IV) concentrations in the leaf solution, by oxidation, the relatively high resistance for SO_2 uptake (when compared with water surfaces) and the photosynthetic feedback control of stomatal resistance.

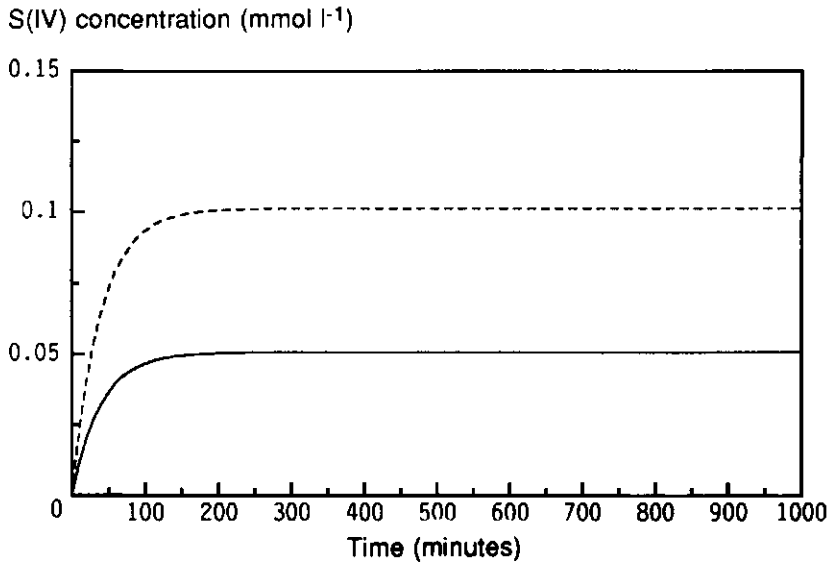


Fig. 3.6 The effect of leaf thickness (d=0.4 mm (——) and d=0.2 mm (- - - -)) on S(IV) concentration in the leaf. Model parameters are listed in Table 3.1.

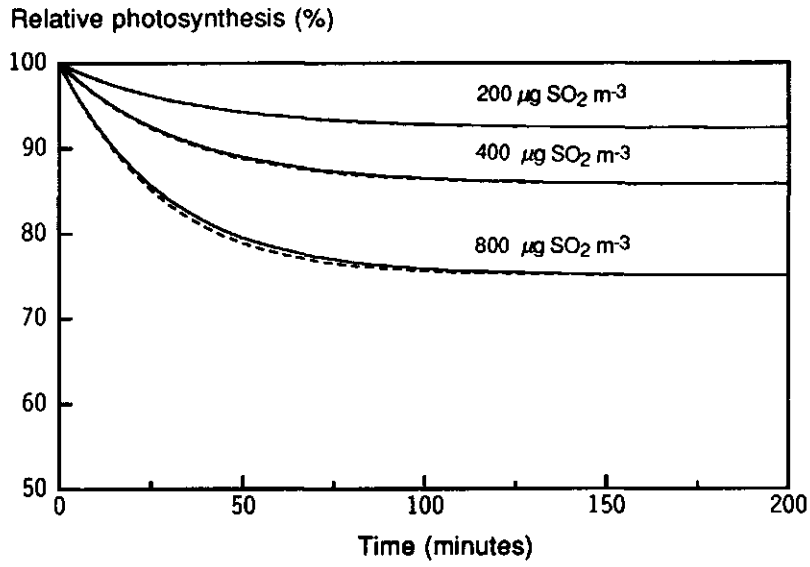


Fig. 3.7 The effect of a delay in stomatal closure when photosynthesis is reduced at 200, 400 and 800 μg SO_2 m^{-3}, respectively. Solid lines represent standard simulation, broken lines indicate the effect of the delay.

The effect of leaf morphology on the sensitivity to SO_2 was analysed by changing the leaf thickness in the model. Fig. 3.6 shows that the leaf S(IV) concentration after 200 minutes is doubled when leaf thickness is reduced to 0.2 mm (which is a typical value for phytotron grown plants). This can be explained by the fact that SO_2 flux per unit leaf volume (which determines the concentration) is doubled whereas the flux density per unit leaf area remains unchanged. This may explain the strong differences in sensitivity between plants grown in glasshouses or outdoors (Darrall, 1986). Thus, the parameter 'flux density' or 'uptake rate' proposed by Black & Unsworth (1979a) to compare species sensitivity, is confusing. Because leaf thickness is an essential dimensional factor, the rate of SO_2 uptake of air pollutants per unit of leaf volume instead of leaf area should be used to compare sensitivity of plant species.

The influence of delayed stomatal closure on the time course of reductions of photosynthesis is shown in Fig. 3.7. A time coefficient of 600 seconds was introduced into the model (Chapter 2) and its effect was analysed at 3 different SO_2 concentrations. The reduction in photosynthesis was slightly increased during the first 100 minutes as a result of a larger uptake, since stomata remain open for a longer time.

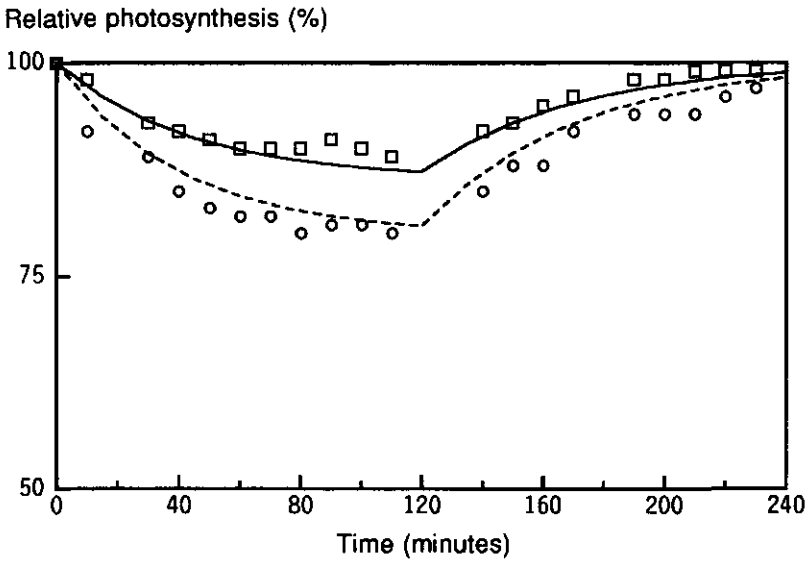


Fig. 3.8 Relative photosynthesis of leaves exposed to different SO_2 concentrations for 2 h followed by a recovery period of 2 hours as simulated with the model using one set of parameters, derived from the data of Bennet & Hill (1973), (for parameters see Chapter 4) at 675 μg SO_2 m^{-3} (□ observed; —— simulated) and 1080 μg SO_2 m^{-3} (○ observed; - - - - simulated).

Parameters on the processes at the biochemical level were estimated from experimental data from the literature with an analytical version of the model, using statistical methods (Chapter 4). To show the behaviour of the model, the time course of photosynthesis (Bennet & Hill, 1973), was simulated using the mean values for biochemical parameters estimated from the two data sets. The results in Fig. 3.8 show that the effect of SO_2 on photosynthesis during exposure and a subsequent recovery period is accurately simulated for two concentrations by using only one parameter set for both SO_2 concentrations. It should be noted here that this is not an evaluation of the model with independent data, since the parameters were estimated from the same data set. However, it can be concluded that the pattern of photosynthetic reduction and recovery is well described by the model, as well as the effect of different concentrations. The model mechanistically explains the rapid reduction in photosynthesis, the quick photosynthetic equilibrium rates and rapid recovery after termination of fumigation based on only a few biochemical parameters. The variation in these parameters and possibilities for application of the model are discussed in Chapter 4.

Extrapolation of short-term effects to longer periods should be made carefully, because acclimation of the leaves may occur (Mooney *et al.*, 1988), like changes in the time coefficient for sulphite oxidation. Other effects may also play a role in long-term effects like a decrease in cellular pH or an inhibited translocation of sugars from the leaves to other organs, with a possible feedback effect on photosynthesis when sugars accumulate.

Symbols used in Chapter 3

C_a	ambient CO_2 concentration	(μg CO_2 m^{-3})
C_i	internal CO_2 concentration	(μg CO_2 m^{-3})
d	leaf thickness	(mm)
ε	initial light use efficiency of leaf photosynthesis	(μg CO_2 J^{-1})
F	rate of SO_2 uptake	(mmol SO_2 m^{-2} s^{-1})
H	solubility constant for SO_2	(-)
I	absorbed radiation	(J m^{-2} s^{-1})
k	parameter for effect of S(IV) on photosynthesis	((mmol S(IV) l^{-1})$^{-1}$)
K_1	equilibrium constant	(mol l^{-1})
K_2	equilibrium constant	(mol l^{-1})
P	rate of photosynthesis during fumigation	(μg CO_2 m^{-2} s^{-1})
P_0	rate of photosynthesis before fumigation	(μg CO_2 m^{-2} s^{-1})
P_{max}	maximum rate of photosynthesis at light saturation	(μg CO_2 m^{-2} s^{-1})
$P_{max,0}$	maximum rate of photosynthesis before fumigation	(μg CO_2 m^{-2} s^{-1})
$P_{max,s}$	maximum rate of photosynthesis during exposure to SO_2	(μg CO_2 m^{-2} s^{-1})
r_b	boundary layer resistance to CO_2	(s m^{-1})
r_s	stomatal resistance to CO_2	(s m^{-1})
$r_{b,s}$	boundary layer resistance to SO_2	(s m^{-1})
$r_{s,s}$	stomatal resistance to SO_2	(s m^{-1})
S_a	ambient SO_2 concentration	(mmol SO_2 m^{-3})
S_i	internal SO_2 concentration	(mmol SO_2 m^{-3})
τ_2	time coefficient for S(IV) removal	(s)

Chapter 4

Quantification of biochemical characteristics determining the effect of SO_2 on leaf photosynthesis

Abstract A summary version of a model for the SO_2 flux into leaves and effects of SO_2 on the rate of photosynthesis was used to analyse experimental data on the effects of SO_2 on the rate of photosynthesis with standard statistical techniques. Values for the two key parameters of the model, a sensitivity parameter relating intracellular S(IV) concentrations (SO_2, bisulphite and sulphite) to photosynthetic reduction, and a time coefficient for S(IV) oxidation, were estimated from data on photosynthesis during fumigation and the subsequent recovery period, by combined non-linear regression of both equations.

The pattern of rapid photosynthetic reduction by SO_2 and rapid recovery following fumigation was accurately described with the model for several data sets. Parameter estimates agree very well with experimentally determined values. It is concluded that differences in photosynthetic sensitivity of plants are mainly due to differences in the time coefficient for sulphite oxidation. Variation in leaf thickness may also have contributed to the differences in sensitivity. This approach can be used to parameterize the model for short-term effects of SO_2 on leaf photosynthesis for specific species and environmental conditions from easily obtained data.

4.1 Introduction

In Chapter 3 a model was presented, which simulated SO_2 flux from the atmosphere into leaves and the effects of toxic SO_2 metabolites on the rate of photosynthesis and on stomatal resistance. The model contained two key parameters, a sensitivity parameter relating photosynthetic reduction to intracellular S(IV) concentration, and a time coefficient for S(IV) oxidation determining the rate of S(IV) removal to the less toxic sulphate. The sensitivity parameter was derived from experimental data on photosynthetic reduction in relation to the intracellular S(IV) concentration from Sakaki & Kondo (1985) who used isolated protoplasts and chloroplasts of *Vicia faba*, and Alscher *et al.* (1987) who studied the effects of SO_2 exposure on two cultivars of *Pisum sativum* L. Other data sets in which both effects on photosynthesis and S(IV) concentrations are reported were not

available in literature. The same relationship between reduction in the rate of photosynthesis and internal sulphite concentration was derived for both data sets (Chapter 3). Especially the results of Alscher *et al.* (1987) suggest that differences in sensitivity for SO_2 between plants are not based on differences in the sensitivity of photosynthesis to internal S(IV). Instead, the data suggest that these differences are primarily based on the capacity of leaves to remove the toxic S(IV) by oxidation, or on differences in leaf thickness, which determine the internal concentration at a given SO_2 flux density. The time coefficient for S(IV) oxidation varies strongly between species and cultivars (Miller & Xerikos, 1979; Alscher *et al.*, 1987). Rothermel & Alscher (1985) observed that the rate of S(IV) oxidation strongly depended on the light intensity, indicating that environmental conditions may influence the rate of S(IV) oxidation.

Quantification of the influence of environmental factors on biochemical parameters is essential for the prediction of SO_2 uptake by leaf canopies and effects on canopy photosynthesis, using crop growth models extended with submodels for the effects of SO_2. Such combination models may help to elucidate and predict the effects of SO_2 on crops and vegetation over an entire growing period.

For experimental determination of S(IV) in plants during the exposure of leaves to realistic SO_2 concentrations (< 200 μg SO_2 m^{-3}), biochemical methods are needed to detect very low S(IV) concentrations in leaf tissue (< 0.05 mmol S(IV) l^{-1}) (Chapter 3; Huber *et al.*, 1987). However, the detection limit is about 0.04 mmol S(IV) l^{-1} (Huber *et al.*, 1987) and experimental determination in leaf tissue is extremely difficult because S(IV) quickly oxidizes to sulphate.

In order to gain more insight in the quantitative values of these two parameters, the model presented in Chapter 3 was fitted to experimental data on the short-term effects of SO_2 on photosynthesis of various species.

4.2 Materials and methods

Theory

Determining biochemical characteristics from the time course of the rate of photosynthesis during exposure of a leaf to SO_2 and subsequent recovery

The SO_2 flux into the leaf, the sulphur balance in the leaf and the effects of toxic SO_2 metabolites on photosynthesis in their simplest form can be described by four equations. A detailed description of the backgrounds is given in Chapter 3.

The sum of resistances to CO_2 (consisting of the stomatal and boundary layer resistance r in s m^{-1}) can be derived from the rate of photosynthesis (P, μg CO_2 m^{-2} s^{-1}) and the difference between ambient and internal CO_2 concentrations (C_a-C_i, μg CO_2 m^{-3}),

which tends to be constant at a given CO_2 concentration, even during SO_2 exposure (Chapter 2):

$$r = \frac{C_a - C_i}{P} \tag{4.1}$$

The SO_2 flux into the leaf (F, mmol SO_2 m^{-2} s^{-1}) can be calculated from the ambient SO_2 concentration (S_a, mmol SO_2 m^{-3}) and the leaf resistance for SO_2 (ca. 1.2 r for CO_2), assuming the internal SO_2 concentration to be zero (Black & Unsworth, 1979; Carlson, 1983; Chapter 3):

$$F = \frac{S_a}{1.2\ r} \tag{4.2}$$

The change in the internal S(IV) (mmol S(IV) l^{-1}) depends on the SO_2 flux into the leaf and rate of S(IV) removal by oxidation of S(IV) to the less toxic sulphate:

$$\frac{dS(IV)}{dt} = \frac{F}{d} - \frac{S(IV)}{\tau_2} \tag{4.3}$$

where τ_2 is the time coefficient (s) for S(IV) oxidation (first order reaction) and d is the leaf thickness (mm). The effects of internal S(IV) on the rate of photosynthesis can be described with a linear relation for S(IV) concentrations up to 0.8 mmol S(IV) l^{-1} (Chapter 3), or reductions in the rate of photosynthesis of less than 60% (Chapter 3):

$$P = P_0\ (1 - k\ S(IV)) \tag{4.4}$$

P_0 denotes the rate of photosynthesis before fumigation, P the actual rate of photosynthesis and the parameter k expresses the sensitivity of the rate of photosynthesis to the S(IV) concentration in (mmol S(IV) l^{-1})$^{-1}$.

Combining these four equations gives an expression for the first derivative of the S(IV) concentration with respect to time:

$$\frac{dS(IV)}{dt} = \frac{S_a\ P_0}{1.2\ (C_a - C_i)\ d} - \left(\frac{S_a P_0\ k}{1.2\ (C_a - C_i)\ d} + \frac{1}{\tau_2}\right) S(IV) \qquad t < t_e \tag{4.5}$$

where t_e is the time at which fumigation is stopped. Equation (4.5) can be abbreviated by introducing another time coefficient (τ_1 (s)):

$$\tau_1 = \frac{1.2\ (C_a - C_i)\ d}{S_a\ P_0\ k} \tag{4.6}$$

Equation (4.5) then becomes:

$$\frac{d\,S(IV)}{d\,t} = \frac{1}{\tau_1 k} - \left(\frac{1}{\tau_1} + \frac{1}{\tau_2}\right) S(IV) \qquad t < t_e \qquad (4.7)$$

Equation (4.7) can be analytically solved (with an initial S(IV) concentration of zero) giving an expression for the time course of S(IV) during exposure of the leaf to SO_2:

$$S(IV) = \frac{\frac{1}{\tau_1 k}}{\frac{1}{\tau_1} + \frac{1}{\tau_2}}\left[1 - \exp\left\{-t\left(\frac{1}{\tau_1} + \frac{1}{\tau_2}\right)\right\}\right] \qquad t < t_e \qquad (4.8)$$

in which t is the time after the start of fumigation. In combination with Equation (4.4) this gives an expression for the relative rate of photosynthesis (P/P_0):

$$\frac{P}{P_0} = \frac{\tau_1}{\tau_1 + \tau_2} + \frac{\tau_2}{\tau_1 + \tau_2} \exp\left\{-t\left(\frac{\tau_1 + \tau_2}{\tau_1 \tau_2}\right)\right\} \qquad t < t_e \qquad (4.9)$$

where P_0 denotes the rate of photosynthesis before fumigation and P is the actual rate of photosynthesis after the start of fumigation. When SO_2 exposure is terminated, another relationship for the rate of photosynthesis has to be used. Equation (4.5) is much simpler when applied to clean air since the ambient SO_2 concentration is zero:

$$\frac{d\,S(IV)}{d\,t} = \frac{S(IV)}{\tau_2} \qquad t > t_e \qquad (4.10)$$

An expression for the S(IV) concentration can be derived from analytical solution of Equation (4.10):

$$S(IV) = S(IV)_{t_e} \exp\left(-\frac{t - t_e}{\tau_2}\right) \qquad t > t_e \qquad (4.11)$$

where t_e is the moment at which fumigation is stopped. Combination of Equation (4.11) with Equation (4.4) gives an expression for the relative rate of photosynthesis during the recovery period:

$$\frac{P}{P_0} = 1 - \left\{1 - \left(\frac{P}{P_0}\right)_{t_e}\right\} \exp\left(-\frac{t - t_e}{\tau_2}\right) \qquad t > t_e \qquad (4.12a)$$

where t is the time since the onset of the exposure and $(P/P_0)_{t_e}$ is the relative photosynthesis at the end of the exposure period. $(P/P_0)_{t_e}$ can be found from Equation (4.9).

The following expression for P/P_0 can then be derived:

$$\frac{P}{P_0} = 1 - \left\{ 1 - \frac{\tau_1}{\tau_1+\tau_2} + \frac{\tau_2}{\tau_1+\tau_2} \exp\left(- t_e \frac{\tau_1+\tau_2}{\tau_1 \tau_2} \right) \right\} \exp\left(- \frac{t - t_e}{\tau_2} \right) \qquad t > t_e \qquad (4.12b)$$

Using a non-linear regression procedure ('OPTIMIZE' in GENSTAT IV, Alvey *et al.*, 1982), the two Equations (4.9) and (4.12b) can simultaneously be fitted to experimental data in which gas exchange is measured during a fumigation period and a subsequent recovery period. This procedure gives estimated values for τ_1 and τ_2. From the estimated value of τ_1 the value of the sensitivity parameter k can be calculated with Equation (4.6), when P_0, S_a, d, C_a and C_i are known (k in (mmol S(IV) $l^{-1})^{-1}$ or in mmol S(IV) m^{-2} leaf)$^{-1}$ when the leaf thickness is not known).

When stomata do not respond to a reduced photosynthesis (*cf.* Alscher *et al.*, 1987), a different set of equations has to be used (see Appendix of this Chapter).

Relating biochemical parameters and the relationship between the rate of photosynthesis and SO_2 uptake in equilibrium situations

The presented set of equations and parameters can be easily related to direct measurements of SO_2 uptake rate and relative photosynthesis in equilibrium situations. The two important parameters τ_2 and k ((mmol S(IV) $m^{-2})^{-1}$) can be included in one expression by combining Equation (4.3) (in which the change in S(IV) concentration is set to zero, since the S(IV) concentration is stable) with Equation (4.4) which relates SO_2 uptake rate and the effect on relative photosynthesis:

$$\frac{P}{P_0} = 1 - k \tau_2 F \qquad (4.13)$$

where F is the rate of SO_2 uptake in mmol SO_2 m^{-2} s^{-1}. In Chapter 2 it was shown that the relationship between SO_2 uptake and reduction of photosynthesis in equilibrium at light saturation of *Vicia faba* leaves can be described by:

$$\frac{P}{P_0} = 1 - q F \qquad (4.14)$$

where q is the slope and equals $k \tau_2$, when k is expressed in (mmol S(IV) $m^{-2})^{-1}$.

Experimental data

Several experimental data sets on relative photosynthesis during a short fumigation period and subsequent recovery, were used to analyse model behaviour and to quantify biochemical characteristics. Bennet & Hill (1973) measured the effect of SO_2 on photosynthesis of 4-8 week old barley plants (*Hordeum vulgare* L., cv. Trebi). The effect of 675 and 1080 μg SO_2 m^{-3} was measured for two hours after the start of fumigation and a recovery period of two hours. Sij & Swanson (1974) analysed the effects of much higher SO_2 concentrations (2700-13500 μg SO_2 m^{-3}) on bean leaves (*Phaseolus vulgaris* L.) for a one-hour fumigation period and a subsequent recovery period of two hours. An extensive data set on photosynthesis of several plant species (*Vicia faba* L., cv. Three fold white and cv. Blaze; *Lolium perenne* L. , cv S23; *Hordeum vulgare* L., cv. Sonja; *Triticum aestivum* L., cv. Virtue and *Brassica napus*, cv. Rafal), influenced by a large range of SO_2 concentrations (270, 540, 810, 1080 and 1350 μg SO_2 m^{-3} respectively) was published by Darrall (1986). The first three species were grown in the glasshouse and the second group was grown outdoors. The plants grown outdoors were much less sensitive to SO_2 than plants grown in the greenhouse. Finally, a data set on leaves of *Vicia faba* L., cv. Minica exposed to 810 and 1600 μg SO_2 m^{-3} was used (Kropff & Smeets, unpublished; for methods see Chapter 2). Absolute rates of photosynthesis before the start of fumigation (P_0) and internal CO_2 concentrations needed to calculate the sensitivity parameter k, were estimated when they were not reported.

4.3 Results and discussion

Determining biochemical characteristics from the time course of the rate of photosynthesis during exposure of a leaf to SO_2 and subsequent recovery

The combination of Equations (4.9) and (4.12b) was fitted to experimental data on photosynthesis during fumigation and recovery to estimate values for the parameters τ_1 and τ_2. These parameter values can also be estimated from the rate of photosynthesis during fumigation only. However, when both the fumigation and recovery period are used, more information on model behaviour in relation to the real system is obtained and the interdependence of the estimated parameter values is reduced.

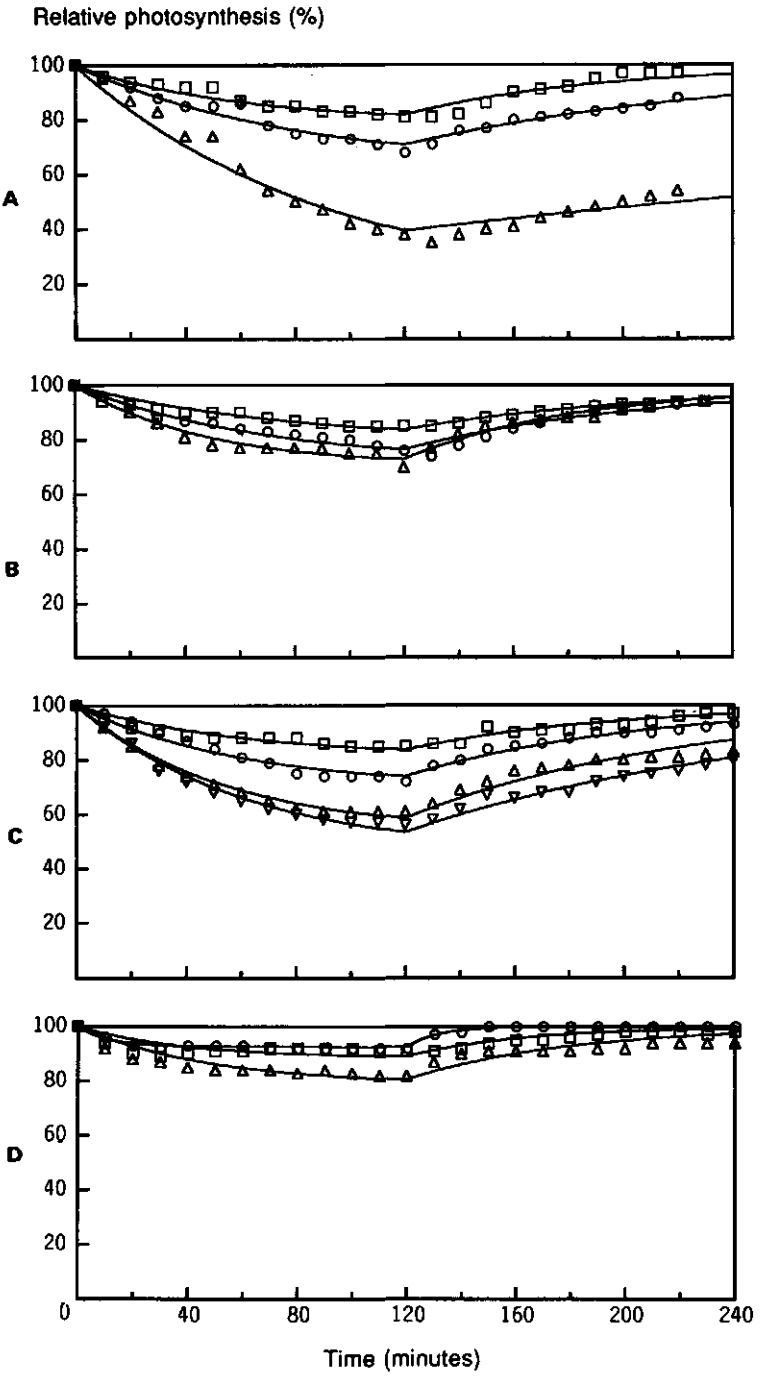
The results of fitting the model to the experimental data from Darrall (1986), Bennet & Hill (1973), Sij & Swanson (1974) and an own data set are presented in Fig. 4.1. The pattern of a rapid reduction after the start of the exposure and the fast recovery after the fumigation period was described very satisfactorily by the model for all data sets. Both the time course of the rate of photosynthesis during and after exposure were accurately described by the model. This indicates that short-term effects can be well described on the basis of the proposed mechanism. Unreliable estimates of the parameters were determined

when reductions in the rate of photosynthesis were very low (< 5%). Relative photosynthesis during the recovery period was not described correctly only at very high SO_2 concentrations, indicating that mechanisms other than a reversible inhibition of photosynthesis by S(IV) played an important role as well (*Vicia faba*, cv. Blaze at 1350 μg SO_2 m^{-3} and *Phaseolus vulgaris* at 13500 μg SO_2 m^{-3}).

The estimated parameter values are given in Table 4.1. Since leaf thickness is unknown for the experimental data used here, k expresses the relative reduction of photosynthesis per unit of S(IV) per unit leaf area, instead of per unit leaf volume. The values for k, are in the same range as the 0.832 (mmol S(IV) $l^{-1})^{-1}$ (Chapter 3), determined from the measurements of Alscher *et al.* (1987) and Sakaki & Kondo (1985): When leaf thickness is 0.5 mm, this value of k will be 1.664 (mmol S(IV) $m^{-2})^{-1}$ and when leaf thickness is 0.2 mm, k will be 4.16 (mmol S(IV) $m^{-2})^{-1}$. This indicates that differences in the determined values of k may possibly be explained on the basis of differences in leaf thickness, i.e. the *Hordeum* plants of Darrall (1986) were grown outside, whereas the other species were grown indoors, where plants normally have thinner leaves.

Large differences were observed in the time coefficient for S(IV) oxidation, which confirms the results of Alscher *et al.* (1987) that the rate of sulphite oxidation determines the sensitivity of the species. The differences in sensitivity between *Hordeum vulgare* and the other species examined by Darrall (1986) seems to be caused by large differences in the rate of S(IV) oxidation in combination with differences in leaf thickness. This illustrates the necessity to work in well defined conditions, since both oxidation potentials of leaves and leaf thickness are determined by growth conditions. The time coefficient for S(IV) oxidation in some plant species (*Vicia faba* cv. Blaze, *Lolium perenne* and *Phaseolus vulgaris*) increases with increasing SO_2 concentration, indicating an additional (irreversible) effect. The sensitivity of plants clearly increases with increasing time coefficient for S(IV) oxidation (Table 4.1; Fig. 4.1).

The estimation of the parameters from literature data is inaccurate, because only means are reported in literature, without standard errors, and the data necessary to calculate k (see Equation (4.6)) are rarely reported accurately. The best procedure for estimation of the parameters will be the determination of the parameters for data on individual plants, which allows the estimation of the variation in k and τ_2. This procedure will be followed in Chapter 5, which deals with the effect of temperature and humidity on photosynthetic depression during SO_2 exposure.


Relative photosynthesis (%)
100
80
60
40
20
A
B
C
D
0
40
80
120
160
200
240
Time (minutes)

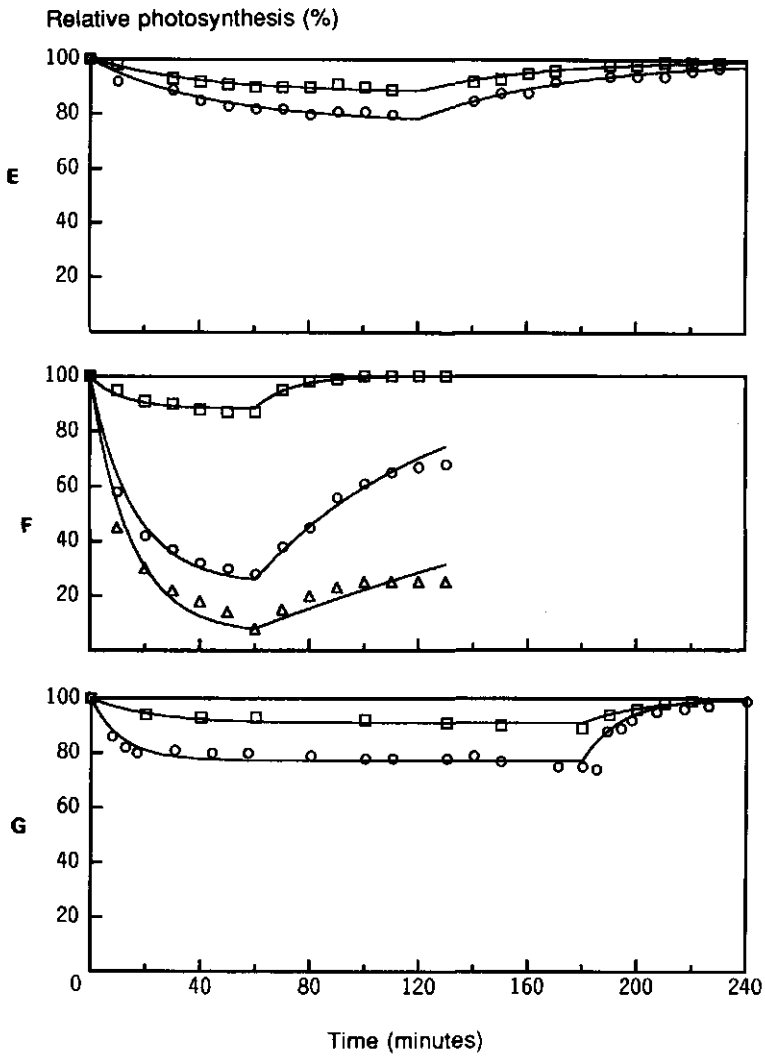


Fig. 4.1 Observed relative photosynthesis of single leaves during fumigation period (1-3 h) and a subsequent recovery period (markers), and fitted relations with the model (lines). Parameter values are listed in Table 4.1. Data from Darrall (1986) (A, B, C, D), Bennet & Hill (1973) (E), Sij & Swanson (1974) (F), and Kropff *et al.*(unpublished data) (G). Concentrations in $\mu g\ SO_2\ m^{-3}$.

A. *Vicia faba* cv. Blaze (□ 810; o 1080; Δ 1350)

B. *Vicia faba* cv. Three fold white (□ 810; o 1080; Δ 1350)

C. *Lolium perenne* cv. S23 (□ 540; o 810; Δ 1080; ∇ 1350)

D. *Hordeum vulgare* cv. Sonja (□ 810; o 1080; Δ 1350)

E. *Hordeum vulgare* cv. Trebi (□ 675; o 1080)

F. *Phaseolus vulgaris* (□ 2700; o 8100; Δ 13500)

G. *Vicia faba* cv. Minica (□ 810; o 1600)

Table 4.1 Estimated values for τ_1 and τ_2 (minutes) with standard error and k ((mmol S(IV) $m^{-2})^{-1}$), calculated from τ_1.

		minutes				(mmol S(IV)$m^{-2})^{-1}$
	$\mu g\ SO_2\ m^{-3}$	τ_1	SE_{τ_1}	τ_2	SE_{τ_2}	k
Vicia faba cv. Blaze (Darrall, 1986)	810	277.2	20.3	72.7	6.5	2.1
	1080	213.0	9.1	128.2	8.6	1.9
	1350	110.4	5.1	533.9	98.5	3.3
Vicia faba cv. Threefold white (Darrall, 1986)	810	367.7	18.5	96.5	6.7	1.1
	1080	237.3	11.8	93.1	6.0	1.4
	1350	159.1	6.9	66.5	3.3	1.7
Lolium perenne (Darrall, 1986)	540	320.9	19.5	74.9	5.6	2.1
	810	196.4	7.9	84.4	4.2	2.3
	1080	117.1	4.8	101.9	5.1	2.9
	1350	114.0	3.2	135.5	5.1	2.3
Hordeum vulgare (1) (Darrall, 1986)	810	335.1	60.2	43.1	8.8	1.2
	1080	150.9	10.5	12.7	0.9	2.2
	1350	224.3	23.9	59.5	7.4	1.2
Hordeum vulgare (2) (Bennet & Hill, 1973)	675	339.6	16.2	46.0	2.5	2.1
	1080	188.3	8.6	56.1	2.9	2.3
Phaseolus vulgaris (Sij & Swanson, 1974)	2700	99.1	11.4	13.3	1.6	1.7
	8100	20.7	1.2	66.1	3.4	2.7
	13500	15.9	1.3	238.2	26.8	2.1
Vicia faba cv. Minica (unpublished results)	810	231.7	24.1	23.1	2.3	2.2
	1600	48.7	5.7	14.4	1.7	2.9

Relating biochemical parameters and the relationship between the rate of photosynthesis and SO_2 uptake in equilibrium situations

The parameter values derived from own data on the rate of leaf photosynthesis during SO_2 exposure in broad bean using Equations (4.13) and (4.14) (Chapter 3), are in close agreement with the observed relationship between the rate of photosynthesis and SO_2 uptake rate after two hours of exposure. The value for q (Equation (4.14)) was estimated to be 0.05 (μg SO_2 m^{-2} $s^{-1})^{-1}$ for this series of measurements. The values for τ_2 and k are given in Table 4.1 (τ_2=1380 seconds and k=2.25 (mmol S(IV) $m^{-2})^{-1}$, resulting in (k τ_2 =) 3105 (mmol SO_2 m^{-2} $s^{-1})^{-1}$ or 0.048 (μg SO_2 m^{-2} $s^{-1})^{-1}$). Leaf thickness was 0.4 mm for the plants used. The k value calculated from Table 4.1 (2.25 x 0.4 =0.915 (mmol S(IV) $l^{-1})^{-1}$) is in agreement with k estimated from data of Alscher *et al.* (1987) and Sakaki & Kondo (1985) in Chapter 3.

Appendix

A model for the effects of SO_2 on photosynthesis when stomata do not respond to a reduced photosynthesis.

When photosynthesis is depressed, but stomatal resistance remains unchanged during SO_2 exposure (*cf.* Alscher *et al.*, 1987), Equations (4.9) and (4.12b) cannot be used to analyse experimental data, because stomatal resistance is assumed to be controlled by photosynthetic feedback in this set of equations. The main difference in the basic set of Equations (4.1)-(4.4) is the substitution of P by P_0 in Equation (4.1). Following the same procedure as described in the text, Equation (4.9) then becomes:

$$\frac{P}{P_0} = 1 - \frac{\tau_2}{\tau_1}\left\{1 - \exp\left(-\frac{t}{\tau_2}\right)\right\} \qquad t < t_e \qquad (4.15)$$

Equation (4.12a) remains unchanged. For Equation (4.12b) the following expression can be derived by combination of Equations (4.12a) and (4.15):

$$\frac{P}{P_0} = 1 - \left[\frac{\tau_2}{\tau_1}\left\{1 - \exp\left(-\frac{t_e}{\tau_2}\right)\right\}\right]\exp\left(-\frac{t - t_e}{\tau_2}\right) \qquad t > t_e \qquad (4.16)$$

The value of k can be calculated using Equation (4.6).

Symbols used in Chapter 4

C_a	ambient CO_2 concentration	(μg CO_2 m^{-3})
C_i	internal CO_2 concentration	(μg CO_2 m^{-3})
d	leaf thickness	(mm)
F	rate of SO_2 uptake	(mmol SO_2 m^{-2} s^{-1})
k	parameter for effect of S(IV) on photosynthesis	((mmol S(IV) l^{-1})$^{-1}$)
P	rate of photosynthesis during fumigation	(μg CO_2 m^{-2} s^{-1})
P_0	rate of photosynthesis before fumigation	(μg CO_2 m^{-2} s^{-1})
r	leaf resistance to CO_2	(s m^{-1})
S_a	ambient SO_2 concentration	(mmol SO_2 m^{-3})
t	time since onset fumigation	(s)
t_e	time since end of fumigation	(s)
τ_1	time coefficient (Equation 4.6)	(s)
τ_2	time coefficient for S(IV) removal	(s)
q	slope of relation photosynthesis and SO_2 uptake	((mmol SO_2 m^{-2} s^{-1})$^{-1}$)

Chapter 5

Effects of SO_2 on photosynthesis: The role of temperature and humidity

Abstract The effect of temperature and humidity on SO_2 induced photosynthetic depression was determined in gas exchange experiments with leaves of *Vicia faba*. Stomatal behaviour was sensitive to humidity resulting in higher uptake rates of SO_2 and stronger reductions of photosynthesis at low VPD (vapour pressure deficit). After a fumigation period of 2 h, when the photosynthetic rate had stabilized, photosynthesis of leaves exposed to SO_2 at 8 °C was reduced more (factor 2.7) than at 18 °C at the same rate of SO_2 uptake. Data analysis with a mechanistic model revealed that this effect was due to the slower rate of S(IV) oxidation at lower temperatures (time coefficient increased by a factor 2.5), resulting in a stronger accumulation of S(IV) and thus stronger reduction of photosynthesis. These results were confirmed by experimental analyses of the S(IV) concentration in leaves following fumigation, which showed that more S(IV) accumulated in leaves exposed at a lower temperature. These findings may explain the high sensitivity of plants exposed to SO_2 under winter conditions, when both VPD and temperature are low.

5.1 Introduction

The magnitude of SO_2 effects on plant growth depends upon the environmental conditions at which the plants are grown (see reviews by Black, 1982; Hållgren, 1984; Roberts, 1984; Unsworth & Ormrod, 1982; Winner *et al.*, 1985). It has been demonstrated that depressing SO_2 effects on plant growth were most severe during winter periods, when temperature and irradiance are low and humidity is high (Cowling & Lockyer, 1978; Davies, 1980; Whitmore & Mansfield, 1983; Pande & Mansfield, 1985; Baker *et al.*, 1986; Baker, Fullwood & Collins, 1987). The reported growth reductions are at least partly due to direct effects on photosynthesis and/or respiration, because no strong effects on leaf area were detected (Whitmore & Mansfield, 1983; Pande & Mansfield, 1985; Baker *et al.*, 1986, 1987).

The environmental conditions in winter (low temperature, low irradiation, high humidity) may directly influence the uptake, metabolism and effects of SO_2 metabolites on

photosynthesis. McLaughlin & Taylor (1981) demonstrated that pollutant uptake is significantly higher when air humidity is high, which can be understood from the well-known observation that stomatal behaviour depends upon air humidity (Losch & Tenhunen, 1981; Schulze, 1986; Morison, 1987; Schulze *et al.*, 1987; Woledge, Bunce & Tewson, 1989). When SO_2 dissolves in the aqueous phase of the leaf, the sulphite and bisulphite formed are quickly oxidized to sulphate (Alscher *et al.*, 1987; Miller & Xerikos, 1979). The rate of S(IV) oxidation in aqueous solutions is very sensitive to temperature (Martin, 1984; Clarke & Radojevic, 1987; Ibusuki & Takeuchi, 1987). When sulphite oxidation is slowed at low temperatures in leaf solutions, more toxic S(IV) compounds will accumulate. Because photosynthetic depression is related to the S(IV) concentration (Alscher *et al.*, 1987; Miller & Xerikos, 1979; Chapter 3), stronger reductions of photosynthesis can be expected at lower temperatures. However, the role of temperature in SO_2 metabolism and its implications for SO_2 effects on photosynthesis has not yet been analysed.

In this study the influence of both humidity and temperature on the effect of SO_2 on leaf photosynthesis was determined by gas exchange measurements, taking into account the strong relation between temperature and VPD. The physiological backgrounds behind the effect of these environmental factors on SO_2 induced photosynthetic depression were analysed using a model for foliar SO_2 uptake, SO_2 metabolism in the leaf and effects of SO_2 metabolites on photosynthesis (Chapters 3 and 4). This theoretical analysis was evaluated with experimental data on the S(IV) content of leaves, exposed to SO_2 at two temperatures.

5.2 Materials and methods

Plant material

Broad bean plants (*Vicia faba* L.) were grown in a greenhouse at 16 °C and 60-80% RH. Artificial supplementary illumination provided a photoperiod of 16 hours. Measurements started when the plants had about 10 leaves on the youngest fully unfolded leaves.

Experimental equipment

Materials which have been used for the construction of existing systems for measurement of photosynthesis absorb or react with pollutants like SO_2 and O_3 (van Hove *et al.*, 1989). Quick changes in pollutant concentrations cannot be realized and analysis of the rate of foliar pollutant uptake is impossible in these systems. Another problem is the cooling system in which water vapour often condensates readily absorbing pollutants like SO_2. To overcome these limitations a new system for gas exchange analysis during exposure to air pollutants at low concentrations was designed. This computer controlled sys-

tem consisted of an air supply unit, two leaf chambers and a unit for gas analysis.

Air was dried and purified by passing the air through several filters, containing MSTE kernels for removal H_2O and CO_2 (Gietart, Hengelo), active charcoal (RL II-Norit), and a molecular sieve 5A. Air was mixed with CO_2 from a cylinder by mass flow controllers. Air humidity was controlled by injection of water vapour into the air flow with computer controlled ultrasonic nebulizers (modified Heyer, USE77) with a droplet size of 0.5 - 4 micron. Aerosols were removed from the air flow by a series of static mixers (Sulzer). Humidity in the chamber could be changed almost instantaneously, without affecting the chamber temperature. The humidified air was mixed in a teflon manifold with SO_2 using thermal mass flow controllers (Brooks instruments). SO_2 was supplied from a cylinder containing a gas mixture of 1000 ppm SO_2 in N_2. All tubing and mixing units, in contact with SO_2 were constructed from teflon or glass. These materials have a relatively small absorption capacity for pollutants and hardly react with the pollutants. All processing was controlled by a Hewlett Packard personal computer (HP 9122) in combination with a data acquisition unit (HP 3497 A).

The leaf chamber was illuminated by a combination of high pressure vapour lights (two SON-T (sodium) and three HPI-T (iodine) lights, each 400 W (Philips)), yielding a maximum PPFD (Photosynthetic Photon Flux Density) of 1400 μE m^{-2} s^{-1}, equal to about 300 J m^{-2} s^{-1} PAR (Photosynthetically Active Radiation). Running cold water through a water bath between the light source and leaf chamber reduced the heating of the chamber. PPFD was measured by a PAR quantum sensor (Technical and Physical Research Service, Wageningen). Air humidity in the chamber was measured by Rotronic hygrometers and the difference in water vapour concentration between the inlet and the outlet of the chamber was measured with an infrared gas analyser (ADC 225 Mk3). CO_2 concentration at the inlet of the chamber was measured with an infrared gas analyser (ADC D6Y34H). The difference in CO_2 concentration between chamber inlet and outlet was measured with a second CO_2 analyser (ADC 225 Mk3). SO_2 concentrations at the inlet and the outlet of two chambers were analysed using a TECO 43A SO_2 monitor. Samples for the analysis of H_2O, CO_2 and SO_2 were drawn from the main air lines entering and leaving the chambers.

The flow rate of the air through the system was 15.5 *l* min^{-1}, giving a residence time of the air in the chamber of 0.54 minutes. Air in the leaf chamber was mixed by recirculation through a heat-exchanger, bathed in alcohol and temperature controlled by a cooling unit (Braun Frigro mix R) which was modified to permit computer control. A teflon fan recirculated the air at a flow rate of 0.8 m^3 min^{-1}, which resulted in a low boundary layer resistance (15 s m^{-1} for H_2O). All data were collected every 5 minutes by the HP microcomputer. Rates of SO_2 uptake, transpiration and photosynthesis were calculated from the difference in the concentration between inlet and outlet of the leaf chamber and the leaf area. Stomatal resistance and internal CO_2 concentration were calculated according to Goudriaan & van Laar (1978).

Experimental procedure

The influence of SO_2 at various combinations of temperature, PPFD and air-humidity on photosynthesis was measured for individual leaves of *Vicia faba* L. The effect of temperature (8 °C and 18 °C) was studied at two levels of radiation (PPFD of 450 or 900 μE m^{-2} s^{-1}) at 70% RH. The effect of air humidity (30, 50 and 70 % RH) was studied at a PPFD of 900 μE m^{-2} s^{-1}, at 18 °C. At 8 °C, photosynthesis was light saturated at 450 μE m^{-2} s^{-1}, and at 18 °C photosynthesis was saturated at 900 μE m^{-2} s^{-1}. Depending on the treatment, SO_2 concentrations ranged from 350 to 1500 μg SO_2 m^{-3}, in order to obtain reductions in photosynthesis in the range of 10-30%, necessary for reliable estimation of biochemical parameters with the model (Chapter 3). The average CO_2 concentration of the ingoing air was 345 ppm.

Leaf thickness was determined by measuring both leaf area and volume. The leaf volume was measured by submerging the leaf in a measuring glass filled with water.

Two plants were placed in controlled climate rooms in the afternoon previous to the day the measurements were conducted, to allow adaptation to the experimental conditions (temperature and humidity). The youngest fully unfolded leaf was enclosed in a leaf chamber. The light intensity was gradually increased to the desired PPFD of 450 or 900 μE m^{-2} s^{-1}. When rates of photosynthesis were stable, the enclosed leaf of one of the plants was fumigated for about two hours. The other plant was used as a control. Photosynthesis and transpiration were measured during the fumigation period and a subsequent recovery period of about 2 hours.

Model analysis of experimental data

A model for foliar SO_2 uptake, SO_2 metabolism in the leaf and effects of SO_2 metabolites on photosynthesis (Chapters 3 and 4) was used to analyse the biochemical backgrounds of temperature effects on the depression of photosynthesis by SO_2. Two model parameters determine the magnitude of SO_2 effects on photosynthesis: the time coefficient for S(IV) oxidation (τ_2, s) and a sensitivity parameter (k), which describes the reduction of photosynthesis in relation to the S(IV) concentration in the leaf (mmol S(IV) $l^{-1})^{-1}$ (Chapter 3). These two parameters can be estimated by fitting the model to data on the time course of photosynthesis during a fumigation period and a subsequent recovery period (Chapter 4).

In the present study, the parameters k and τ_2 were determined for all measured time series of photosynthesis separately. At 8 °C and high PPFD, photosynthesis of the control plants showed a continuous decrease during the day. This is possible due to photoinhibition, which is assumed to be based upon inactivation of the electron transport system in the thylakoids (Öquist, Greer & Ögren, 1987; Krause, 1988; Powles, 1984;

Kyle & Ohad, 1986). Before model analysis, the data on photosynthesis of the fumigated plants were corrected for the time trend in photosynthesis observed in the control plants. The decrease in photosynthesis over a 4 hour period varied between 20 and 50%. Because reliable estimation of model parameters is only possible when photosynthesis is reduced by 5% or more, plants which showed reductions of less than 5% were not analysed by the model. These data were not excluded from the analysis of the relation between the rate of SO_2 uptake and photosynthetic reduction.

Detection of S(IV) in leaves

To verify conclusions from model analysis of gas exchange measurements at the biochemical level, S(IV) concentrations were analysed in leaves of *Vicia faba* after a two hour fumigation period (1200 μg SO_2 m^{-3}). The West & Gaeke (1956) procedure for the determination of SO_2 was adapted to plant tissue for this purpose following Miller & Xerikos (1979). Samples of leaves (10 g fresh weight) were immediately frozen and ground in liquid nitrogen. The powder was added to 100 ml 0.2 M TCM (0.2 M HgCl and 0.4 M NaCl) and the suspension was ultrasonically vibrated for 3 minutes and centrifuged at 12500*g*. Formaldehyde (0.2 ml 0.2%) and 0.5 ml color solution (0.48 mM pararosanaline in 1 mM phosphoric acid) were added to 1.8 ml of the supernatant. The final pH of the solution was 1.2. After 25 minutes in the dark (20 °C) the absorbance was measured at 575 nm. Standard additions of S(IV) to non-fumigated leaf material established the reliability of the technique and were used to calculate S(IV) concentrations in $\mu g\ g^{-1}$ fresh weight glutathion, which may accumulate after SO_2 exposures (Maas, 1987), did not interfere with the measurements, since it was stabilized by TCM at the pH of 1.2.

5.3 Results and discussion

The effect of relative humidity and temperature on photosynthesis during a short fumigation period and a subsequent recovery period is illustrated in Fig. 5.1. These data illustrate the pattern of photosynthesis during short fumigation experiments. Photosynthesis quickly decreases after the onset of fumigation, followed by a stabilization within two hours of fumigation and a recovery when fumigation is stopped. Generally, the depression of photosynthesis was more severe at higher humidity and at lower temperature (Table 5.1). Information on photosynthesis, leaf thickness, SO_2 concentration and uptake rate at the end of the fumigation period for the different treatments is given in Table 5.1.

Table 5.1 Experimental details on plants of the different treatments and results of the model analysis.

Temperature (°C)	8	8	18	18	18	18
Radiation (μE m^{-2} s^{-1})	450	900	450	900	900	900
Relative humidity (%)	70	70	70	70	50	30
Number of replicates (n)	6	6	2	6	2	2
Photosynthesis at start fumigation (μg CO_2 m^{-2} s^{-1})	236 ± 26	213 ± 33	395 ± 15	468 ± 23	349 ± 32	348 ± 3
Stomatal resistance at start fumigation (s m^{-1})	104 ± 6	125 ± 18	160 ± 15	107 ± 8	203 ± 42	194 ± 26
SO_2 concentration (μg m^{-3})	869 ± 79	697 ± 140	1080 ± 10	1210 ± 97	1461 ± 16	1485 ± 13.5
Leaf thickness (mm)	0.33 ± 0.01	0.34 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0..33 ± 0.01	0.33 ± 0.03
SO_2 uptake rate at the end of fumigation (μg m^{-2} s^{-1})	3.43 ± 0.44	2.60 ± 0.38	3.00 ± 0.01	4.73 ± 0.43	3.20 ± (0.10	3.35 ± 0.25
Relative photosynthesis (at the end of fumigation (%))	53.3 ± 7.7	76.0 ± 3.5	79.5 ± 1.5	76.5 ± 1.9	88.0 ± 7.0	82.0 ± 6.0
k ((mmol S(IV) l^{-1}) $^{-1}$)	1.00 ± 0.11	0.77 ± 0.07	0.95 ± 0.05	0.88 ± 0.05	1.08 ± 0.02	0.98 ± 0.06
τ_2 (minutes)	51.3 ± 9.4	47.0 ± 10.6	23.0 ± 0.1	21.2 ± 1.9	16.0 ± 2.0	16.5 ± 3.5

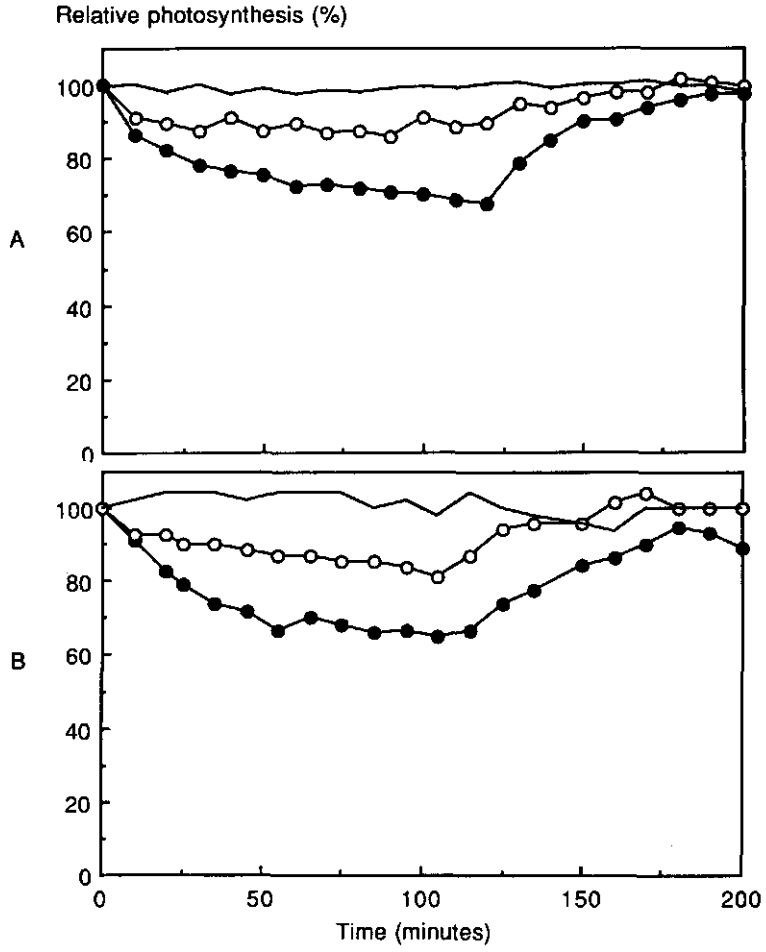


Fig. 5.1 Relative photosynthesis of *Vicia faba* leaves during a fumigation period of 120 minutes and a recovery period of 80 minutes. (A) control (——); fumigated with 1500 μg SO_2 m^{-3} at 18 °C and 30% RH (o); fumigated with 1500 μg SO_2 m^{-3} at 18 °C and 70% RH (●). (B) control (——); fumigated with 1000 μg SO_2 m^{-3} at 18 °C and 70% RH (o); fumigated with 1000 μg SO_2 m^{-3} at 8 °C and 70% RH (●).

The effect of humidity

The relationship between the rate of SO_2 uptake and relative photosynthesis for the different treatments two hours after the onset of fumigation is presented in Fig. 5.2. No effect of humidity (30-70% RH) on the relation between the rate of SO_2 uptake and relative photosynthesis was observed at 18 °C (Fig. 5.2A). However, the rate of SO_2 up-

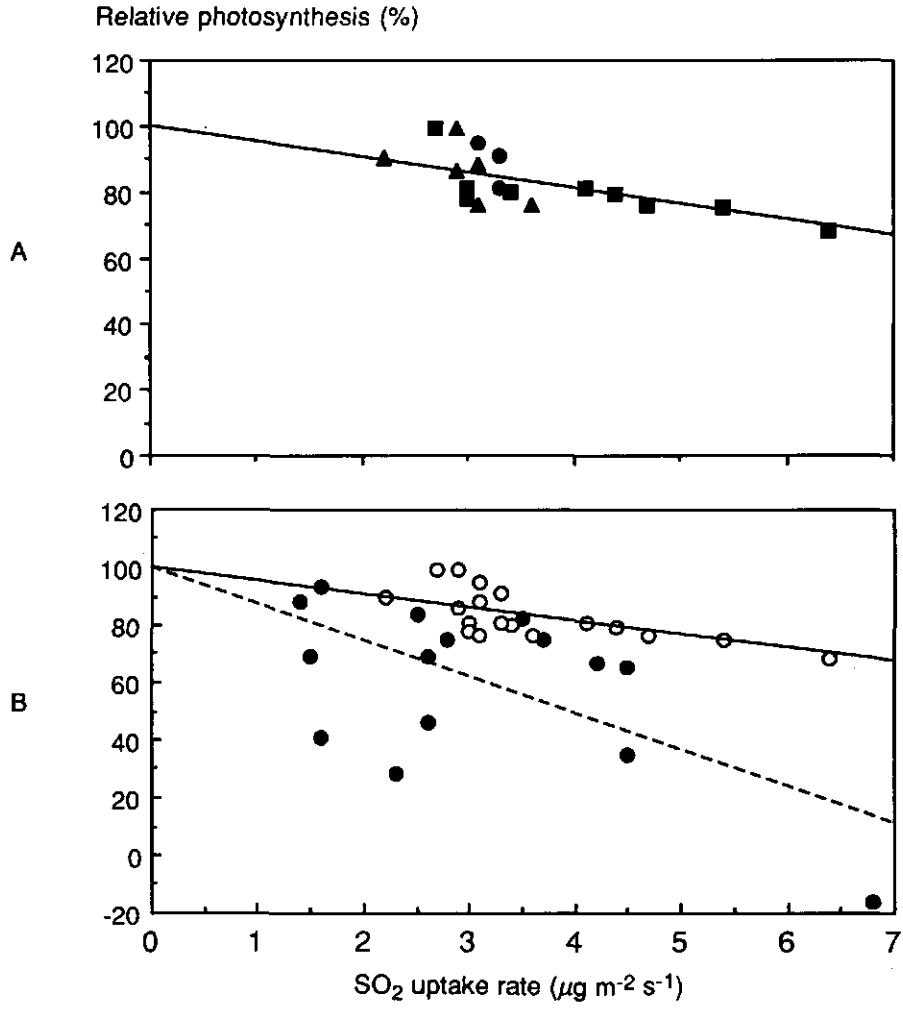


Fig. 5.2 Relative rate of photosynthesis of *Vicia faba* leaves after 120 minutes of SO_2 exposure in relation to the rate of SO_2 uptake (F in $\mu g\ m^{-2}\ s^{-1}$). (A) fumigated at 18 °C and 30% RH (▲), 50 % RH (●) and 70% RH (■). Relative photosynthesis (18 °C) (%)= 100 - 4.7 F ; $r^2 = 0.46$, n=18. (B) fumigated at 18 °C (○), solid line (data from A); fumigated at 8 °C (●), broken line; Relative photosynthesis (8 °C) (%)= 100 - 12.7 F ; $r^2 = 0.35$, n=15.

take increased strongly at a higher humidity, reducing photosynthesis even more. For the data of the 18 °C treatment, a linear relation was found, with a slope of 4.7 ($\mu g\ SO_2\ m^{-2}\ s^{-1})^{-1}$. Similar conclusions were drawn from preliminary experiments at the same conditions in the equipment for routine measurement of photosynthesis at the Centre for Agrobiological Research (Kropff & Smeets, unpublished). This significant role of

humidity in plant responses to SO_2 was demonstrated earlier by McLauglin & Taylor (1981).

The effect of air humidity on the rate of SO_2 uptake can be easily understood from the response of stomata to humidity. Stomatal behaviour can be characterized by the ratio between internal and ambient CO_2 concentration (C_i and C_a, respectively), because this ratio tends to be constant over a wide range of conditions in many plant species (Goudriaan & van Laar, 1978; Wong *et al.*, 1979; Louwerse, 1980; Bell, 1982; Farquhar & Sharkey, 1982; Morison, 1987). It was demonstrated in Chapter 2 that this ratio was not altered when leaves were fumigated with SO_2, although a time lag of 5 - 10 minutes was observed between photosynthetic reduction and stomatal response, resulting in an initial increase of C_i. The effect of air humidity on stomatal behaviour of the plants used in this study is illustrated in Table 5.2. The ratio C_i/C_a was determined at the high radiation conditions from control and fumigated plants when photosynthesis rates were stable previous to fumigation and increased with increasing air humidity. At 70% RH, the ratio was much higher at low temperature than at high temperature. This confirms the observation that VPD (Vapour Pressure Deficit), instead of relative humidity is the driving power behind stomatal reactions to humidity (Morison, 1987). The relationship between internal and ambient CO_2 concentration (C_i and C_a, μg CO_2 m^{-3}), photosynthesis (P, μg CO_2 m^{-2} s^{-1}), and the stomatal and boundary layer resistance (r_s and r_b, s m^{-1}) can be described by resistance analogy: $P = (C_a - C_i) / (r_s + r_b)$ (Gaastra, 1959). From this equation it follows that a higher C_i/C_a ratio, reflecting a smaller difference in $C_a - C_i$, is coupled to a lower stomatal resistance at a given rate of photosynthesis. The rate of SO_2 uptake will be correspondingly higher, because the stomata form the main entrance for SO_2 (Unsworth *et al.*, 1976).

The effect of temperature

Photosynthetic depression at the end of the fumigation period at a given rate of SO_2 uptake was much stronger at 8 °C when compared to leaves exposed to SO_2 at 18 °C (Fig. 5.2B). This effect was either due to an increased sensitivity of photosynthesis to S(IV) metabolites or to a reduced rate of S(IV) oxidation. The slope of this line can be expressed in terms of the model by the product of the sensitivity parameter k and the time coefficient for S(IV) oxidation τ_2 (Chapter 4).

The effect of temperature over all treatments on the parameters k and τ_2, estimated by model analysis of the data is presented in Fig. 5.3. The strong effect of temperature on the relationship between SO_2 uptake and relative photosynthesis was explained by an effect of temperature on the rate of S(IV) oxidation. The time coefficient for S(IV) oxidation (τ_2) was increased by a factor 2.5, while the sensitivity parameter (k) was not significantly affected. An increase of the slope relating SO_2 uptake to relative photosynthesis by a factor 2.5 is expected, because this slope is proportional to $k\,\tau_2$. This is

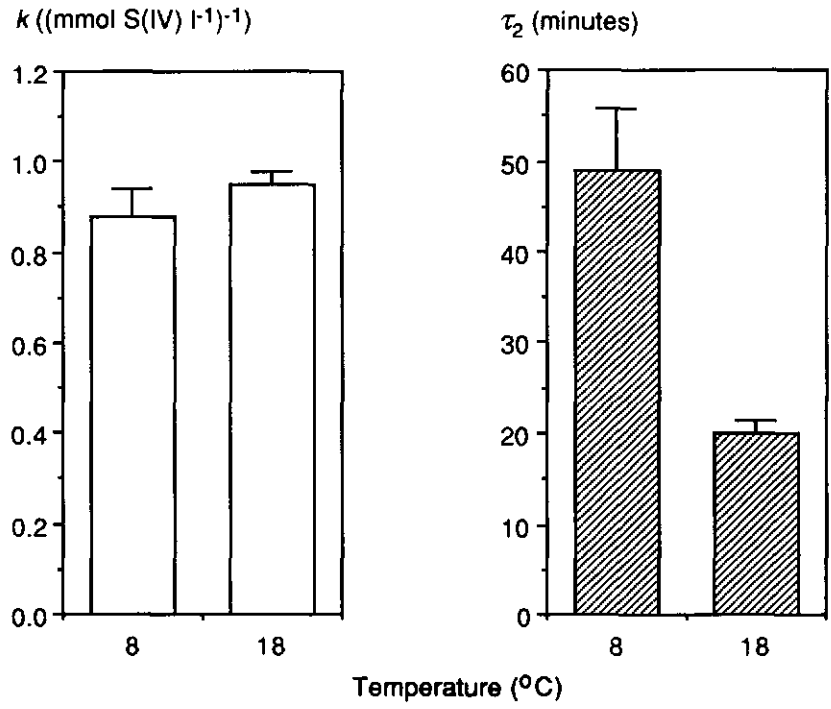


Fig. 5.3 The effect of temperature on (A) the sensitivity parameter (k, (mmol S(IV) $l^{-1})^{-1}$ and (B) the time coefficient for S(IV) oxidation (τ_2, minutes), determined by model analysis of gas exchange measurements on *Vicia faba* leaves during SO_2 exposure. Values represent means of 12 data sets (SE of means are indicated).

close to the factor 2.7, which was observed (Fig. 5.2). These results indicate that the higher sensitivity of the plants to SO_2 at 8 °C (Fig. 5.2) was probably caused by the stronger accumulation of S(IV) as a result of a slower rate of S(IV) oxidation. Similar or higher effects of temperature on S(IV) oxidation were reported for S(IV) in aqueous solutions (Martin, 1984; Ibusuki & Takeuchi, 1987; Clarke & Radojevic, 1987). More detailed information on the model parameters, estimated for the different treatments is given in Table 5.1. The sensitivity parameter k at the low temperature and high radiation level (when photoinhibition was observed) was lower than k at the low temperature and lower radiation. Because the data on photosynthesis were corrected for the time course of photosynthesis in the control plants, interpretation of the results from the photoinhibited plants requires research at the biochemical level. The absence of an effect of photoinhibition on τ_2 contradicts the assumption that active oxygen species are formed when the electron transport is inhibited, which should enhance the oxidation rate of S(IV) (Öquist *et al.*, 1987 ; Krause, 1988; Powles, 1984; Kyle & Ohad, 1986).

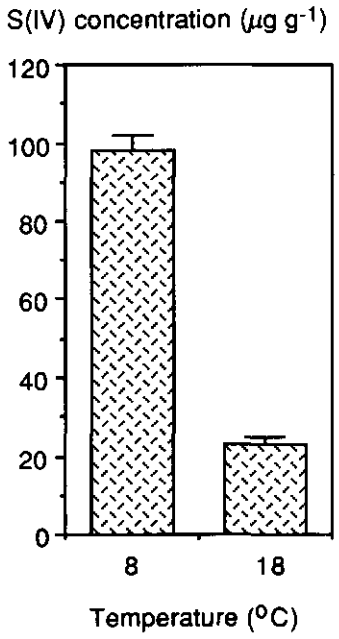


Fig. 5.4 The effect of temperature on S(IV) concentration (μg S(IV) g fresh weight) in *Vicia faba* leaves after exposure to 1200 μg SO_2 m^{-3} for 2 hours (n=12 for 8 °C and n=6 for 18 °C). Values represent means (SE of means are indicated).

From the model analysis, it directly follows that the S(IV) concentration at the end of the fumigation period, in the stationary state, should be higher at lower temperatures. S(IV) measurements in leaves of *Vicia faba*, exposed to 1200 μg SO_2 m^{-3} for 2 hours at 7 and 20 °C, are presented in Fig. 5.4. These results indeed indicate a stronger accumulation of S(IV) in the leaves at the lower temperature. The S(IV) concentration is a factor 4.3 higher at 7 °C than in leaves exposed at 20 °C. This is higher than the factor 2.5 derived from the model analysis. This may be partly explained by the greater difference in temperature between the treatments. Another reason for the difference may be that for S(IV) measurements five leaf numbers were sampled from the plants, which differed in age to obtain enough material for chemical analysis, whereas the youngest fully unfolded leaves were used in the gas exchange measurements.

To analyse the possible contribution of a reduced assimilate availability at the low temperature to the increase of the time coefficient for S(IV) oxidation, the relationship between the rate of net photosynthesis at the beginning of the fumigation period and τ_2 is given in Fig. 5.5 which indicates that τ_2 is not related to the rate of photosynthesis.

Table 5.2 The effect of humidity (RH in %) and vapour pressure deficit (VPD, mbar) on the ratio between internal (C_i) and ambient (C_a) CO_2 concentration at high radiation levels. n is number of replicates.

Temperature (°C)	n	RH (%)	VPD (mbar)	C_i / C_a
8	14	70	3.3	0.93 ± 0.01
18	12	70	6.2	0.85 ± 0.01
18	4	50	10.3	0.81 ± 0.01
18	8	30	14.4	0.81 ± 0.01

The value for the sensitivity parameter k, about 0.9 (mmol S(IV) $l^{-1})^{-1}$ (Table 5.1) was very close to the value determined from experimental data of Alscher *et al.* (1987) and Sakaki & Kondo (1985) in Chapter 3, which was 0.825 (mmol S(IV) $l^{-1})^{-1}$.

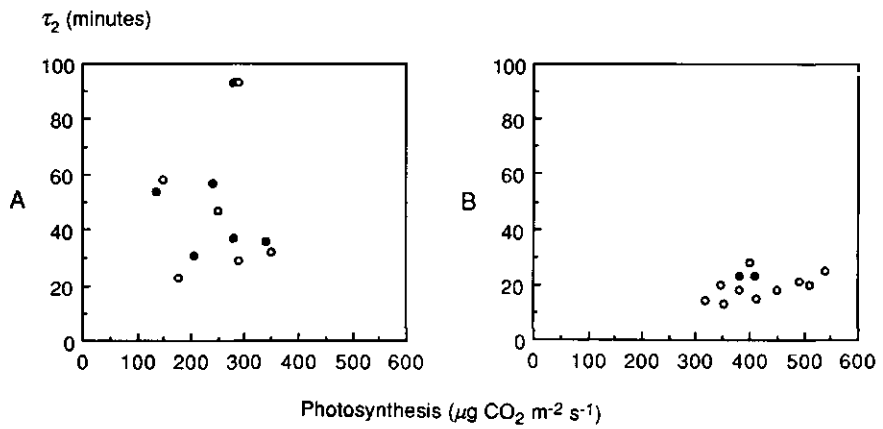


Fig. 5.5 Relation between the time coefficient for S(IV) oxidation (τ_2, minutes) as determined by model analysis and the rate of photosynthesis at the onset of fumigation at 8 °C (A) and 18 °C (B). Data were obtained at a PPFD of 900 μE m^{-2} s^{-1} (○) and a PPFD of 450 μE m^{-2} s^{-1} (●).

Separating effects of temperature and humidity on photosynthesis

Analysis of the backgrounds of temperature effects on photosynthetic depression at a given SO_2 concentration is confounded by temperature effects on photosynthesis as well as VPD, because both factors influence stomatal conductance, and thus SO_2 uptake rates. At 8 °C photosynthetic rates were lower than at 18 °C, which likely resulted in stomatal closure, but the VPD was also lower at 8 °C (at the same relative humidity), which causes stomatal opening. Stomatal resistance was almost the same for plants at the two temperatures at 70% RH, indicating that both effects were compensatory. Therefore, the observed difference in effect between 8 and 18 °C was mainly due to the difference in the time coefficient for S(IV) oxidation in this experiment.

A factor complicating interpretation of the data was that stomatal responses to SO_2 induced changes in photosynthesis were small in plants fumigated at 8 °C, which resulted in an increase of C_i/C_a throughout the fumigation period. This effect is important when SO_2 exposure results in severe reductions of photosynthesis, because the rate of SO_2 uptake is much higher when stomata do not close as a result of a decreasing photosynthesis. An illustration of this phenomenon is given in Fig. 5.6, where the simulated effect of 400 and 1200 μg SO_2 m^{-3} respectively on leaf photosynthesis is presented for a leaf in which the stomata respond to the lowered rate of photosynthesis, and for a leaf in which stomatal conductance remains unchanged. The effects of SO_2 are much more severe when the stomata do not close as a result of the reduced rates of photosynthesis when photosynthetic depression is high.

Most studies in which the effect of SO_2 on photosynthesis is analysed, reported very small or no effects of SO_2 at low concentrations (0 - 400 μg SO_2 m^{-3}) (Darrall, 1989). However, the data presented in this study demonstrate that growth reductions due to reduced photosynthesis may occur at these low concentrations in winter periods. When the results obtained in this study are extrapolated to low concentrations, a photosynthetic reduction of 5.5 % is expected at 8 °C at 100 μg SO_2 m^{-3} (uptake 0.5 μg SO_2 m^{-2} s^{-1}) (Fig. 5.2B). However, since the values for τ_2 showed a large variation (from 23 to 93 minutes at 8 °C), the expected range of photosynthetic reductions for the plants used in this study at 8 °C exposed to 100 μg SO_2 m^{-3} is 2 - 10%.

Implications for plant growth at winter conditions

The results presented in this study may explain the severe effects of SO_2 on plant growth in winter conditions, observed in many studies (Davies, 1980; Baker, Unsworth & Greenwood, 1982; Baker *et al.*, 1986; Cowling & Lockyer, 1978; Whitmore & Mansfield, 1983; Pande & Mansfield, 1985). Besides low temperatures, low radiation levels also reduce the rate of S(IV) oxidation in plant material (Rothermel & Alscher, 1985), possibly explaining the extreme effect of low radiation on plant growth described by Davies (1980).

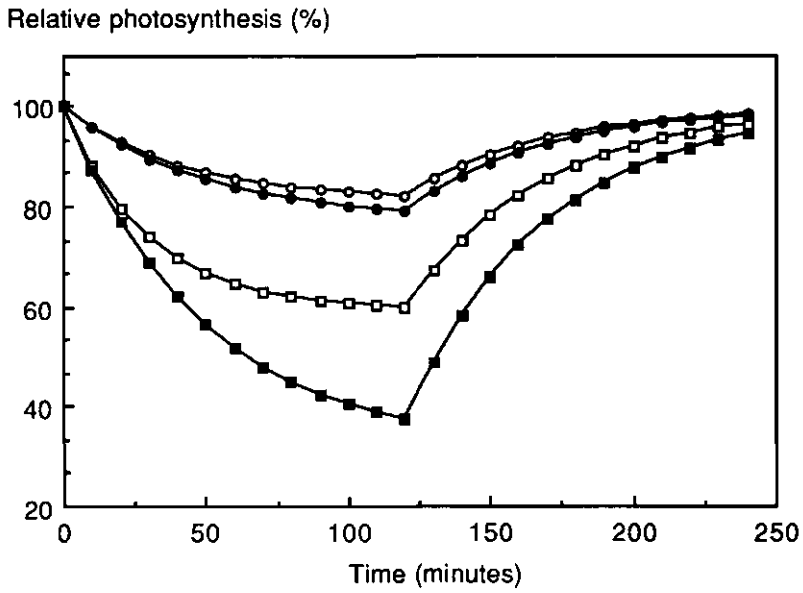


Fig. 5.6 Simulated effect of SO_2 on relative photosynthesis during a fumigation period of 120 minutes and a subsequent recovery period of 120 minutes at 8 °C; leaves exposed to 400 μg SO_2 m^{-3} with stomatal feedback (○) and without stomatal feedback (●); leaves exposed to 1200 μg SO_2 m^{-3} with stomatal feedback (□) and without stomatal feedback (■). Leaf characteristics obtained from the experimental data of *Vicia faba* leaves.

When plants with thin leaves and a low capacity for S(IV) oxidation are exposed to low SO_2 concentrations at low temperatures, significant reductions of photosynthesis have to be expected. The approach presented in this paper may help to quantify growth depressions in plants exposed to SO_2 at winter conditions, by incorporation of the submodel for SO_2 effects on leaf photosynthesis in crop growth models (Chapters 6 and 9).

Symbols used in Chapter 5

C_a	ambient CO_2 concentration	(μg CO_2 m^{-3})
C_i	internal CO_2 concentration	(μg CO_2 m^{-3})
k	parameter for effect of S(IV) on photosynthesis	((mmol S(IV) l^{-1})$^{-1}$)
P	rate of photosynthesis during fumigation	(μg CO_2 m^{-2} s^{-1})
r_b	boundary layer resistance to CO_2	(s m^{-1})
r_s	stomatal resistance to CO_2	(s m^{-1})
τ_2	time coefficient for S(IV) oxidation	(s)

Chapter 6

Modelling effects of SO_2 on photosynthesis of leaf canopies

Abstract The effect of short-term SO_2 exposures on photosynthesis of a broad bean crop was analysed with mobile equipment in the field. Canopy photosynthesis was only affected at high radiation levels and reduced by 2-6% during fumigation with 800 $\mu g\ SO_2\ m^{-3}$.

The experimentally obtained data were used to evaluate the performance of a model for the effects of SO_2 on leaf canopy photosynthesis. The model was based on a calculation procedure for canopy photosynthesis, extended with a submodel for SO_2 uptake by leaves and effects of SO_2 on leaf physiology. Diurnal photosynthesis and the effect of SO_2 on canopy photosynthesis were approximated closely with the model. Possibilities for the application of this approach in crop growth models (operating at a time step of integration of 1 day) are presented and evaluated.

6.1 Introduction

Effects of SO_2 on photosynthesis of leaves and whole plants have been studied extensively in order to find a mechanistic explanation for growth reductions in plants exposed to SO_2 in the absence of visible injury. Most workers observed depressing effects of SO_2 on leaf and plant photosynthesis (Bennet & Hill, 1973; Black, 1982; Black & Unsworth, 1979a, b; Darrall, 1986; Chapter 2). The general pattern of photosynthetic reduction after the onset of fumigation is a rapid decline in photosynthesis, followed by stabilization within 30-120 minutes (Chapter 2). A model was described earlier (Chapter 3) to simulate SO_2 uptake, the balance of toxic metabolites in the leaf and effects of toxic S(IV) compounds on rates of photosynthesis. To simulate leaf photosynthesis during fumigation with SO_2, the parameters to be quantified are the time coefficient for S(IV) oxidation and the parameter which describes the sensitivity of photosynthesis with respect to the S(IV) concentration. Values for these two parameters are relatively easy to obtain from data on photosynthesis during a fumigation period and a subsequent period of recovery (Chapter 3). The model provides a good description of the behaviour of leaf

photosynthesis during short-term SO_2 exposure periods (Chapters 3 and 4).

To evaluate the impact of atmospheric pollutants on crops and vegetation, models for the effect of air pollutants on leaf physiology can be incorporated into models for growth and production of crops and vegetation. Such combination models enable the estimation of effects without doing expensive, and in the case of forests almost impossible, experiments. However, realistic prediction demands that such models are based upon thorough insight in the effects of air pollutants on crop physiology. The effects of SO_2 on photosynthesis of leaf canopies based on observations on single plants, was first modelled by Black & Unsworth (1979a). They predicted 65% reduction in daily total net photosynthesis of a *Vicia faba* canopy with a leaf area index of 4 when exposed to 95 μg SO_2 m^{-3}. This is not likely to be a realistic prediction for field crops, since only small yield reductions have been observed when crops were exposed to low SO_2 concentrations in the field. In 1985, we exposed field crops of *Vicia faba* to elevated SO_2 concentrations (100-200 μg SO_2 m^{-3}) and analysed growth and production. Crop growth was reduced by 17% at the end of the growing season, but effects on the growth rate were only observed at the end of the growing season, in the pod filling period (Chapter 7). No effects could be detected in the linear growth phase. In other field studies with barley, no clear effects on total dry matter growth and only small yield reductions were observed at low SO_2 concentrations (< 150 μg SO_2 m^{-3} as a seasonal average) (Baker *et al.*, 1986; McLeod *et al.*, 1988). This illustrates the importance of validation of models used to predict air pollutant effects on leaf canopies based on observations on laboratory grown plants.

The aim of this study was to experimentally analyse the short-term effects of SO_2 on photosynthesis and transpiration of a broad bean crop and to evaluate the performance of a model for the effects of SO_2 on photosynthesis of leaf canopies by comparing simulation results with experimental data.

6.2 Materials and methods

Equipment for measurement of photosynthesis of leaf canopies

Measurement of photosynthesis and transpiration of enclosed canopies were performed with a mobile system, described by Louwerse & Eikhoudt (1975) and Louwerse (1980). Data logging and processing was performed by a PDP 11 minicomputer. The enclosure chamber (80x80x80 cm) consisted of transparent acrylic, sealed on a metal container (80x80x65 cm) in which the plants were grown. The measurement system was placed outdoors to obtain a natural time course of radiation. The air flow containing ambient CO_2 concentration (340 ppm) through the whole system was about 0.03 m^3 s^{-1}, which corresponds with a residence time of about 20 seconds (volume=0.6 m^3). The enclosure system operates as an open system with overpressure to avoid effects of leakage and soil respiration.

Total global radiation was measured with a Kipp solarimeter, air temperature with copper/constantan thermocouples and air humidity with Vaisala probes. SO_2 was bled into the air inlet (unfiltered air) of the chambers regulated by mass flow controllers from a cylinder (2000 ppm SO_2 in N_2). Since the flow of circulating air in the chamber was 10 times as high as the net replacement flow, SO_2 was well mixed. SO_2 concentration in the chambers was measured with a fluorescent SO_2 analyser (Monitor Labs, model 8850). Air was conducted from the chambers through teflon tubing by a teflonized pump. The incoming SO_2 flow was continuously adjusted manually to prevent large changes in SO_2 concentration in the chambers. The air temperature was maintained at 20 °C during the photoperiod and at 15 °C during the night.

Plant material

Vicia faba L. (cv. Minica) was sown in the glasshouse in pots filled with a commercial potting soil in the beginning of April 1988. One week before the measurements started, the plants were placed in metal containers and placed outdoors under a transparent roof to prevent frost damage.

Measurement procedure

Net photosynthesis of 2 enclosed canopies was measured simultaneously between 18-27 April 1988, at the Centre for Agrobiological Research in Wageningen. After one week of measurement new containers with plants were placed in the chambers. Since not enough containers were available, the chambers had no surrounding plants. Leaf area was determined after the measurements. Two extra containers were harvested in the first week. Development of LAI during the measurements was estimated by non-linear interpolation.

The canopies were fumigated on two consecutive Tuesdays and Thursdays. SO_2 was supplied to one chamber from 8.00 h - 13.00 h and to the other one from 13.00 h - 20.00 h. Because of the expected variation in radiation conditions, the crops were fumigated with 800 μg SO_2 m^{-3} in all experiments. This concentration was based on preliminary experiments.

Dynamic simulation of SO_2 effects on canopy photosynthesis

In order to compare simulated effects of SO_2 on canopy photosynthesis with measurements, photosynthesis of a block-leaf canopy was modelled by an adapted version of the procedure for the calculation of photosynthesis of row canopies, developed by Goudriaan (1977) and improved by Gijzen & Goudriaan (in prep.), which is basically

identical to the procedure for the calculation of photosynthesis of horizontally homogeneous canopies given by Goudriaan (1982, 1986) and Spitters (1986).

The fraction of diffuse radiation was calculated according to Spitters, Toussaint & Goudriaan (1986), who related this fraction to the ratio of the observed global radiation and the calculated extra-terrestrial global radiation. The solar position was calculated from the latitude and time of measurement (day and hour). The fraction of photosynthetically active radiation (400-700 nm) was assumed to be 50% of the global radiation. Absorption of light by the acrylic chamber was about 20%.

The path length of a single light beam from the margin of the block to a given point in the block canopy was calculated according to Goudriaan (1977) by using a converted co-ordinate system, to characterize the direction of the beam with respect to the spatial position of the block. By multiplying this path length by the leaf area density, it could be expressed as the leaf area traversed by the beam. The leaf area in the block was assumed to be homogeneously distributed over the block volume.

The average leaf projection in any direction was assumed to be 0.5 (spherical leaf angle distribution, Goudriaan (1977)). Taking into account reflection of the canopy, scattering by the leaves (transmission and reflection by the leaves in the canopy) and the path length, the radiation flux from a certain point at the hemisphere at a given point in the block, can be calculated. The absorbed radiation at a point in the canopy equals the radiation decrease in the direction of the beam. Integration of absorbed diffuse radiation over all angles of incidence, was performed with the Gaussian integration principle (Goudriaan, 1988). Calculation of absorption and scattering of direct radiation is analogous to the procedures given by Goudriaan (1982), Spitters (1986) and Spitters *et al.* (1986) for horizontally homogeneous canopies.

At a given point in the block canopy, SO_2 uptake, S(IV) oxidation and the amount of S(IV) in the leaf (influencing the rate of photosynthesis at light saturation) was calculated in a submodel for leaf photosynthesis (Chapters 3 and 4). The rate of photosynthesis was calculated on the basis of the absorbed amount of radiation and the S(IV) concentration in the leaves at that point in the canopy. On the basis of earlier observations, effects on respiration were neglected (Chapter 2).

Total photosynthesis of the block was obtained by a 5-point Gaussian integration (Goudriaan, 1986) over length, width and height of the block. The rate of photosynthesis and the balance of S(IV) in the leaf at 125 points in the block was calculated every timestep of integration for sunlit (divided over 3 leaf angle classes) and shaded leaves separately. The model was run with a time interval of 1 minute.

Modelling the effects of SO_2 on daily canopy photosynthesis

Other approaches than the one described above which operate with timesteps of one minute, have to be used when submodels for the uptake and effects of SO_2 are included in crop growth simulation models with integration intervals of one day. In the existing

simulation models for crop growth, daily photosynthesis of crop canopies is calculated from an assumed daily pattern for weather data, calculated from daily totals (radiation) or averages (temperature, rainfall) (Spitters *et al.*, 1986). The rate of photosynthesis is calculated for a selected number of moments per day and integrated over the day by using the Gaussian integration principle, which enables a strong reduction of the number of calculations (Goudriaan, 1986). When the effects of air pollutants are included, processes with time coefficients of several minutes have to be approached with equilibrium models.

The basic equation for the calculation of daily canopy photosynthesis is the photosynthesis light response curve for individual leaves (Goudriaan, 1982), described by:

$$P = P_{max} \left\{ 1 - \exp\left(\frac{-\varepsilon I}{P_{max}} \right) \right\} \qquad (6.1)$$

where P is the rate of leaf photosynthesis (μg CO_2 m^{-2} s^{-1}), P_{max} is the rate of leaf photosynthesis at light saturation (μg CO_2 m^{-2} s^{-1}), ε is the initial light use efficiency (μg CO_2 J^{-1}) and I is the absorbed radiation (PAR, J m^{-2} s^{-1}). It has been shown that SO_2 only influences the rate of photosynthesis at high levels of radiation (Black, 1982; Hållgren, 1984; Chapter 2). The effect of SO_2 on the maximum rate of photosynthesis is linearly related to the amount of toxic SO_2 metabolites in the mesophyll (S(IV) (mmol l^{-1})) when photosynthetic reduction is not extremely high (Chapter 3):

$$P_{max,s} = P_{max,0} (1 - k \, S(IV)) \qquad (6.2)$$

where $P_{max,0}$ is the maximum rate of leaf photosynthesis in clean air, $P_{max,s}$ is the maximum rate of leaf photosynthesis during exposure to SO_2, and k is the effect parameter in (mmol S(IV) l^{-1})$^{-1}$.

Photosynthetic reduction stabilizes soon after the start of exposure of leaves to SO_2 (Black & Unsworth, 1979b; Chapter 2) which can be explained by a rapid establishment of equilibrium concentrations of S(IV) in the leaf (Chapter 3). The supply of new metabolites by SO_2 uptake (Flux (F, mmol m^{-2} s^{-1}) divided by leaf thickness (d, mm)) then equals the rate of S(IV) removal by oxidation (which can be approached as a first order reaction with a time coefficient τ_2):

$$\frac{F}{d} = \frac{S(IV)}{\tau_2} \qquad (6.3)$$

The combination of Equations (6.2) and (6.3) gives an expression for the maximum rate of leaf photosynthesis during fumigation:

$$\frac{P_{max,s}}{P_{max,0}} = 1 - \frac{k \, \tau_2 \, F}{d} \qquad (6.4)$$

The stomatal resistance (r_s, s m^{-1}) to CO_2 can be calculated from the rate of leaf photosynthesis (P, μg CO_2 m^{-2} s^{-1}), the difference between ambient and internal CO_2 concentrations (C_a-C_i, μg CO_2 m^{-3}), which tends to be constant at a given CO_2 concentration even during SO_2 exposure (Chapter 2), and the boundary layer resistance(r_b in s m^{-1}):

$$r_s = \frac{C_a - C_i}{P} - r_b \qquad (6.5)$$

The flux of SO_2 into the leaf (F, mmol SO_2 m^{-2} s^{-1}) can be calculated from the ambient SO_2 concentration (S_a, mmol SO_2 m^{-3}), assuming the internal SO_2 concentration to be 0 (Black & Unsworth, 1979a; Carlson, 1983; Chapter 3), and the stomatal (r_s) and boundary layer (r_b) resistance to SO_2 (r_s for SO_2 equals about 1.21 x r_s for CO_2 and r_b for SO_2 is about 1.13 x r_b for CO_2). The expression for the flux is:

$$F = \frac{S_a}{1.21\, r_s + 1.13\, r_b} \qquad (6.6)$$

With the Equations (6.1), (6.4), (6.5) and (6.6) the effect of SO_2 on leaf photosynthesis in equilibrium can be calculated. However, this has to be done iteratively, since there is an internal loop in the equations: photosynthetic rate determines the stomatal resistance and thus SO_2 uptake which reduces photosynthesis in turn.

6.3 Results and discussion

Measured SO_2 effects on canopy photosynthesis

Table 6.1 gives some data on the four enclosed crop canopies exposed to SO_2 on four days in April, 1988, under the following environmental conditions with varying leaf area indices. On 19 and 28 April the sky was almost continuously clear, while on 21 and 26 April the sky was cloudy, resulting in strongly fluctuating radiation levels (Fig. 6.1). The extremely high peak levels of radiation on 26 April were caused by reflecting white clouds. Due to occasional equipment failure, radiation data were not always logged. Relative humidity in the chambers varied between 36 and 47%.

Net photosynthesis as measured in the two chambers on 19 April is given in Fig. 6.2. Both the LAI and photosynthesis of the crops in both chambers was almost equal (Table 6.1). The effect of SO_2 fumigation was only detectable around noon, when photosynthetic rates were high. In the morning, when chamber 2 was fumigated, photosynthesis in chamber 2 was lower than photosynthesis in chamber 1. When fumigation was switched to chamber 1 at noon, photosynthesis in chamber 2 became higher than photosynthesis in chamber 1. Fig. 6.2 clearly illustrates that the SO_2 effect was smaller in

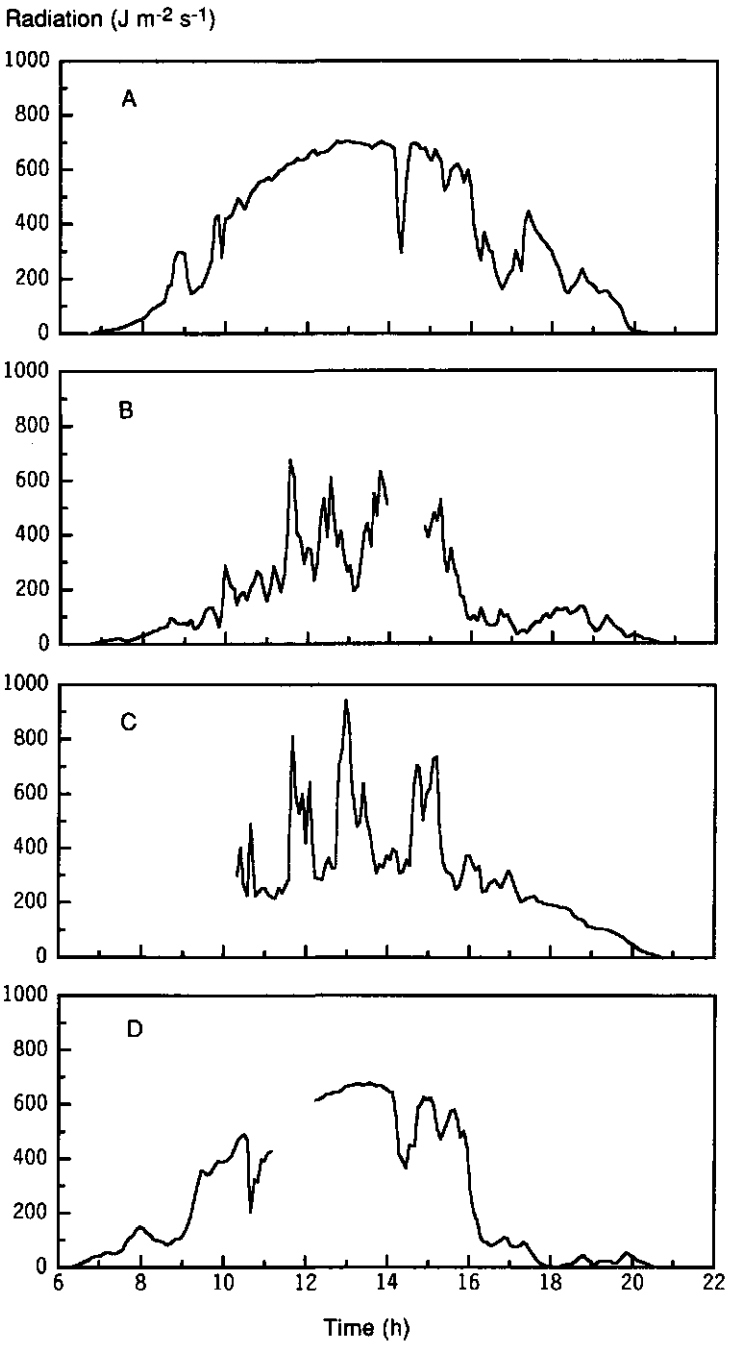


Fig. 6.1 Diurnal time course of total global radiation (J m^{-2} s^{-1}) during fumigation experiments (A. 19 April ; B. 21 April; C. 26 April; D. 28 April). No data were available for some periods due to equipment failure.

Table 6.1 Environmental conditions and leaf area indices (LAI) of 4 *Vicia faba* canopies during the fumigation experiments in 1988 at Wageningen.

Date	19 April	21 April	26 April	28 April
Daily total radiation (MJ m^{-2} d^{-1})	16.15	8.87	16.80	14.71
Hours sunshine	7.3	0.9	5.9	4.7
Relative humidity (%) (inside chamber)	47	39	36	39
LAI chamber 1	1.6	1.8	1.9	2.1
LAI chamber 2	1.6	1.8	2.1	2.3
Chamber fumigated in the morning	2	1	1	2
Chamber fumigated in the afternoon	1	2	2	1

periods with low photosynthesis and reduced radiation levels due to clouds (i.e. at 14.00 h, Fig. 6.1A). These results are consistent with experimental data on the effects of SO_2 on leaf and plant photosynthesis, which indicated effects on photosynthesis at light saturation only (Black, 1982; Hållgren, 1984; Chapter 2).

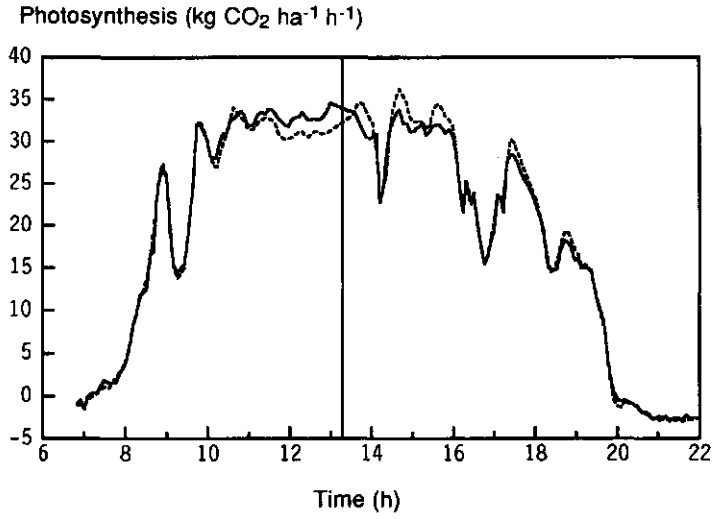


Fig. 6.2 Diurnal canopy photosynthesis (kg CO_2 ha^{-1} h^{-1}) on 19 April during SO_2 exposure. Chamber 2 (- - - -) was fumigated in the morning and chamber 1 (——) was fumigated in the afternoon. Moment of fumigation switch between the chambers is indicated with a vertical line.

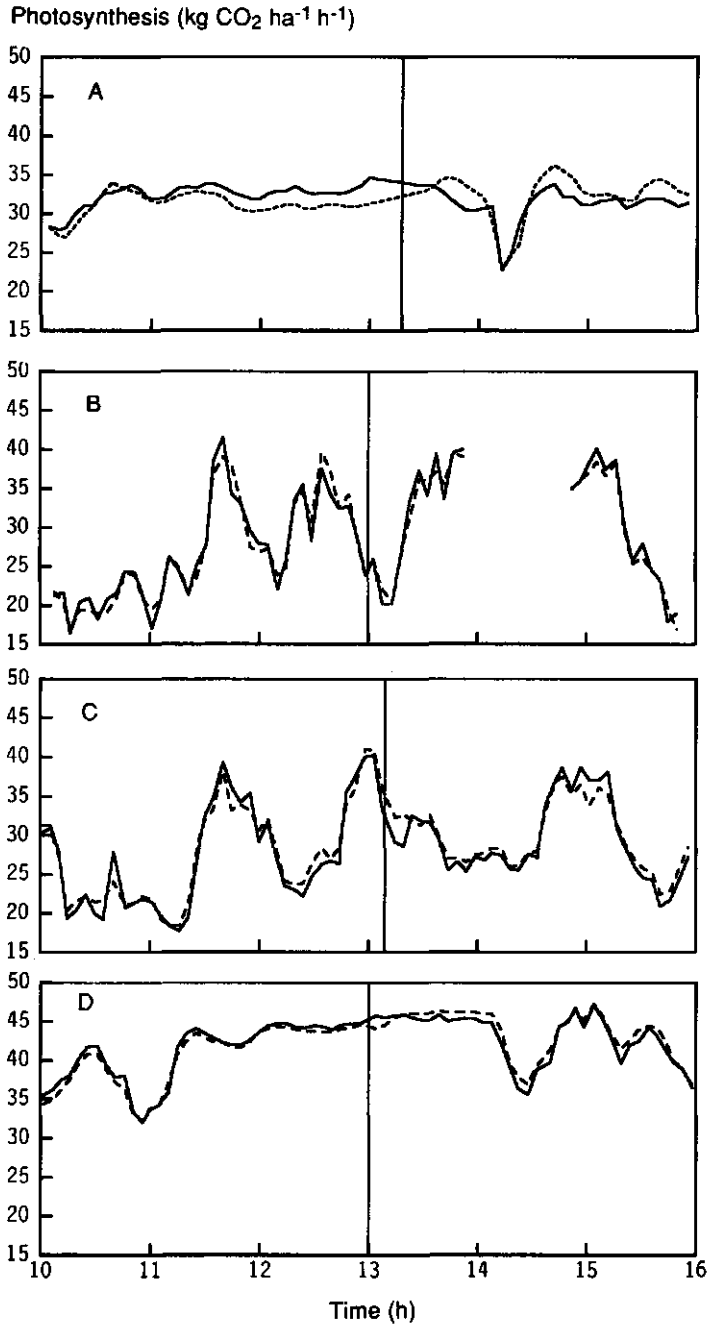


Fig. 6.3 Canopy photosynthesis (kg CO_2 ha^{-1} h^{-1}) from 10.00 h to 16.00 h during fumigation experiments (A. 19 April; B. 21 April; C. 26 April; D. 28 April). Moment of fumigation switch between the chambers is indicated by a vertical line. Chamber 1 was fumigated in the morning (——) and chamber 2 in the afternoons (- - - - -) on 21 and 26 April. Chamber 2 was fumigated in the morning and chamber 1 in the afternoon, on 19 April and 28 April.

The rates of photosynthesis from 10.00 h - 16.00 h on the four measurement days are given in Fig. 6.3 for the period around the fumigation switch. In the second week the crop in chamber 2 had a higher LAI than the crop in chamber 1 resulting in higher rates of photosynthesis. To enable graphical comparison of the data of both chambers, the rate of photosynthesis in chamber 2 was corrected for this difference in LAI by scaling with the mean ratio between photosynthesis of chamber 2 and chamber 1 (determined by linear regression). The effect of fumigation was clearly observed on 19 and 28 April, when the sky was clear for a long period. Canopy photosynthesis was reduced by about 6% on 19 April and by about 2% on 28 April by fumigation with 800 μg SO_2 m^{-3}.

No effects of SO_2 on canopy photosynthesis were detected on 21 and 26 April, when radiation continually changed. However, interpretation of these results was difficult as a result of the time lag between gas analysis of the chambers (72 seconds) and the sampling interval of data by the computer (5 minutes). When radiation and photosynthesis quickly changed, the difference between photosynthesis of the two chambers fluctuated. This effect is illustrated in Fig. 6.4, where both the rate of photosynthesis of one of the enclosed canopies and the difference in the rate of photosynthesis between the two chambers on 26 April is plotted. The difference between chamber 1 and chamber 2 was

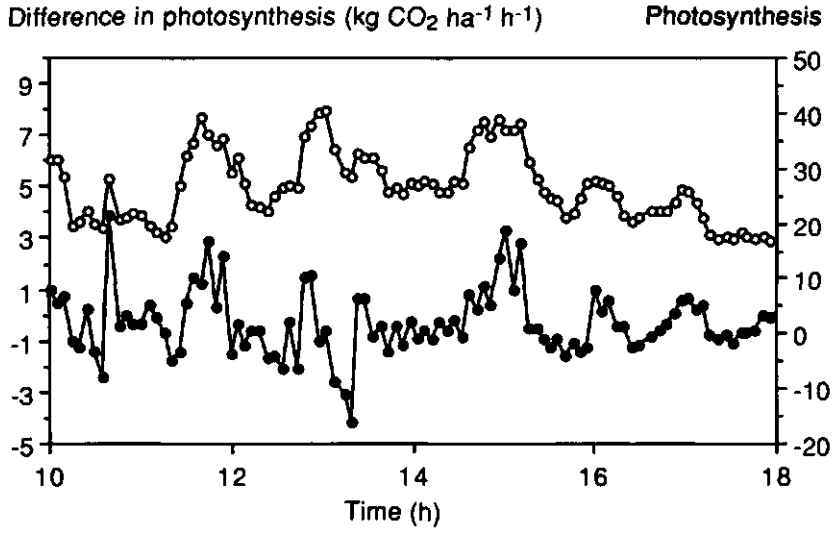


Fig. 6.4 Canopy photosynthesis in chamber 1 (kg CO_2 ha^{-1} h^{-1}) (o) and the difference in photosynthesis between both chambers (kg CO_2 ha^{-1} h^{-1}) (●) on 26 April during SO_2 exposure.

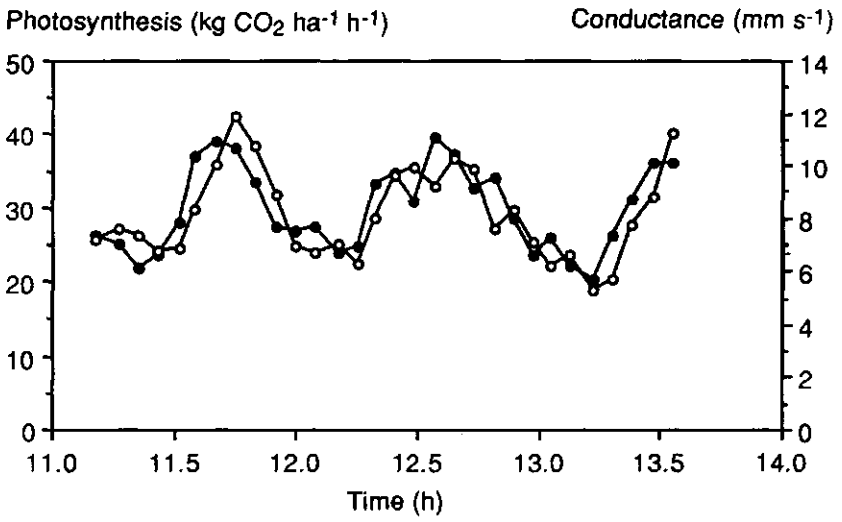


Fig. 6.5 Time course of canopy photosynthesis (●) (kg CO_2 ha^{-1} h^{-1}) and canopy conductance (○) (mm s^{-1}) on 21 April.

positive when photosynthesis increased and was negative when photosynthesis decreased, because chamber 2 was sampled first. The only periods on the cloudy days when photosynthesis was more or less stable for 2 subsequent measurements at high radiation levels was on 26 April at 13.00 h, when chamber 1 was fumigated and around 15.00 h when chamber 2 was fumigated. The pattern in photosynthetic reduction during these periods confirms the effects observed on the clear days.

The conductance of the canopy appeared to follow the assimilation rate with a delay of 5 minutes (Fig. 6.5). A similar delay with respect to photosynthesis was observed by Goudriaan, Louwerse & van Kleef (in prep.) for broad bean crops. This is of importance for the uptake of SO_2 by leaf canopies when radiation fluctuates, since the uptake of SO_2 and the pool of SO_2 metabolites will also follow photosynthesis with a delay. When radiation fluctuates, highest S(IV) levels will occur when photosynthesis is already lower and will thus have less influence on photosynthesis.

Model performance

The time course of photosynthesis in both chambers was simulated for the four days on which the crops were exposed to about 800 μg SO_2 m^{-3} for half a day. The measured data on radiation and SO_2 concentration were input in the model. Model parameters are

Table 6.2 Parameters used in the simulation model for the effects of SO_2 on canopy photosynthesis.

C_i/C_a	ratio between internal and ambient CO_2	0.75
$P_{max,0}$	maximum rate of photosynthesis (μg CO_2 m^{-2} s^{-1})	830
ε	initial light use efficiency (μg CO_2 J^{-1})	12.5×10^{-6}
k	sensitivity parameter ((mmol S(IV) $l^{-1})^{-1}$)	0.9
d	leaf thickness (mm)	0.4-0.5*
τ_2	time coefficient for S(IV) oxidation (s)	1200
τ	time coefficient for reaction of stomata (s)	300

* Leaf thickness of the plants in the first week was 0.4 mm and in the second week 0.5 mm.

listed in Table 6.2. The time course of photosynthesis of the enclosed crops on all four days was accurately simulated with the model (Fig. 6.6), where the observed and simulated photosynthesis in chamber 1 is plotted for a clear (19 April) and a cloudy day (21 April). Because the effects of SO_2 were very small, the accuracy of simulation of the effects of SO_2 can best be analysed by plotting the simulated and observed difference in photosynthesis between the chambers. The measured differences on the cloudy days cannot be interpreted due to the delay of 72 seconds between the measurements of the chambers, which causes strong fluctuations in the difference in photosynthesis between the chambers (see Fig. 6.4). Therefore only the results for the 2 clear days (19 and 28 April) are given in Fig. 6.7. For the construction of Fig. 6.7B (28 April) the data on measured and simulated photosynthesis of chamber 2 were corrected for differences in LAI. The time course of the difference in photosynthesis between the chambers was simulated very well on the basis of effect parameters derived in earlier studies of the effects of SO_2 on leaf photosynthesis (Table 6.1; Chapter 4). The magnitude of the effect was simulated correctly on 19 April, but was slightly overestimated on 28 April. This may have been caused by the different conditions at which the plants had been grown before the measurements started. The plants which were measured in the first week had been grown outdoors for one week at daily average temperatures of 10 °C and the plants measured in the second week had been grown outdoors for two weeks at high radiation levels and low temperatures (daily average temperature of about 5 °C in the second week when the first crop was measured). This resulted in thicker leaves of the second group of plants (0.5 mm compared to 0.4 mm). This effect was accounted for in the model.

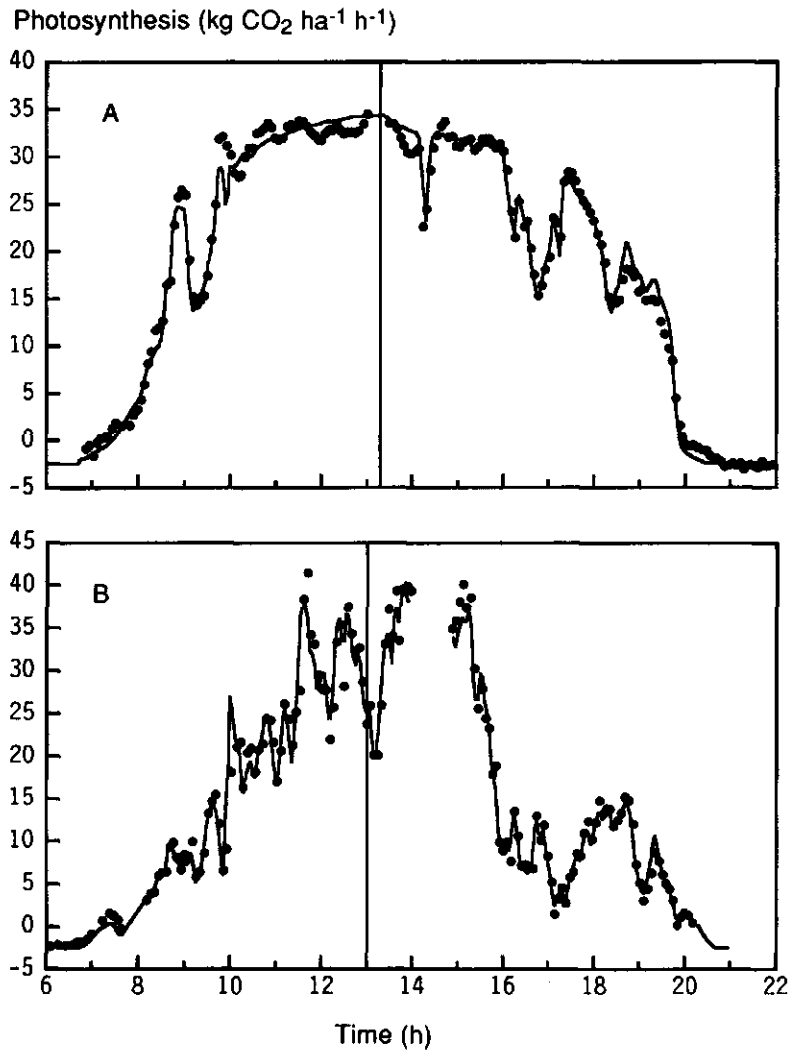


Fig. 6.6 Simulated (———) and observed (●) diurnal canopy photosynthesis (kg CO_2 ha^{-1} h^{-1}) on (A) 19 April and (B) 21 April in chamber 1. Moment of fumigation switch between the chambers is indicated with a vertical line.

Effects of SO_2 on daily photosynthesis of canopies

To analyse the errors made by neglecting the effect of quick changes on S(IV) concentration in the leaf in equilibrium models, the time course of photosynthesis during SO_2 exposure on 19 April was simulated with both the detailed and the equilibrium approach

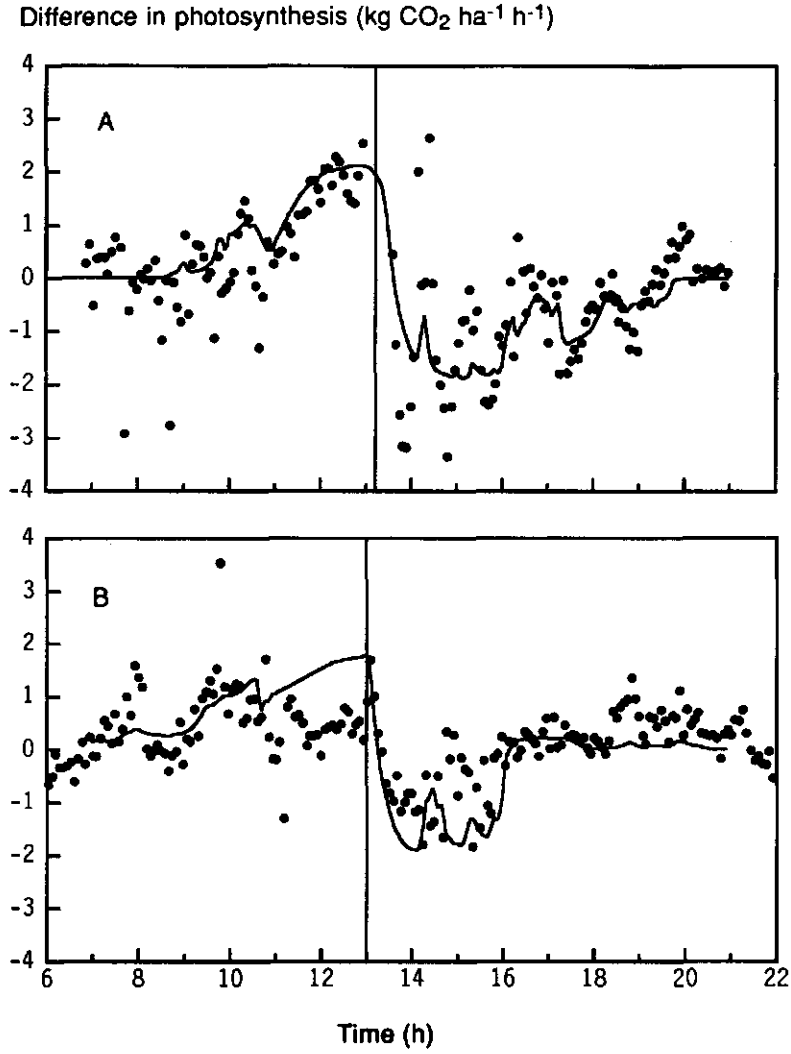


Fig. 6.7 Simulated (——) and observed (●) difference in canopy photosynthesis (kg CO_2 ha^{-1} h^{-1}) between both chambers on 19 April (A) and 28 April (B). On both days chamber 2 was fumigated in the morning and chamber 1 in the afternoon. Moment of fumigation switch between the chambers is indicated by a vertical line.

for the block canopy. Fig. 6.8 shows that the effect of SO_2 is overestimated with the equilibrium approach when radiation or SO_2 concentration quickly changes, since the dynamics in the processes determining the S(IV) pool in the leaf are ignored in the equilibrium approach. The difference between both versions of the model are small, especially

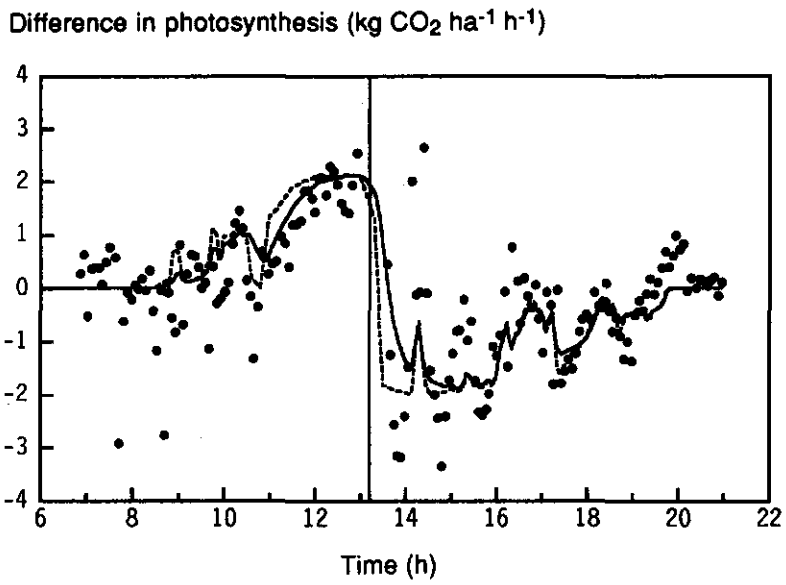


Fig. 6.8 *Simulated and observed difference in diurnal canopy photosynthesis* (kg CO_2 ha^{-1} h^{-1}) between the chambers on 19 April with the detailed version of the model (——) and the equilibrium approach (- -). Moment of fumigation switch between the chambers is indicated by a vertical line.

when compared to the actual rates of canopy photosynthesis. The overestimation of the effect will be strongest on days in which radiation strongly fluctuates. The equilibrium approach can be used in models operating with time steps of integration of one day which use data on daily total radiation as input. It should be noted that the overestimation of effects when radiation fluctuates will be compensated by the underestimation of effects during bright periods, because daily total radiation is smoothly distributed over the day in these models.

The simulated uptake of SO_2 by a normal crop canopy (no block form) and its effect on gross canopy photosynthesis in dependence of daily global radiation at 800 μg SO_2 m^{-3} is presented in Fig. 6.9 (for the maximum rate of leaf photosynthesis $P_{max,0}$ the value of 1100 μg CO_2 m^{-2} s^{-1} was used, since this is more realistic for field grown plants). This figure clearly shows that effects on daily canopy photosynthesis only occurred at high radiation levels, and hardly increased with LAI. Since canopy conductance is closely related to the rate of photosynthesis, the daily uptake of SO_2 by the canopy followed the same pattern as the rate of photosynthesis. Fig. 6.10 shows that the relative effect of SO_2 on canopy photosynthesis was smaller at higher LAI, because more leaves were functioning at low levels of radiation. This also explains the smaller effect of SO_2 on canopy photosynthesis when compared to the effect of SO_2 on leaf photosynthesis (for the same conditions a reduction of 10% was observed (Chapter 4)).

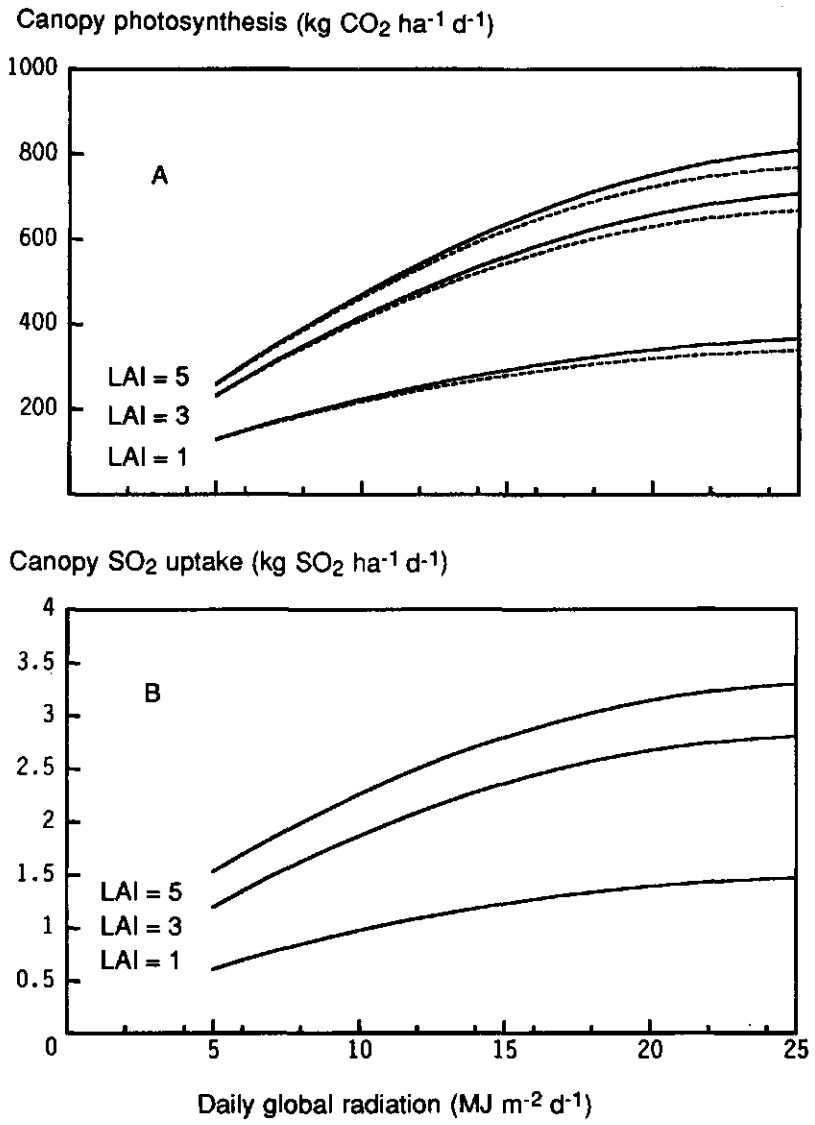


Fig. 6.9 A. Simulated relation between canopy gross photosynthesis and daily global radiation for LAI = 1, 3 and 5, respectively, without SO_2 (——) and with 800 μg SO_2 m^{-3} (- - - -). B. Simulated relation between SO_2 uptake by the canopy and daily global radiation. Parameters for *Vicia faba* (see text).

The effects presented in Figs. 6.9 and 6.10 are small. When the plants are more sensitive (thinner leaves, larger time coefficients for S(IV) oxidation) the effects will be more pronounced. Table 6.3 shows the effect of SO_2 on daily canopy photosynthesis for leaf

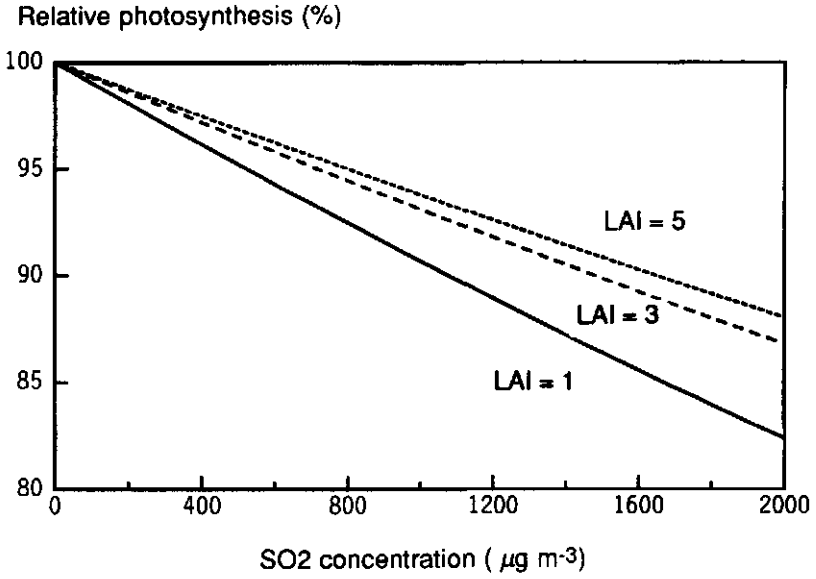


Fig. 6.10 Simulated effect of SO_2 on relative gross canopy photosynthesis for LAI = 1, 3 and 5 respectively, on a clear day in June. Parameters for *Vicia faba* (see text).

canopies, differing in average leaf thickness (0.2-0.4 mm) and the time coefficient for S(IV) oxidation. Two realistic extreme values were chosen: 20 minutes and 100 minutes (Chapter 4). Considerable effects on leaf canopy photosynthesis were only observed at the high SO_2 concentration of 800 μg SO_2 m^{-3}, even when the leaves were very sensitive (thin leaves and a slow rate of S(IV) oxidation). These results explain the findings of several workers who studied the effect of SO_2 on crop growth in field situations, and observed no effects on crop growth in the linear growth phase of the crop at concentrations <150 μg SO_2 m^{-3} (Baker *et al.*, 1986; McLeod *et al.*, 1988; Chapters 7 and 8). The small effects of low SO_2 concentrations on crop growth during winter may be explained by differences in the oxidation characteristics at low temperatures (Chapter 5).

Symbols used in Chapter 6

C_a	ambient CO_2 concentration	(μg CO_2 m^{-3})
C_i	internal CO_2 concentration	(μg CO_2 m^{-3})
d	leaf thickness	(mm)
ε	initial light use efficiency of leaf photosynthesis	(μg CO_2 J^{-1})
F	rate of SO_2 uptake	(mmol SO_2 m^{-2} s^{-1})
I	absorbed radiation	(J m^{-2} s^{-1})
k	parameter for effect of S(IV) on photosynthesis	((mmol S(IV) l^{-1})$^{-1}$)
P	rate of photosynthesis during fumigation	(μg CO_2 m^{-2} s^{-1})
P_{max}	maximum rate of photosynthesis at light saturation	(μg CO_2 m^{-2} s^{-1})
$P_{max,0}$	maximum rate of photosynthesis before fumigation	(μg CO_2 m^{-2} s^{-1})
$P_{max,s}$	maximum rate of photosynthesis during exposure to SO_2	(μg CO_2 m^{-2} s^{-1})
r_b	boundary layer resistance to CO_2	(s m^{-1})
r_s	stomatal resistance to CO_2	(s m^{-1})
S_a	ambient SO_2 concentration	(mmol SO_2 m^{-3})
τ_2	time coefficient for S(IV) removal	(s)

Chapter 7

Effects of long-term open-air fumigation with SO_2 on a field crop of broad bean.

I. Depression of growth and yield

Abstract In an open-air field exposure system for the controlled release of air pollutants, broad bean (*Vicia faba* L.) crops were exposed to elevated SO_2 concentrations in three growing seasons, in order to analyse the effects on crop growth under field conditions. The treated plots were exposed to a mean concentration of 165 μg SO_2 m^{-3} in 1985, 62 μg SO_2 m^{-3} in 1986 and 74 μg SO_2 m^{-3} in 1988. The background concentration was about 10 μg SO_2 m^{-3}.

A reasonably uniform distribution of SO_2 concentration was obtained over an area of 8 m x 8 m and concentrations exceeding the target concentration were rare. In 1985 and 1988, the growth rate of the crop was depressed at the end of the pod filling period. This resulted in a reduction of total dry matter production of 17% in 1985 and 9% in 1988, and a seed yield reduction of 23% in 1985 and 10% in 1988. In 1986, dry matter growth was not analysed up to the end of the growing season due to a severe infection of *Botrytis fabae* (Chocolate spot disease) infection in the control plot in the middle of the pod-filling period. Slight *B. fabae* infections in the control plots only were also observed in 1985 and 1988. No significant reductions of dry matter growth were observed in the vegetative and early reproductive phases.

7.1 Introduction

In the past decade, research efforts on the effects of air pollutants on plants have gradually changed from analyses of acute visible leaf injury caused by short exposures to high concentrations, to studies on chronic effects of airborne pollutants at lower concentrations. The first type of study gives information on effects which may occur occasionally as a result of accidents or on effects observed near point sources. Studies on chronic effects at lower concentrations which do not cause acute effects, may provide information on the effects of pollutants on crops and vegetation in extended areas. For SO_2, Fowler & Cape (1982) estimated the rural area in Europe exposed to concentrations exceeding 50

$\mu g\ SO_2\ m^{-3}$ (annual means) to be about 6%. Concern that agricultural crops are adversely affected by SO_2 is based on evidence that sulphur dioxide may reduce plant growth at concentrations as low as 40 $\mu g\ SO_2\ m^{-3}$ without causing visible injury (Bell *et al.*, 1979).

Most experimental work on the effects of air pollutants on plants has been carried out in laboratory or greenhouse growth chambers. In such facilities, the microclimate strongly differs from the field, which results in plants with a different physiological and morphological status (*e.g.* photosynthetic capacity of leaves and leaf thickness). As there is a strong interaction between climatic factors and the uptake and effects of air pollutants, it is hard to extrapolate observed effects in these facilities to field situations (Black, 1982; Unsworth & Mansfield, 1980). In addition, it is difficult to grow plants at realistic densities to maturity in indoor facilities. Results from such studies, therefore, only indicate the sensitivity of plants in the early vegetative growth period and cannot be related to effects on crop growth. Exponentially growing plants may be much more sensitive to effects on physiological processes than linearly growing plants in closed canopies, since reduced growth rates may cause reductions in leaf area development as well, which in turn affects the growth rate of exponentially growing plants. The growth rate of a closed canopy, however, is hardly affected by small changes in leaf area development. Effects occurring in early stages sometimes decrease or disappear during the growing season (Baker *et al.*, 1986; McLeod *et al.*, 1988; Pande & Mansfield, 1985) or increase (Agrawal, Nandi & Rao, 1985; Wright, 1987).

Field studies of the effects of pollutants on crops have been carried out in open-top chambers to overcome such problems (Davis, 1972; Heagle *et al.*, 1974). Although these facilities permit the study of long-term effects on crops and vegetation, there are still problems in extrapolating the effects to the field, since environmental factors (temperature, radiation, windspeed) are affected by the chambers, resulting in a modification of pollutant effects. Olszyk *et al.* (1986) demonstrated that there were dramatic differences in the effects of pollutants on plant growth between plants grown in open-top chambers and air-exclusion systems, because air-exclusion systems influenced microclimate less than open-top chambers did. Wheat yield decreased as a result of exposure to SO_2 in open top chambers (405 $\mu g\ SO_2\ m^{-3}$) but was not affected in the air-exclusion system. For lettuce, the opposite reaction was observed. Plant growth in both systems differed much from plants grown in outside plots.

Only a few systems for open-air exposure of vegetation without any form of plant enclosure have been developed in the past decades, ranging from single point sources to computer-controlled square or circular line sources (see review by McLeod *et al.*, 1985). Two open-air fumigation systems have been developed in which a reasonably uniform distribution of gas over the treated area is obtained, without affecting the environmental conditions (Greenwood *et al.*, 1982; McLeod *et al.*, 1985). In both systems, experiments were conducted with winter barley. McLeod *et al.* (1988) observed an enhanced dry weight and leaf area production of barley in plots fumigated with 62 μg

SO_2 m^{-3} and a reduced yield at 157 μg SO_2 m^{-3}, but interpretation of their results is difficult, due to a rust infection. Baker *et al.* (1986, 1987) observed growth reductions in winter barley exposed to SO_2 (108-540 μg SO_2 m^{-3}) in winter and early spring, but a recovery during spring and summer. However, grain yield was reduced at all concentrations in both years. They observed higher leaf area indices in the fumigated plots and no acceleration of leaf senescence. The few studies with dicot crop species exposed to SO_2 in the field were carried out in field fumigation systems in which the SO_2 concentration was not homogeneously distributed in time and/or space (McLeod *et al.*, 1985). Strong yield reductions of soybeans (up to 48%) were reported by Sprügel *et al.* (1980), who fumigated the crop during 18 days (4.2 hours per day) with 243-972 μg SO_2 m^{-3}. However, extremely high peak concentrations were measured during short periods (Miller *et al.*, 1980).

In this study, a recently designed open-air fumigation system in which crops or vegetation can be exposed to a reasonably uniform distribution of SO_2 concentrations, was used to analyse the effects of an elevated SO_2 concentration on the growth and development of broad bean. In this Chapter we report on the performance of the exposure system and present data on dry matter growth and seed yield. In Chapter 8, detailed data on the effects of exposure on growth components, leaf area development and chemical composition will be discussed.

7.2 Materials and methods

The open-air fumigation system

A new open-air exposure system was designed for the assessment of crop or vegetation responses to elevated concentrations of air pollutants. The requirements needed to overcome the limitations of earlier developed systems were:
1. Spatially uniform concentrations over an area of at least 100 m^2 in order to have enough plants to allow growth analysis by periodical harvesting.
2. The possibility to maintain predetermined concentrations of the pollutants and to avoid peaks in concentration.

To meet these requirements, a circular line source system was developed in which gas release was controlled by a computer on the basis of information on windspeed and gas concentrations in the sampling area. Fig. 7.1 shows a schematic representation of the system. SO_2 supply from gas cylinders was determined by computer directed mass flow controllers. The gas was mixed with dried air, to avoid condensation of SO_2 at low temperatures in the supply tubes. Two circular pipelines with a diameter of 30 m (at 0.6 and 2.1 m height) were connected by vertical gas release tubes with three emission holes (1 mm diameter) per tube. The circular system was divided into 16 independent segments, each segment containing six vertical gas release tubes. All segments were connected with the main supply tube by computer directed valves.

Based upon the measured wind direction, the valves of three upwind segments were opened to release SO_2 gas. The SO_2 concentration was maintained at the desired concentration by computer-controlled adjustment of the rate of SO_2 gas release. The rate of gas release was primarily regulated in relation to the measured windspeed, combined with feed-back control on measured SO_2 concentration in the central plot, since the delay in the sampling and measurement procedure was about 6 minutes. SO_2 was only released when the windspeed exceeded 1 m s^{-1}, to avoid too high concentrations caused by poor gas dispersion. Sulphur dioxide release and data collection (measured windspeed, wind direction and SO_2 concentrations) were controlled and processed by a Hewlett Packard 85 microcomputer which was linked-up with a data acquisition system (HP 3497 A). To prevent excessive SO_2 concentrations a number of safety precautions were included in the hardware and software of the system. At the centre of the fumigated plot (at 1.5 m height), the concentration of SO_2 was measured with a Monitor Labs model 8850 SO_2 pulsed fluorescence analyser. For 1986 and 1988, data from a nearby station were used (station Barneveld). Data were collected by the National Institute for Public Health and Environmental Protection (RIVM, Bilthoven). To assess the horizontal distribution of the SO_2 concentration, SO_2 was monitored at four additional points at the edges of the 8 m x 8 m exposed plot. The background concentration of SO_2 was only monitored in 1985 with a Philips PW 9700/00 analyser. Wind velocity and direction were measured at a height of 3 m. The exposure system operated continuously and automatically throughout the growing season, except for periods in which the wind velocity was lower than 1 m s^{-1} or during equipment failure.

A full technical description of the computer-controlled field fumigation system was given by Mooi & van der Zalm (1986). A similar system has been developed by McLeod *et al.* (1985).

Plant material

To avoid confounding variation due to differences in soil conditions between the plots, plants of *Vicia faba* L. (cv. Minica) were grown in plastic containers (55 x 22 x 25 cm) in the field at a density of 20 plants per m^2. The containers were filled with a commercial potting mixture ((Trio 17) with additional fertilizer; per container: 7 g sporumix A and 14 g triabon), which was inoculated with *Rhizobium*. The amount of available water was kept high enough to avoid water limitation of crop growth with a drip-irrigation system. One plot of 8 m x 8 m (150 containers) with broad bean plants was placed within the exposure system and a similar control plot was located at a distance of 250 m from the exposure system. In 1988 only one third of the fumigation system was used (50 containers). In 1985 plants emerged on 16 April (Day 106), in 1986 on 1 May (Day 121) and in 1988 on 15 May (Day 135). Final harvest in 1985 was on 29 July (Day 210), in 1986 on 21 July (Day 202) and in 1988 on 25 August (Day 237).

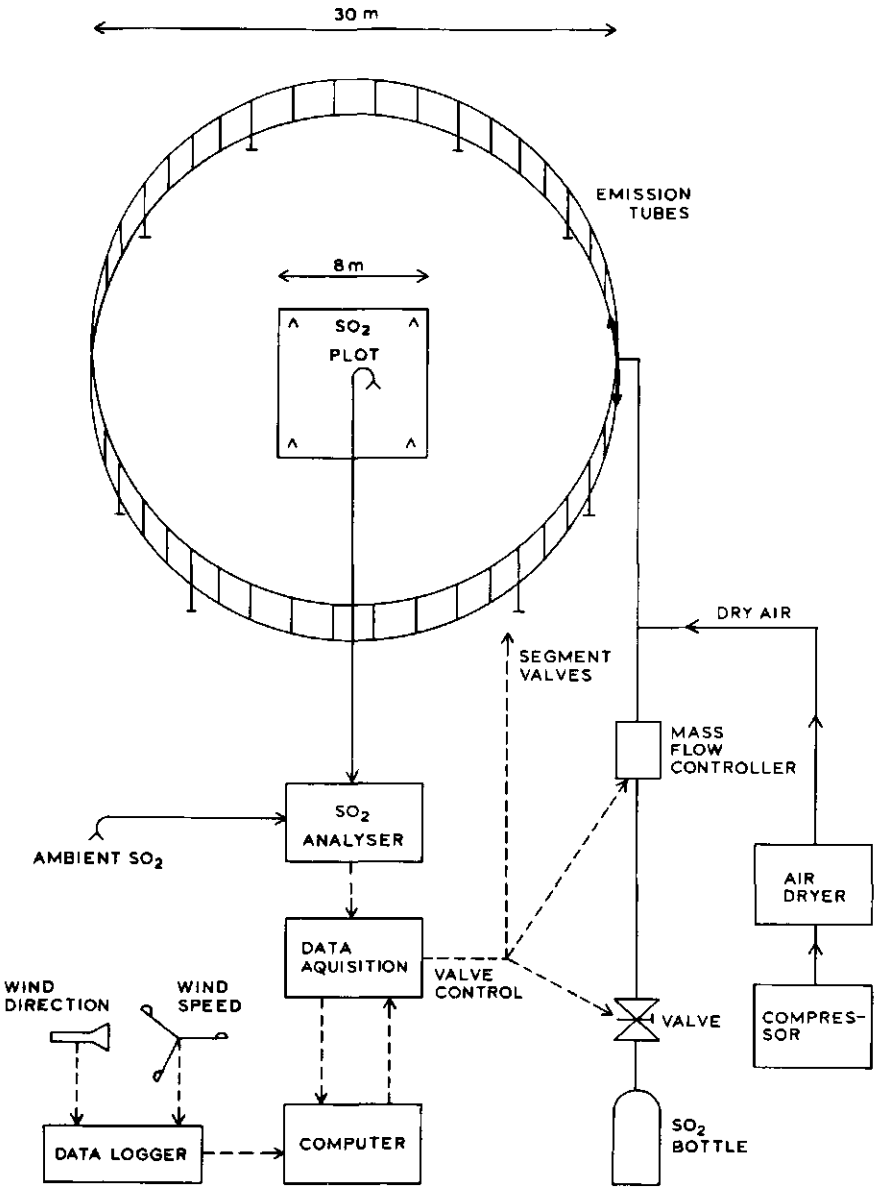


Fig. 7.1 A schematic representation of the field exposure system. Sampling points are indicated.

Experimental procedure

The experiments were conducted in a 1.5 ha grassland at Wageningen, the Netherlands, near the Research Institute for Plant Protection Research. The plots were fenced to prevent damage from rabbits. Meteorological data were collected at 1 km distance from the experimental site at the Meteorological station of the Agricultural University Wageningen (temperature, radiation, windspeed, relative humidity). As equipment for monitoring of O_3 was not available, monitoring data from another comparable area (about 60 km away) were used (data from RIVM, Bilthoven). In 1985 the target concentration in the fumigated plot was 200 μg SO_2 m^{-3} and in 1986 and 1988 100 μg SO_2 m^{-3}. SO_2 release was started as soon as possible after crop emergence and continued until maturity. In 1986, however, gas release was started 32 days after crop emergence and SO_2 measurement 22 days after emergence due to equipment failure. In 1988, gas release was started 20 days after emergence. The plots were divided into 4 blocks each consisting of a number of subplots, corresponding with the number of harvests (6 harvests in 1985 and 5 harvests in 1986). Five half containers per block were harvested from subplots. In 1988, only 3 blocks were available from which one container was harvested. Mean per plant dry matter per container was used as the statistical unit in the analysis of variance. In all experiments *Botrytis fabae* was controlled chemically when the first symptoms were recognized. However, in 1986 a severe *Botrytis fabae* infection could not be prevented in the control plots. In this year, plants were randomly sampled from non-infected blocks in the control plot for detailed analysis at final harvest. Data on dry matter and leaf area at final harvest were not available for harvest 5. The plants were separated into leaves, stems and reproductive organs and the leaf area was measured. Dry weight was determined after a drying period of 24 hours at 105 °C. The chemical composition of the organs was analysed in the laboratory of the Research Institute for Plant Protection and is presented in a subsequent paper (Chapter 8).

The experiment was on a too large a scale to allow replicates of the exposure system within one growing season. Therefore, we repeated the experiment in time and eliminated as many sources of variation in plant growth between the plots as possible (growing the plants in plastic containers, using drip irrigation).

7.3 Results

The hourly mean SO_2 concentration in the fumigated plot during the growing season was 165 μg SO_2 m^{-3} in 1985, 62 μg SO_2 m^{-3} in 1986 and 74 μg SO_2 m^{-3} in 1988. These data include the periods when fumigation was turned off as a result of low wind velocity or equipment failure. The background SO_2 concentration was 16 μg SO_2 m^{-3} in 1985, 7 μg SO_2 m^{-3} in 1986 and 9 μg SO_2 m^{-3} in 1988. The SO_2 concentration in the fumigated plot fluctuated within short time spans when wind velocity fluctuated quickly due to the reaction time of the system. This reaction time consisted of a delay in changing gas con-

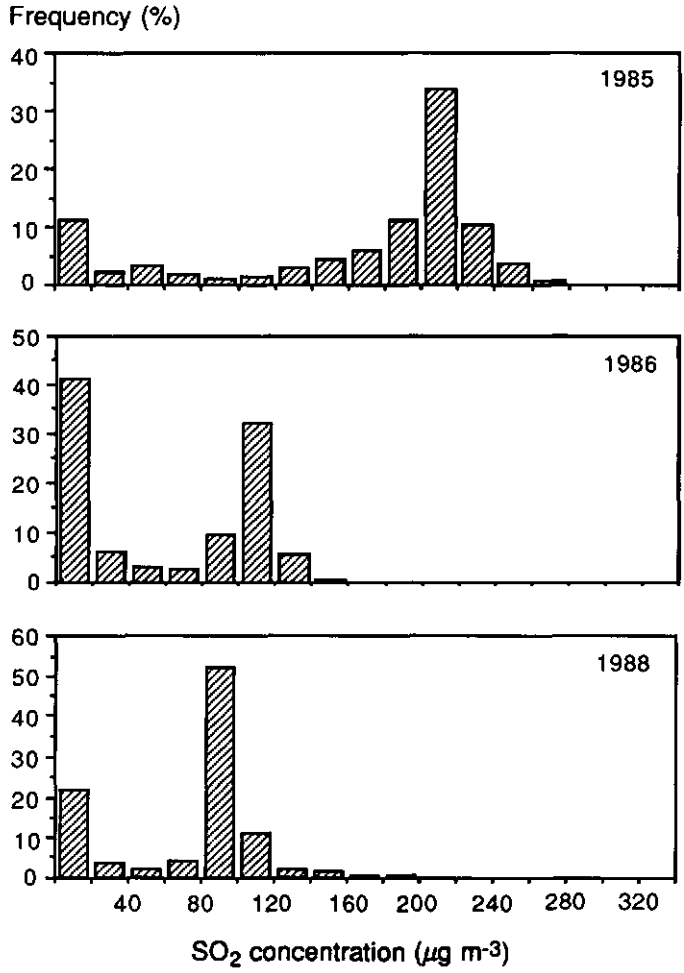


Fig. 7.2 Frequency distribution of the hourly SO_2 concentrations ($\mu g\ m^{-3}$) in the fumigated plot in 1985, 1986 and 1988.

centration in the long gas supply tube, and the reaction time of mass flow controllers and wind speed measurement equipment. The frequency distribution of hourly mean concentrations, measured at the central sampling point in the plot, shows that the desired concentration (200 $\mu g\ SO_2\ m^{-3}$ in 1985 and 100 $\mu g\ SO_2\ m^{-3}$ in 1986 and 1988) was realized during most of the fumigation period (Fig. 7.2). In 1986, the crop was not fumigated for 32 days in the beginning of the growing period as a result of equipment failure and in 1988 gas release started 20 days after emergence, which caused the high frequency of low concentrations.

Table 7.1 Average hourly mean SO_2 concentrations ($\mu g\ m^{-3}$) in the open-air exposure system at 1.5 m height at the edges of the 8 m x 8 m plot.

sampling point	1985	1986
1	191	98
2	178	111
3	167	109
4	175	114

As a consequence of safety precautions peaks in SO_2 concentration were avoided. The measured hourly mean SO_2 concentration did not exceed 400 $\mu g\ SO_2\ m^{-3}$ for more than 2 hours per sampling point in 1985 (with a maximum of 750 $\mu g\ SO_2\ m^{-3}$) and the concentration did not exceed 400 $\mu g\ SO_2\ m^{-3}$ for more than 2 hours in 1986 and 1988 (with a maximum of 645 $\mu g\ SO_2\ m^{-3}$).

The average hourly mean SO_2 concentrations measured at the different sampling points are presented in Table 7.1. Since the points at the edges of the exposed crop were sampled only after readjustment of gas supply on the basis of preceding measurements of the central point, these values are close to the target SO_2 concentration. These data show that the horizontal distribution of hourly SO_2 concentrations was reasonably uniform over the fumigated plot. The time course of radiation and daily average temperature is presented in Fig. 7.3. The main difference between the three experiments was a bright and warm period in June 1986 (day 160-180). O_3 concentrations (5-day averages) are given in Fig. 7.3C. Strong differences between the three years were not observed. The average hourly mean O_3 concentration during the experiments was 65 $\mu g\ O_3\ m^{-3}$ in 1985, 64 $\mu g\ O_3\ m^{-3}$ in 1986 and 57 $\mu g\ O_3\ m^{-3}$ in 1988. Differences in the extremes of concentration (the concentrations exceeded for 50, 10 or 5 % of the time) were almost equal for the experimental periods (Table 7.2).

Table 7.2 Hourly mean O_3 concentration ($\mu g\ m^{-3}$) that was exceeded for 50, 10, 5 or 2 % of the time during the growing season.

	1985	1986	1988
50%	67	63	56
10%	103	106	101
5%	121	124	120
2%	148	147	144

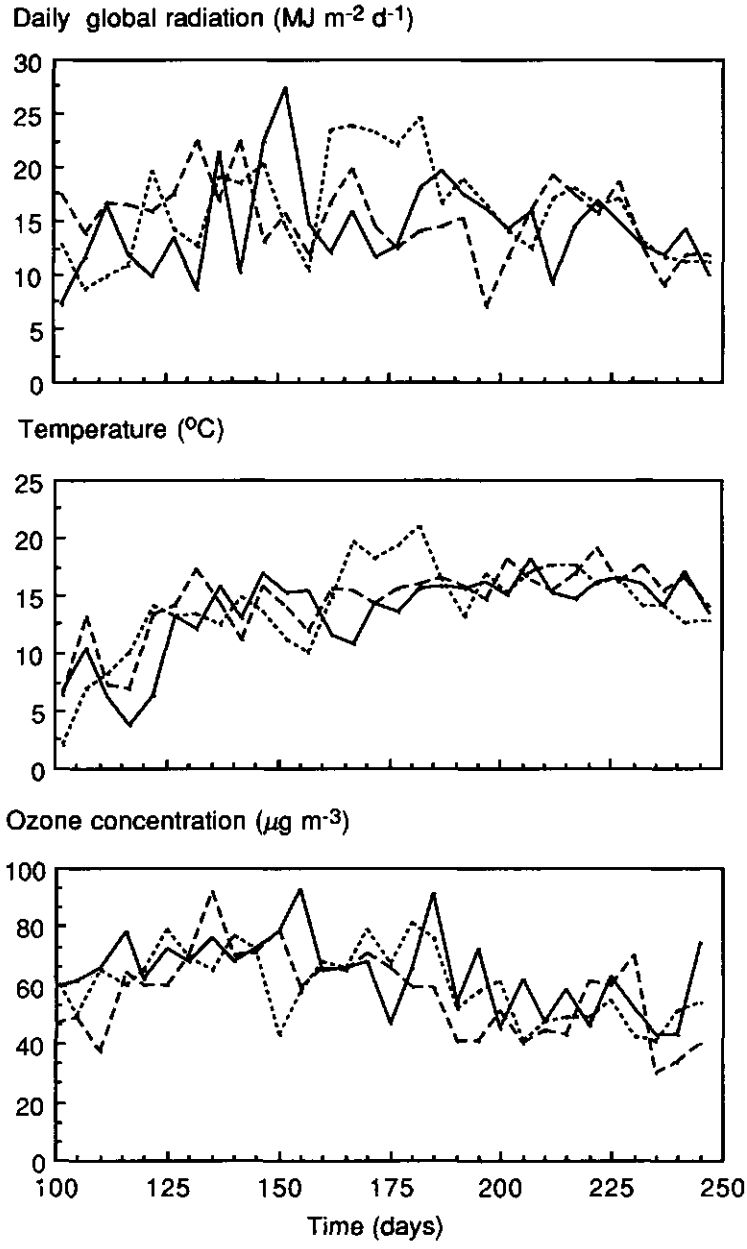


Fig. 7.3 Time course of (A) daily global radiation in MJ m^{-2} day^{-1}, (B) daily average temperature and (C) hourly mean O_3 concentration (μg m^{-3}), in 1985 (——) , 1986 (- - - -) and 1988 (– – –). All data are expressed as 5-days averages.

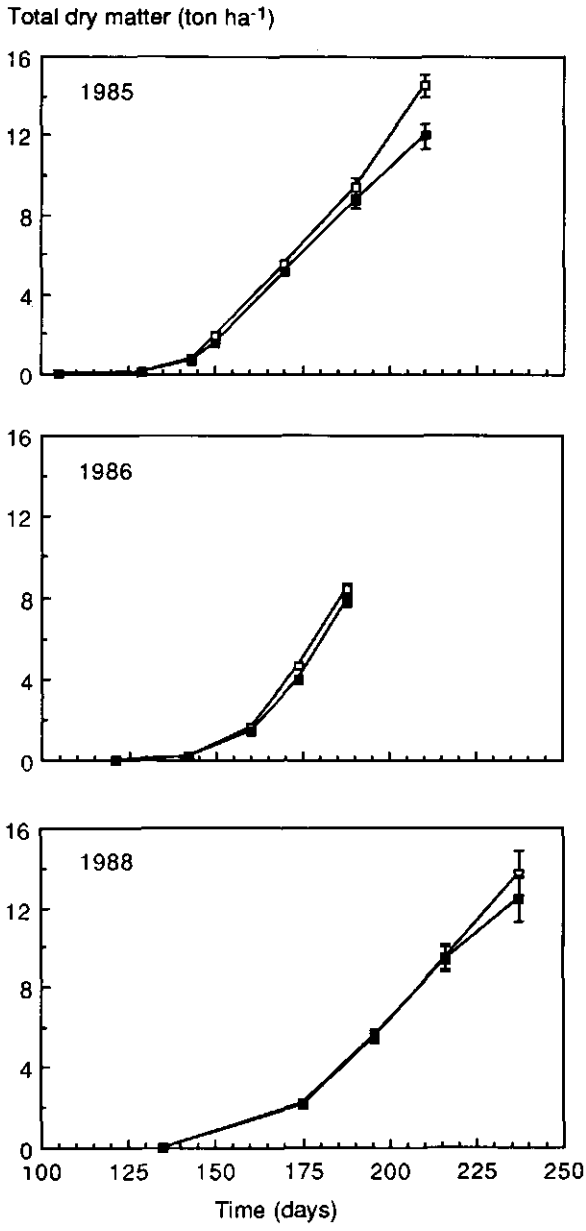


Fig. 7.4 Time course of total above ground dry matter (ton ha^{-1}) of broad bean (*Vicia faba*) in control plots (□) and fumigated plots (■) in 1985, 1986 and 1988. Standard errors are shown by vertical bars.

The time course of dry matter production is given in Fig. 7.4. In all the experiments a slight reduction in dry matter growth was observed in the first part of the growing period (exponential growth phase). In the linear growth phase, growth rates of the fumigated and the control crops were similar in all three experiments. However, at the end of the growing period in 1985, dry matter growth was strongly reduced in the fumigated plots, which resulted in significant reductions of total above ground biomass at final harvest (17% in 1985). A similar pattern was observed in 1988 when a reduction of 9% in total above ground biomass was observed. Due to the small samples in 1988 these effects were not significant. In 1986, the growth rate of the crops was higher in the middle of the growing season. This was probably due to the higher levels of radiation in this year. As a result of a severe *B. fabae* infection in 1986 in the control crop between harvest 4 and harvest 5, no data on final dry matter production are available. A slight *B. fabae* infection in the control plot only was also observed in 1985 and 1988.

Seed yield in 1985 was reduced by 23% at final harvest and in 1988 by 10%. No data are available for 1986, as a result of the *B. fabae* infection.

7.4 Discussion

The target SO_2 concentration in the open-air exposure system was realized during a large part of the experimental period (Fig. 7.2). Strong fluctuations, as observed in the system of Miller *et al.* (1980), were avoided by computer-controlled gas release. Excessive high concentration peaks, which may cause acute effects, were almost completely avoided by safety precautions, incorporated in the software and hardware of the computer-control system. The exposure system provided a reasonably uniform gas dispersion over the treated area, an essential requirement in exposing an area as large as 8 m x 8 m from which sequential harvests are taken to assess the pollutant's effect on plant growth. A theoretical motivation for this type of exposure system, based upon modelling of gas dispersion was given by McLeod *et al.* (1985).

The results presented in this Chapter indicate that exposure of field crops to elevated concentrations of SO_2 may influence the growth and production of crops at concentrations which are realistic for agricultural areas in Europe (1% >100 μg SO_2 m^{-3} and 5% between 50 μg SO_2 m^{-3} and 100 μg SO_2 m^{-3} (annual means), Fowler & Cape, 1982).

The effects of SO_2 on seed yield presented in this paper are in agreement with data from other field studies with leguminous crop species. McLaughlin & Taylor (1985) summarized data from open-air exposure studies with soybean and snapbean in which the methodology ranged from linear gradient sources to plots differing in distance from smelters which emitted large amounts of SO_2. They found a close relationship for these data between yield loss and exposure dose (yield loss (%) =28.9 x log (dose in ppmh) - 15.9, r^2=0.77). We observed smaller yield losses in relation to pollutant dose, although our data are still in the range of observations given by McLaughlin & Taylor (1985). The

observed yield loss in 1985 was 23% and the calculated yield loss from the pollutant dose (when night exposures are neglected) according to the equation was 34%. For 1988 a yield loss of 10% was observed whereas from the relation of McLaughlin & Taylor (1985) a yield loss of 25% would be expected. This difference may be due to differences in exposure technique: in our study the crops were exposed to low, more-or-less constant concentrations throughout the entire growing season, whereas in most studies reviewed by McLaughlin & Taylor (1985) the plants were exposed to higher concentrations for shorter periods (*e.g.* Sprügel *et al.* (1980) exposed the plants only 18 times during 4.2 hours per day in the daytime in the pod filling period).

In field studies in which cereals were exposed to elevated SO_2 concentrations (108-540 μg SO_2 m^{-3}), significant reductions of seed yield have been observed, without clear effects on total dry matter (Baker *et al.*, 1986). Others report a higher grain yield and a total dry matter production at low concentrations for cereal crops exposed to elevated SO_2 concentrations (62 μg SO_2 m^{-3}) and reductions at higher concentrations (157 μg SO_2 m^{-3}, McLeod *et al.*, 1988), but their data cannot be interpreted as a result of a clear interactions between SO_2 effects and a rust infection. The development of the rust infection was strongly reduced in the fumigated plots (McLeod, 1988). A similar interaction between SO_2 exposure and a fungal pathogen *B. fabae* was observed in this study. In a study with soybeans exposed to elevated SO_2 concentrations during 18 days in the pod-filling period (4.2 hours per day), strong reductions in seed yield have been observed (up to 48%) with SO_2 concentrations ranging from 243 to 972 μg SO_2 m^{-3}, mostly without visible injury (Sprügel *et al.*, 1980). Growth and yield reductions of dicot crops have been reported by several workers (reviewed by Roberts, 1984; McLaughlin & Taylor, 1985), but comparison of our data with those from other studies is hindered by the lack of other studies in which crops have been exposed to SO_2 in the open air.

In field studies as presented here, the influence of other air pollutants may play a role. Several authors have observed an O_3/SO_2 interaction (review by Kohut, 1985). Additive, less than additive and more than additive effects have been observed. Kohut (1985) concluded that most of the more than additive effects for SO_2 and O_3 have been observed in laboratory studies where single or short term exposures to high concentrations were applied. He also concluded that dose response research conducted in the field generally indicated that the pollutants do not interact and that their effects are additive.

An interesting observation in this study was the occurrence of a strong infection of the control plot in 1986 (and slight infections in the controls of two other experiments) with the fungal pathogen *B. fabae*. Similar findings were published by Heggestad *et al.* (1986), McLeod *et al.* (1988) and McLeod (1988). Heggestad *et al.* (1986) observed leaf spot disease (*Septoria sp.*) especially in open-top chambers where tomatoes were not exposed to elevated SO_2 concentrations. McLeod (1988) observed a strong interaction between the level of fungal disease infection in winter cereals exposed to SO_2 in the field and exposure to SO_2, resulting in a depressed disease development in the exposed plants. McLeod (1988) concluded that the magnitude of the SO_2-pathogen interactions at ambient concentrations and under field conditions suggests that these have considerable importance

for disease control in agriculture.

The published effects of SO_2 on growth of different crops vary so much that it is difficult to derive dose-response relationships which can be used for prediction of air pollutant effects, especially since the response of crops also varies with species, varieties, microclimate in the exposure system, etc. (Mclaughlin & Taylor, 1985; Roberts, 1984). A more complete description of plant response to SO_2 can only be achieved by defining the qualitative and quantitative basis for the final response of crops to air pollutants.

Chapter 8

Effects of long-term open-air fumigation with SO_2 on a field crop of broad bean.

II. Effects on growth components, leaf area development and elemental composition

Abstract Faba bean crops (*Vicia faba* L.) were exposed to elevated SO_2 concentrations in three different years in an open-air field exposure system for the controlled release of air pollutants. The treated crops were exposed to an average SO_2 concentration of 165 μg SO_2 m^{-3} in 1985, 62 μg SO_2 m^{-3} in 1986 and 74 μg SO_2 m^{-3} in 1988. The ambient SO_2 concentration was about 10 μg SO_2 m^{-3}. Plant height, number of internodes and number of pods were not affected by SO_2. The specific leaf area was reduced in the SO_2 exposed plants at the end of the growing season. Leaf area development was strongly affected during the pod-filling period in 1985 and 1988 as a result of leaf injury and defoliation in the fumigated plots. In 1986 a similar trend in leaf area reduction was observed in the early reproductive phase. N and Mg content of the different organs was unaffected by SO_2. The S content was strongly elevated in the leaves and pods of the fumigated plants. The Ca content of the leaves was reduced by SO_2. Chlorophyll content of different leaf numbers was unaffected by SO_2.

8.1 Introduction

The influence of atmospheric pollutants, especially SO_2, on plant growth has been the subject of numerous studies in the past decades. Most studies have shown that plant growth is depressed as a result of long term exposures to concentrations as low as 40 μg SO_2 m^{-3} (Bell *et al.*, 1979). However, other workers have reported increases in the growth of plants exposed to low levels of SO_2, even when the sulphur nutrition of the plants is adequate (McLeod *et al.*, 1988). One of the main causes of reported differences in responses of plants to air pollutants is the influence of the particular exposure system on the microclimate, since the effect of air pollutants on plants is largely affected by

environmental factors such as windspeed, humidity and radiation (Black, 1982). In most experiments in which the effects of air pollution on plant growth are studied, some form of enclosure is used in controlling the pollutant, ranging from indoor growth cabinets to open-top chambers in the field. The extrapolation of the effects observed in these studies to the field situation may lead to over- or underestimation of the effects (Olszyk *et al.*, 1986).

In Chapter 7 the effects of SO_2 on the growth and seed yield of broad bean exposed to long-term elevated SO_2 concentrations were reported. In three years, the crops were exposed to mean concentrations of 165 μg SO_2 m^{-3} (in 1985), 62 μg SO_2 m^{-3} (in 1986) and 74 μg SO_2 m^{-3} (in 1988). In all years no effects on plant growth were found during the vegetative phase. A final reduction in total dry matter of 17% was observed in 1985 and 9% in 1988. In 1986, a severe infection of the control crop with the fungal pathogen *Botrytis fabae* occurred in the pod filling period. Slight *B. fabae* infections were also observed in the control plots in 1985 and 1988. The objective of this part of the study was to determine the effects of SO_2 on dry matter growth of the different plant organs, morphological parameters and mineral content of plant organs.

8.2 Materials and methods

Broad bean crops (*Vicia faba* L., cv. Minica) were exposed to elevated SO_2 concentrations in three contrasting growing seasons in 1985, 1986 and 1988, using the open-air exposure system developed by Mooi & van der Zalm (1986). Experimental details were given in Chapter 7. In the open-air exposure system, plots of 8m x 8m were exposed to mean concentrations of 165 μg SO_2 m^{-3} in 1985, 62 μg SO_2 m^{-3} in 1986 and 74 μg SO_2 m^{-3} in 1988. The control plot was located at 250 m distance from the system, far enough away to be exposed to SO_2 at background concentrations of SO_2 only which were 16 μg SO_2 m^{-3} in 1985, 7 μg SO_2 m^{-3} in 1986 and 9 μg SO_2 m^{-3} in 1988.

The bean plants were grown at a density of 20 plants per m^2 in plastic containers (55x22x25 cm) filled with a commercial potting mixture (Trio 17) in order to avoid confounding effects of differences in soil conditions between the plots. Water was supplied by a drip-irrigation system. In 1985 the plants emerged on 16 April, in 1986 on 5 May and in 1988 on 15 May. Plant growth was determined up to 29 July in 1985, 21 July in 1986 and 25 August in 1988 by frequent harvesting. The plots were divided into 4 blocks each containing one subplot for each harvest (6 in 1985 and 5 in 1986). In 1988, five plants were harvested from one container of the three blocks. Between the last two harvests in 1986, plants in the control plots became infected with the fungal pathogen *B. fabae*.

After collection of the plants in the field, they were divided into leaves, stems, and pods. Leaf area was measured separately for each plant. As leaf necrosis and yellowing were unevenly distributed over the leaf (mostly starting at the margins), the total area of the green, yellow and necrotic tissue was measured by passing the leaves through a Delta-

T Devices moving belt planimeter. In 1988, the fraction of yellow and necrotic tissue was estimated. The dry weight of the organs was determined after a drying period of 24 hours at (105 °C). Plant height, the number of internodes (which is a measure of phenological development) and the number of pods were determined on all harvested plants. In 1986, the number of leaves was also counted. In 1986, leaf area and leaf dry weight were determined for 4 leaf numbers separately (numbers 5, 10, 15 and 20 counted from the bottom of the plants). From leaf dry weight and leaf area the specific leaf area was calculated (SLA in cm^2 leaf g^{-1} leaf), which is a measure of leaf thickness. In 1986 only plants which were not infected with *B. fabae* were sampled from the control plot at final harvest to permit the analysis of the effects of SO_2 on elemental composition.

Subsamples of the harvested plants were analysed for N, S, Ca and Mg content in the Chemical Laboratory of the Research Institute for Plant Protection. The unwashed leaves were dried and ground in a laboratory mill to pass a 2 mm sieve. Both S and N were analysed with an Carbo Erba Elemental Analyser. Ca was analysed spectrophotometrically with a Technicon Autoanalyser GT II. The chlorophyll a and b content of leaf samples was determined according to Bruinsma (1963).

8.3 Results

The response of the dry weight of plant organs during the growing season to SO_2 exposure is given in Table 8.1. Total dry weight of the plants was significantly reduced in 1985 in the vegetative phase of plant growth and at final harvest when a total dry weight reduction of 17% was observed. In 1986 and 1988, slight reductions in total dry weight were not significant. Although the dry weight of all organs was reduced in the exposed plots at final harvest in 1985, significant reductions were observed only in leaf dry weight (28%), due to defoliation, and in pod dry weight (23%). Pod dry weight was slightly higher in the exposed plants in 1986. In 1986, only a small, but significant, reduction in stem dry weight was observed. In 1988, leaf dry weight was significantly reduced by 47%. Dry matter content of the exposed plants was not influenced by SO_2 (data not shown).

In all years the time course of leaf area development in both the control and the fumigated crop showed the same pattern (Fig. 8.1): a slight depression of the leaf area index (LAI) at the beginning of the growing season and a strong reduction of the LAI at the end of the growing period. This was due to severe leaf injury and defoliation of the oldest leaves. In 1986, only the onset of defoliation in the fumigated plots can be detected from Fig. 8.1B. However, in the field we observed severe leaf injury in the second part of the pod filling period (after harvest 4) in the fumigated plot.

The responses of plant height, number of internodes, number of pods, number of leaves and the SLA to SO_2 fumigation are given in Table 8.2. The effect of SO_2 exposure on plant height was small. The number of internodes was not influenced in 1985 and 1988

Table 8.1 The response of leaf, stem, pod and total dry weight of broad bean (*Vicia faba*) exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), in 1986 (62 $\mu g\ m^{-3}$), and in 1988 (74 $\mu g\ m^{-3}$, in relation to plant age expressed in days after emergence.

		dry weight (kg ha^{-1})							
Plant age		leaf		stem		pod		total	
	days	control	fumigated	control	fumigated	control	fumigated	control	fumigated
1985	24	96	84***	32	26***	-	-	128	110***
	38	494	404***	315	241***	-	-	809	645***
	45	935	814***	960	722***	-	-	1895	1536***
	65	1946	1906	3488	3150*	92	52***	5526	5108*
	85	2432	2296	5066	4952	1936	1392**	9434	8640
	105	2020	1456***	4626	4442	7888	6108***	14534	12006**
1986	21	144	168	59	64	-	-	203	232
	39	817	752	766	647	65	46	1648	1445
	53	1646	1401*	2657	2177**	335	404	4658	3982
	67	1799	1646	3399	2844***	3265	3334	8454	7824
1988	40	968	1065	1147	1119	-	-	2115	2184
	61	1759	1691	3618	3730	129	145	5506	5566
	81	1743	1589	4848	6055*	2897	1728*	9488	9372
	102	1255	662*	4699	4839	7707	6920	13661	12421

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as:
* P<0.05; ** P<0.01; *** P<0.001

Table 8.2 The response of plant height, number of internodes, number of leaf layers, number of pods and SLA of broad bean (*Vicia faba*) exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), in 1986 (62 $\mu g\ m^{-3}$), and in 1988 (74 $\mu g\ m^{-3}$), in relation to plant age expressed in days after emergence.

	Plant age	plant height (m)		number of internodes		number of leaves		number of pods		Specific Leaf Area ($cm^2\ g^{-1}$)	
	days	control	fumigated	control	fumigated	control	fumigated	control	fumigated	control	fumigated
1985	24	0.05	0.04	1	1	-	-	-	-	208	204
	38	0.16	0.11***	6	4	-	-	-	-	236	205
	45	0.38	0.32***	8	6	-	-	-	-	228	221
	65	0.91	0.82***	15	14	-	-	a	a	303	267**
	85	1.26	1.22	19	19	-	-	22	22	300	239***
	105	1.31	1.24***	21	21	-	-	17	15	314	194***
1986	21	-	-	-	-	-	-	-	-	310	256***
	39	-	-	8	7*	8	7	-	-	328	294*
	53	-	-	16	17***	10	13***	23	36***	311	342*
	67	-	-	22	21	17	15*	25	23	341	273***
	81	-	-	22	20***	15	13***	18	18	314	281*
1988	40	53	51	10	10	10	10	-	-	362	363
	61	119	121	17	18	15	14	-	-	377	388
	81	129	138	19	21	13	11*	-	-	310	278
	102	129	124	23	22	15	6*	-	-	189	134

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as:
* P<0.05; ** P<0.01; *** P<0.001
a. number of pods was not counted.

Table 8.3 The response of mineral content of leaves, stems and pods of broad bean (*Vicia faba*) exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), in relation to plant age expressed in days after emergence.

		mg g^{-1} dry weight							
	Plant age	N		S		Ca		Mg	
	days	control	fumigated	control	fumigated	control	fumigated	control	fumigated
Stems	24	61.8	62.0	4.6	5.0	3.0	2.9	2.2	2.2
	38	44.7	50.6***	3.3	3.5	a	a	a	a
	45	31.6	34.1	2.5	2.4	3.3	3.6	1.9	1.4
	65	17.0	17.8	1.2	1.5	3.3	3.3	1.4	1.4
	85	12.7	13.6	1.5	1.8	4.4	3.7	0.9	0.8
	105	7.7	9.6*	1.4	2.3**	4.2	5.1*	1.2	1.2
Leaves	24	71.8	70.9	4.9	6.8***	6.6	6.1*	3.1	3.0
	38	64.3	63.7	4.5	6.5***	a	a	a	a
	45	58.1	57.7	4.2	6.1**	9.9	9.6	2.1	2.1
	65	58.6	61.2	4.2	9.4***	11.7	9.9**	4.0	3.7
	85	52.0	50.3	3.7	9.6***	14.5	9.9*	4.4	3.3*
	105	44.6	45.7	3.3	12.7***	21.1	15.8***	5.3	4.2*
Pods (- seeds)	65	58.8	56.8	3.0	3.9	8.9	9.1	4.0	3.4
	85	44.9	45.5	2.0	2.9***	4.1	3.8	1.5	2.4
	105	26.0	26.9	1.4	2.8***	2.7	3.0	2.2	2.3
Seeds	85	63.0	64.0	3.2	3.8***	2.2	1.6	2.0	a
	105	49.0	48.0	2.2	3.2***	1.3	1.3	1.1	1.2

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as:
* $P<0.05$; ** $P<0.01$; *** $P<0.001$
a. no data available

Table 8.4 The response of mineral content of leaves of broad beans (*Vicia faba*) exposed to ambient or elevated SO_2 concentrations in 1986 (62 $\mu g\ m^{-3}$), in relation to leaf position (leaf number counted from the bottom upwards) and plant age expressed in days after emergence.

		mg g^{-1} dry weight					
	Plant age	N		S		Mg	
	days	control	fumigated	control	fumigated	control	fumigated
leaf number 5	39	51.3	51.2	2.9	5.3***	6.0	4.9
	53	32.8	27.0	2.1	4.7***	6.7	7.9
	67	21.9	24.3	1.7	5.7***	9.8	8.5
leaf number 10	39	59.7	59.8	4.0	4.8**	2.5	2.2
	53	49.0	54.0	3.8	6.7***	3.9	3.7
	67	40.6	40.2	3.7	7.6***	5.6	5.5
	81	36.8	33.6	2.4	7.5***	5.7	5.9
leaf number 15	53	51.5	59.2*	3.5	6.0**	2.7	3.2
	67	52.1	54.0	5.2	8.7***	4.7	4.8
	81	51.1	47.5	3.6	8.5***	5.9	5.8
leaf number 20	67	53.6	61.9	3.7	5.9***	4.8	4.7
	81	53.5	54.1	3.7	8.4***	6.3	6.3

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as:
* $P<0.05$; ** $P<0.01$; *** $P<0.001$

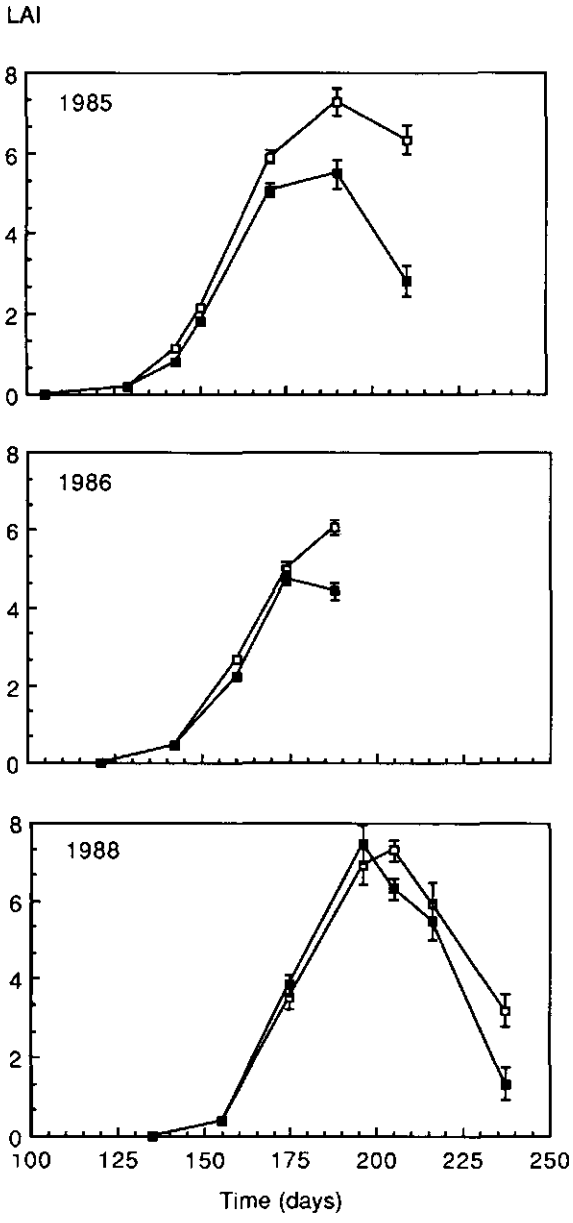


Fig. 8.1 Time course of the Leaf Area Index (m^2 leaf m^{-2} ground) in control (□) and fumigated (■) plots in 1985, 1986 and 1988. Standard errors are given as bars. Time is expressed as the day of the year.

and slightly reduced in 1986. The number of leaves was reduced from about 60 days after emergence onwards, when the leaves in the exposed plots showed the first symptoms of defoliation. Pod numbers were hardly influenced by SO_2 in both years except for an enhanced number of small pods formed in the beginning of the pod filling period in 1986. The specific leaf area was reduced in the reproductive growth phase of the exposed plants in all three years.

The effects of open-air exposure on elemental content of the plant organs in 1985 and of different leaf numbers in 1986 are presented in Table 8.3 and Table 8.4, respectively. These results show no effects of SO_2 on N and Mg content of the different organs during the whole growing season. Differences in Ca content were observed only in the leaves at the end of the growing period in 1985. Ca content of leaves from fumigated plants increased less than Ca content of leaves from control plants. The S content was strongly affected in the leaves, pods and seeds of the exposed plants. The S content of the stem was less influenced. Data in Tables 8.3 and 8.4 indicate a smaller accumulation of S in the leaves of the exposed plants in 1986 when the SO_2 concentration was much lower. In 1986, chlorophyll content was determined throughout the growing season for 4 leaf numbers. No effect of SO_2 on chlorophyll content of the leaves was detected (Table 8.5).

Table 8.5 The response of chlorophyll content of leaves of broad beans (*Vicia faba*) exposed to ambient or elevated SO_2 concentrations in 1986 (62 $\mu g\ m^{-3}$), in relation to leaf position and plant age expressed in days after emergence.

	Plant age	Chlorophyll content (mg g^{-1})	
	days	control	fumigated
leaf number 5	39	13.7	12.2
leaf number 10	39	13.1	12.4
	53	17.8	13.9
	67	11.2	12.6
	81	8.4	6.1
leaf number 15	53	14.6	11.0*
	67	12.1	15.9*
	81	12.7	10.3
leaf number 20	67	15.5	18.7
	81	13.3	11.7

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: * $P<0.05$, ** $P<0.01$; *** $P<0.001$

8.4 Discussion

Total dry weight of the exposed plants was reduced by exposure to SO_2 during the growing period. However, effects were only significant at final harvest and early in the growing season of 1985 (Table 8.1). Reduction of total dry matter early in the season of 1985, may be explained by the leaf epidermal damage observed in 1985, 29 days after emergence of the crop. The contact between the abaxial epidermis of the leaves and the mesophyll cells was broken. This effect was observed both in the control and in the fumigated plot, but the effect was more marked in plants exposed to SO_2. Although the dry weight of almost all organs was reduced in the exposed plots throughout the growing season, significant reductions were observed only in leaf and pod dry weight in 1985, in stem dry weight in 1986 and in leaf dry weight in 1988. The reduction in total dry weight at final harvest in 1985 was mainly due to the depression of pod dry weight.

Reduction of plant growth was accompanied by a large reduction in leaf area at the end of the growing season. This was mainly due to effects on the older leaves, which first showed red brown colored necrotic spots, starting at the margins of the leaf. The symptoms clearly differed from the symptoms which may be observed as a result of known disease infections (Gerlach, personal comm.). In many other studies, reductions in crop growth during fumigation were accompanied by leaf injury (Bell & Clough, 1973; Brisley, Davis & Booth, 1959; Davis, 1972), but growth reductions without visible injury have also been reported (Sprügel *et al.*, 1980; Bleasdale, 1972; Lockyer, Cowling & Jones, 1976; Tingey & Reinert, 1971). Sometimes an enhanced leaf area has been observed during exposure to SO_2 in controlled environments (Whitmore & Mansfield, 1983; Pande & Mansfield, 1985). The timing of reductions of growth and leaf area in this study suggests a causal relationship between the two (Table 8.1, Fig. 7.4 and Fig. 8.1). The observed reduction in leaf area at the end of the experiment in the fumigated plots caused reductions in the amount of absorbed radiation causing reduced rates of canopy photosynthesis. This may partly explain the depression in dry matter growth and seed yield. However, direct effects on photosynthesis and/or translocation may also have contributed to the yield losses observed in this study. Quantitative assessment of the impact of the reduced leaf area on crop growth and production can be determined with the help of simulation models, which calculate potential rates of crop growth at a given leaf area index on the basis of the amount of radiation absorbed by the leaves. Such an analysis of the data presented here will be given in Chapter 9.

Plant height was slightly reduced in the fumigated plots in 1985 and the number of internodes was only reduced slightly in 1986. Kress, Miller & Smith (1986) reported small reductions in the height of soybean in fumigation plots with SO_2 concentrations up to 1500 μg SO_2 m^{-3} for 4 hours a day. Heggestad *et al.* (1986) reported no effects on plant height of tomatoes exposed to 300 μg SO_2 m^{-3} for 5 hours a day for 5 days a week. The final number of pods was not influenced by SO_2 fumigation (Table 8.2). A much larger number of pods per plant was observed in 1986 in the beginning of the pod-filling

period in the fumigated plots. As a result of abortion of young pods, the final number was not influenced by SO_2. Kress *et al.* (1986) report small effects of SO_2 on seed numbers of soybeans produced, but Heggestad *et al.* (1986) reported reduced fruit numbers in exposed tomatoes at the high concentrations they applied. The number of leaves was significantly reduced as a result of an enhanced leaf loss in the fumigated plots.

The SLA (Specific Leaf Area in m^2 leaf m^{-2} ground) is determined by environmental factors and changes during plant development. The SLA in both years was measured from pooled leaf material, and thus encompasses the effects of plant development, senescence or SO_2 on leaf size and dry weight. In 1985, the SLA of the leaves in the control plot increased with time, which indicates the formation of thinner leaves at the end of the growing season. The SLA was nearly constant in the control plots in 1986. Its value was comparable with the SLA of the 1985 crop at the end of the growing season. The SLA was reduced at the end of the growing season in the fumigated plots. This effect was more marked in 1985. The reduction of the SLA in the fumigated plots at the end of the growing season may have been caused by the necrosis which reduced the leaf size, in the lower leaf numbers in the fumigated plants, or it may reflect a reduced translocation of assimilates from the leaves to other organs.

The concentration of N and Mg in the tissue of the different organs was not influenced by fumigation of the plants. Hardly any differences between the fumigated plants and the control plants were observed for any organs and at all harvest dates in 1985 (Table 8.3.). The sulphur content was strongly enhanced in the SO_2-treated plots, especially in the leaves and pods. Uptake of SO_2 by leaves and sulphur accumulation in leaves are well known phenomena (Guderian, 1977). The sulphur level in the plants, however, may be affected by many factors such as translocation, dilution by new growth, losses through leaching, gaseous emission and exudation by roots (Garsed, 1984). Heggestad *et al.* (1986) observed no enhanced S levels in tomato fruits, but Sprügel *et al.* (1980) found higher S concentrations in the seeds. In the present work, the calcium content of leaves at the end of the growing season was significantly lower in the fumigated plots. This may be a result of the increased rate of defoliation instead of gradual senescence. For the different leaf numbers the same conclusions can be drawn with respect to the effect of SO_2 exposure on N, Mg and S content. The highest sulphur levels were found in relatively young leaves (number 15) in 1986, obviously as a result of the delay of 32 days after emergence in the start of fumigation. The chlorophyll content was unaffected in the treated plants in 1986, which suggests that foliar injury was not preceded by a slow process of chlorophyll breakdown.

Chapter 9

Effects of long-term open-air fumigation with SO_2 on a field crop of broad bean.

III. Quantitative analysis of damage components

Abstract Effects of SO_2 on growth and production of broad bean observed in three open-air fumigation experiments, were interpreted in terms of damage components with a mechanistic simulation model. The model consisted of an elementary model for crop growth, extended with submodels for the microclimate in the crop and with a submodel for uptake of SO_2 by leaves and for effects on leaf photosynthesis.

The major part of the observed reduction in total dry matter production could be largely explained by leaf injury observed in the fumigated plants. The effect consisted of dry matter loss through leaf abscission and a reduced growth rate at the end of the growth period due to reduction of the amount of absorbed radiation by the canopy. Direct effects of SO_2 on leaf photosynthesis explained an extra 10 % of the observed yield loss (which ranged from 7 to 17% of control yield). This small effect was confirmed by field measurements which showed no detectable effects of SO_2 on leaf and canopy photosynthesis. Increased leaf respiration, which was observed in the 1988 experiment, explained another 10% of the observed yield reduction.

Total SO_2-sulphur uptake by the fumigated crop, which is an important component of dry deposition of SO_2, was accurately simulated by the model.

9.1 Introduction

Quantitative assessment of the effects of air pollutants, especially SO_2, on crop yield has been a major issue of research on air pollutant effects on ecosystems. The extensive published data on crop loss in fumigation experiments have been compiled and summarized with simple descriptive dose-response models for effects of SO_2 on grasses (Roberts, 1984) and dicotyledonous crops (McLaughlin & Taylor, 1985). They noted a considerable variability in responses to SO_2 between different species, between years within the same species and between different exposure systems, ranging from laboratory growth chambers to open-air exposure systems. Results obtained by controlled laboratory

or greenhouse studies, representing a very broad range of species and exposure conditions, provided no indication of a consistent relationship between pollutant dose and plant response (McLaughlin & Taylor, 1985).

Accurate prediction of SO_2 effects on crop production in field situations with descriptive dose-response relationships fitted to experimental data is impossible because plant responses to SO_2 are strongly influenced by environmental factors (*e.g.* windspeed, radiation, temperature) and by the physiological status of the plant (Black, 1982).

In descriptive empirical models like dose response relationships, the mechanisms by which the crop responds to its environment are considered to be a black box. Since the variation in plant responses to SO_2 is likely to be related to metabolic mechanisms, more accurate predictions of the impact of air pollutants on crops should be based upon understanding of the effects of air pollutants on physiological processes which determine crop growth and production. Such knowledge can be incorporated into existing deterministic models for crop growth and production which have been developed in the past decades (de Wit *et al.*, 1978; Penning de Vries & van Laar, 1982). These models have been successfully applied to growth and production of forest stands (Mohren, 1987). Models for the effects of SO_2 on crop growth and production should simulate the uptake of SO_2 by the canopy, the metabolism of SO_2 and the effect of these metabolites on physiological processes. Such deterministic models may help to identify the complex relations between plant growth and effects of air pollutants at the metabolic level.

Much experimental work has been conducted on the analysis of air pollutant effects, and in particular SO_2, on crops and vegetation at various levels of resolution ranging from the biochemical to the ecosystem level (*cf.* Ziegler, 1975; Hållgren, 1978; Unsworth & Ormrod, 1982). However, relatively few attempts have been made to integrate this extensive amount of information into mechanistic models, and to interpret the various observed phenomena (Kercher & King, 1985; Krupa & Kickert, 1987).

The aim of this study is to interpret SO_2 effects on crop yield with a mechanistic model and to quantify the contributions of different physiological effects to the loss in crop yield which have been observed in three field experiments with broad beans exposed to SO_2 in the field (Chapters 7 and 8). For this purpose we extended a crop growth model with submodels for the microclimate in the crop canopy and a submodel for the uptake of SO_2 by leaves and subsequent effects of SO_2 on leaf physiology (Chapters 3, 4, 5 and 6).

9.2 Materials and methods

Experimental design

In 1985, 1986 and 1988, the effect of an elevated SO_2 concentration on field crops of broad bean (*Vicia faba* L.) was studied by using a computer controlled open-air exposure system (Mooi & van der Zalm, 1986). The system consisted of a circular network of

stainless steel piping with a diameter of 30 m. SO_2 was released from the upwind section exposing broad bean to 165 μg SO_2 m^{-3} in 1985, 62 μg SO_2 m^{-3} in 1986 and 74 μg SO_2 m^{-3} in 1988 (seasonal means). The control plot was located at 250 m from the system. The ambient background concentrations were 16 μg SO_2 m^{-3} in 1985, 7 μg SO_2 m^{-3} in 1986 and 9 μg SO_2 m^{-3} in 1988. Details on the spatial and temporal distributions of the SO_2 gas in the system, weather conditions and O_3 concentrations are given in Chapter 7. The plants were grown in plastic containers (55x22 cm, with a height of 25 cm) filled with a commercial potting mixture. Water was supplied with a drip irrigation system.

Plant growth was analysed by frequent harvesting. In 1986, the experiment was terminated following a severe *Botrytis* infection in the control plants. Total sulphur was analysed in the different organs with a Carbo Erba Elemental Analyser. Further experimental details are given in Chapter 7.

Measurement of photosynthesis and respiration

Long-term effects of SO_2 exposure on leaf photosynthesis were measured in 1985, 1986 and 1987 with portable equipment consisting of a leaf chamber, an air supply unit and a portable leaf chamber analyser (Analytical Development Co. Ltd., Herts, England). Photosynthesis of the youngest fully unfolded leaf was measured 79 days after crop emergence in 1985, while in 1986 photosynthesis in 2 leaf numbers (leaf numbers 10 and 15, from the bottom of the canopy) was measured 63 and 78 days after emergence. In 1987, plants with about 10 leaves were placed in the open-air fumigation experiment to measure the semi long-term effects of SO_2 on leaf photosynthesis. Photosynthesis of one leaf number was measured by repeated measurements in the field during 8 days at high radiation levels.

Dark respiration (5 different leaf numbers) was measured in 1988 around the onset of the pod filling period, when the first leaf injury symptoms had been observed. Dark respiration was measured according to de Visser, De Kock & Spitters (in prep), using Warburg equipment for the analysis of O_2 consumption of leaf discs (Umbreit, Burris & Stauffer, 1957). Dark respiration was measured 3 hours after incubation.

Canopy photosynthesis of control and fumigated plants was measured around the onset of pod filling in 1988 with a mobile system described by Louwerse & Eikhoudt (1975) and Louwerse (1980). Two containers, with seven plants were placed in each of two enclosure chambers (80x80x80 cm) consisting of transparent acrylic, sealed onto a metal frame. Photosynthesis was measured under natural radiation in four replicates. The temperature in the chambers was 20 °C during the day and 14 °C at night. Global radiation was measured with a Kipp solarimeter near the chambers. SO_2 was injected into the air inlet of the chambers with a mass flow controller, which was adjusted manually. Air from the chambers was sampled through teflon tubing, and SO_2 concentrations were analysed with a fluorescent SO_2 analyser, Monitor Labs (model 8850).

9.3 Model description

General structure of the model

The model simulates the effects of SO_2 on crop growth in potential production situations where crop growth is only determined by the incoming radiation, temperature and some physiological characteristics of the species. Water, nitrogen and other nutrients are assumed to be abundant and the crop is assumed to be free of pests, diseases and weeds. The model consisted of an elementary model for crop growth (Spitters, van Keulen & van Kraalingen, 1989; de Wit *et al.*, 1978; Penning de Vries & van Laar, 1982), a submodel

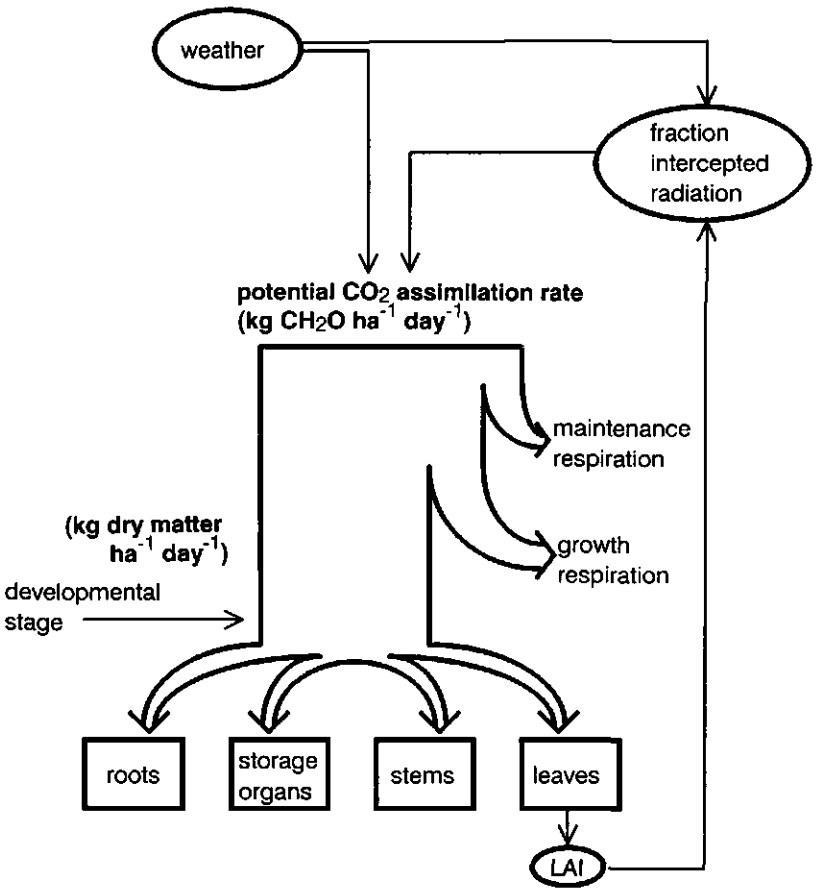


Fig. 9.1 A schematic representation of the simulation model for crop growth. Boxes represent amounts and arrows indicate flows of material. Lines indicate flows of information.

Table 9.1 Experiment-specific model inputs.

1. Daily maximum and minimum temperature	(°C)
2. Daily total global radiation	(MJ m^{-2} d^{-1})
3. Date of emergence and final harvest	(day of the year)
4. Hourly values of SO_2 concentration	(μg m^{-3})
5. Measured Leaf Area Index as a function of time	(m^2 m^{-2})

for the microclimate inside the canopy and a leaf submodel for the uptake of SO_2 by the leaf, the metabolism of S(IV) in the leaf and effects on stomatal conductance and photosynthesis (Chapters 2, 3 and 6). The model operates with time steps of one day, but allows for daily variation in radiation. A schematic representation of the elementary model for crop growth is given in Fig. 9.1. The core of this model is formed by the calculation of canopy photosynthesis and respiration on the basis of processes at the organ level. The daily dry matter production is distributed over the various plant organs dependent of the developmental stage. Numerical integration in time gives the time course of dry matter. The inputs required by the model are listed in Table 9.1.

Simulation of canopy photosynthesis

Daily gross canopy photosynthesis was calculated from daily total global radiation, daily maximum and minimum temperature and the Leaf Area Index (LAI) of the crop, following the procedure given by Goudriaan (1982, 1986), Spitters (1986) and Spitters *et al.* (1986).

Daily photosynthesis was calculated from an assumed diurnal weather pattern based on daily totals of radiation or means of temperature and windspeed (Spitters *et al.*, 1986). The rate of photosynthesis was calculated each hour from the absorbed photosynthetically active radiation and the photosynthesis light response of individual leaves. The hourly values of canopy photosynthesis were obtained by using the Gaussian integration technique for integration of photosynthesis over the LAI of the canopy (Goudriaan, 1986).

From the windspeed above the canopy, the turbulent resistance of the canopy and the boundary layer resistance of the leaves in the canopy were calculated as a function of height according to Goudriaan (1977). Stomatal conductance of leaves at a certain height in the canopy was calculated from the rate of leaf photosynthesis (determined by the amount of absorbed radiation) and the boundary layer resistance at that height in the canopy. Uptake of SO_2 and effects on leaf photosynthesis was calculated according to the stationary state version of the leaf submodel which is described in Chapter 6.

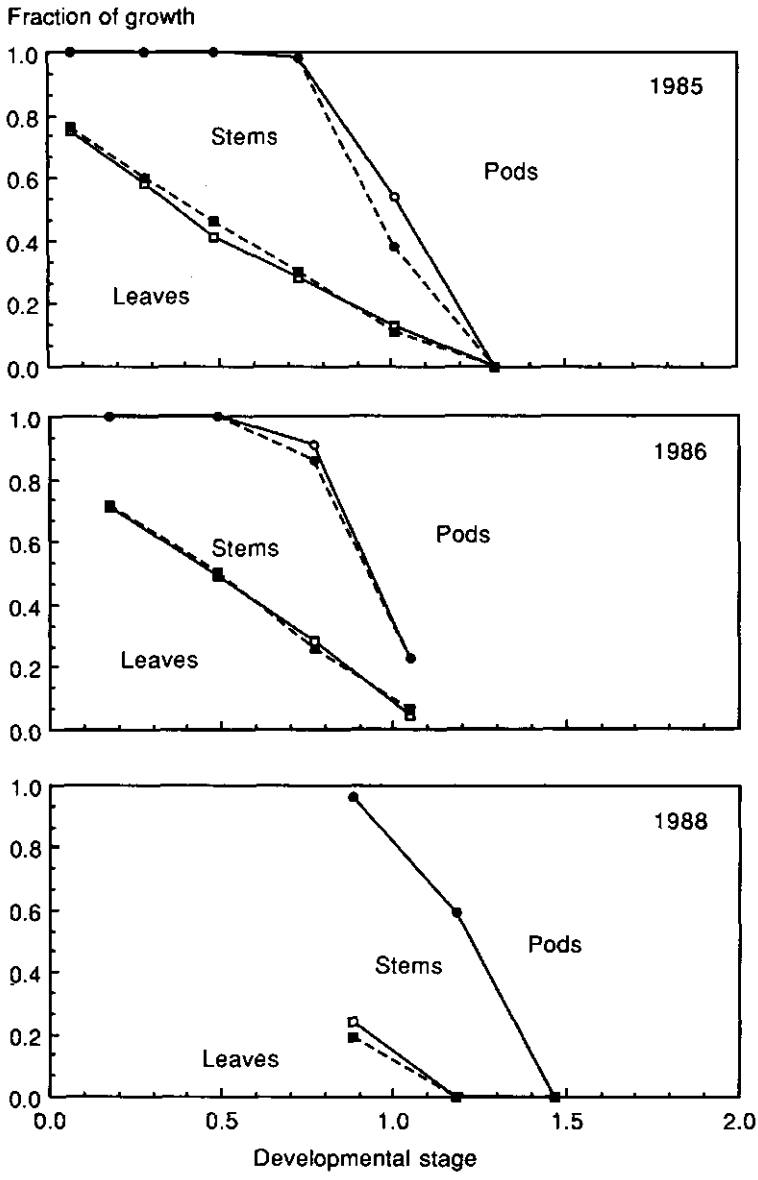


Fig. 9.2 Dry matter distribution over the plant organs of *Vicia faba* as a function of developmental stage (0=emergence, 1=onset of pod filling and 2=maturity), for control (open symbols and solid lines) and fumigated plants (closed symbols and dashed lines) in 1985, 1986 and 1988.

Simulation of dry matter growth

The growth rate of the crop canopy (dY/dt, dry matter in kg ha^{-1} d^{-1}) was then calculated according to:

$$\frac{dY}{dt} = (A - R_m) / G$$

where A denotes the gross rate of canopy photosynthesis (kg CH_2O ha^{-1} d^{-1}), R_m the maintenance respiration (kg CH_2O ha^{-1} d^{-1}), and G the glucose requirement for the formation of structural dry matter from sugars (kg CH_2O kg^{-1} dry matter). The dry matter produced was distributed over the organs depending on the developmental stage, according to empirically determined distribution functions. Phenological development was linearly scaled from emergence to the onset of pod filling, and from the onset of pod filling to maturity. At stage 0 the crop emerged, at stage 1 pod filling started and maturity was reached at stage 2. The developmental rate was calculated as a function of daily mean temperature.

Parameterization

The parameters for the photosynthesis light response curve and their dependence on temperature and development stage were derived from Grashoff, Klein Hulze & Smid (1987), and from own data (Kropff, unpublished). Commonly used values for maintenance respiration coefficients were used (Spitters *et al.*, 1989). Allowance was made for the decrease of these coefficients with plant development by relating them to the nitrogen content of the stems and leaves. The relation between developmental rate and temperature was derived from Grashoff *et al.* (1987). The dry matter distribution functions for above-ground organs in the model were based on the first two field experiments (Chapters 7 and 8). The dry matter distribution pattern in the 1988 experiment clearly differed from the other two experiments for both the control and the fumigated crop. In 1988, the pod filling period started at a later calculated developmental stage than in 1985 and 1986. A slight water stress at the onset of pod filling may have favorable effects on pod production, and especially on early pod growth (Grashoff *et al.*, 1987). It is likely that such a slight water stress occurred around the beginning of the pod filling period in 1985 and 1986, because the capacity of the drip irrigation system was lower in 1985 and 1986 than in 1988 because less plants were grown in the 1988 experiment.

The fractions of dry matter allocated to the different above-ground plant organs were not affected by SO_2 in any of the experiments (Fig. 9.2). Therefore, SO_2 effects on the distribution pattern of dry matter were not included in the model. Since root growth was not determined in the experiments, effects on dry matter distribution between shoot and root were not accounted for. The relative death rate of the leaves (d^{-1}) in dependence of

developmental stage and exposure regime was estimated for the control and the fumigated crops in all experiments. Experiment specific relative death rates were input in the model, because SO_2 strongly influenced the relative death rate of the leaves.

Procedure of quantitative interpretation of SO_2 effects observed in the field experiments

Model performance was first evaluated by comparing its results with the three experimental data sets on broad bean growth in the control plots, with one set of parameters and functions describing species characteristics. Since the model was used for a quantitative explanation of the backgrounds of SO_2 effects on crop growth rates, the LAI was not simulated in the model, but the measured LAI was input in the model. This enables the separation of SO_2 effects on crop growth as a result of a reduced light interception caused by leaf necrosis from direct effects of SO_2 metabolites on physiological processes.

The observed effect on green leaf area was first introduced in the model to analyse the contribution of only the reduction of LAI to the observed yield loss. The leaf submodel for SO_2 effects on photosynthesis was then introduced followed by the analysis of the effect of increased leaf maintenance respiration with the model.

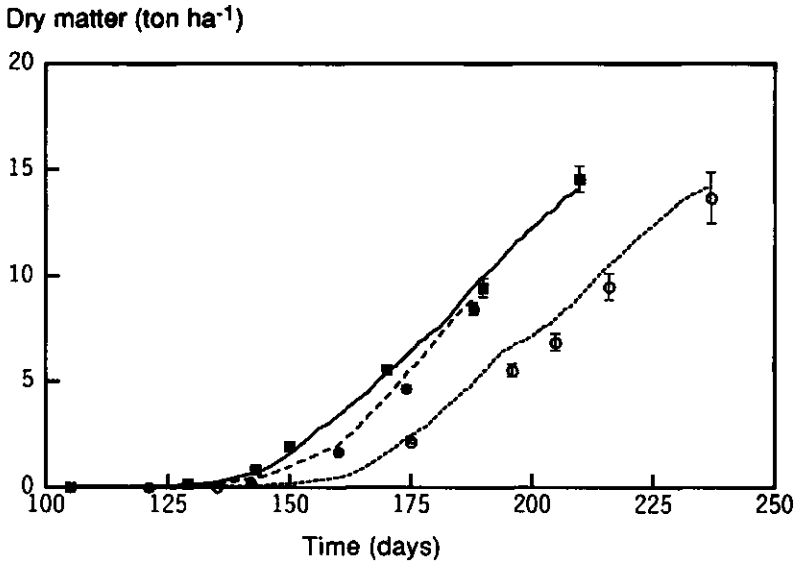


Fig. 9.3 Observed and simulated total dry matter production of the control crops in 1985 (■, observed; ——, simulated), 1986 (●, observed; – – –, simulated) and 1988 (○, observed; - - - - simulated).

9.4 Results and discussion

Simulation of control crops

Observed and simulated production of total above ground dry matter of control crops are presented in Fig. 9.3. With one set of parameters for species characteristics, growth and production of the broad bean crops was accurately simulated for all experiments.

Yield reduction due to leaf injury in fumigated crops

Leaf injury was observed in the fumigated plots in each experiment, resulting in strong reductions of LAI at the end of the growing period (Chapter 8). As leaf injury proceeded from older to younger leaves, it was unnecessary to account for shading of green leaves by yellow or dead leaves in the model. Introduction of the measured (green) leaf area index of fumigated crops explained much of the reduction in total dry matter in all three experiments: 69% in 1985, 69% in 1986 and 86% in 1988 (Table 9.2, simulation experi-

Table 9.2 Damage components of reduction in total dry matter kg ha^{-1}, at final harvest in three field experiments with *Vicia faba* exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), 1986 (62 $\mu g\ m^{-3}$) and 1988 (74 $\mu g\ m^{-3}$).

	kg dry matter ha^{-1}		
Experiment	1985	1986	1988
Observed control yield	14534	8454	13661
Observed reduction	2528	630	1240
Simulated reduction caused by individual components:			
1. Effects on leaf area development			
- leaf abscission	479	229	426
- reduced light interception	1256	210	641
2. Direct effects on photosynthesis	212	70	92
3. Effects on leaf respiration	202	145	201
1+2+3.	2149	654	1360

Table 9.3 Damage components of yield reduction in kg dry matter (pods) ha^{-1}, at final harvest in three field experiments with *Vicia faba* exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), 1986 (62 $\mu g\ m^{-3}$) and 1988 (74 $\mu g\ m^{-3}$).

	kg dry matter ha^{-1}	
Experiment	1985	1988
Observed control yield	7888	7707
Observed yield reduction	1780	787
Simulated yield reduction caused by individual components:		
1. Effects on leaf area development	841	719
2. Direct effects on photosynthesis	116	69
3. Effects on leaf respiration	126	140
1+2+3.	1083	928

ment 1). The effect of reduced leaf area in fumigated crops, resulting in reduced absorbed radiation, was largely responsible for this effect in 1985 and 1988 (Table 9.2). The reduction of the seasonal amount of absorbed radiation was 7.2% in 1985, 3.8% in 1986 and 4% in 1988. The simulated effect of the damage components on pod production is given in Table 9.3 for the experiments in 1985 and 1988. These results yield the same picture as the results from the analysis of reductions in total dry weight. The 1986 experiment was omitted, since the crops were not harvested because of a severe *Botrytis* infection in the control plots (Chapter 7).

The time course of differences in total dry matter between control and exposed plants was simulated accurately until the final harvest, by only taking into account the differences in leaf area development between the treatments (Fig. 9.4). This illustrates that leaf injury was largely responsible for the reductions in total above ground dry matter. Possible mechanistic explanations for leaf injury as a result of long-term fumigation are described in Chapter 10.

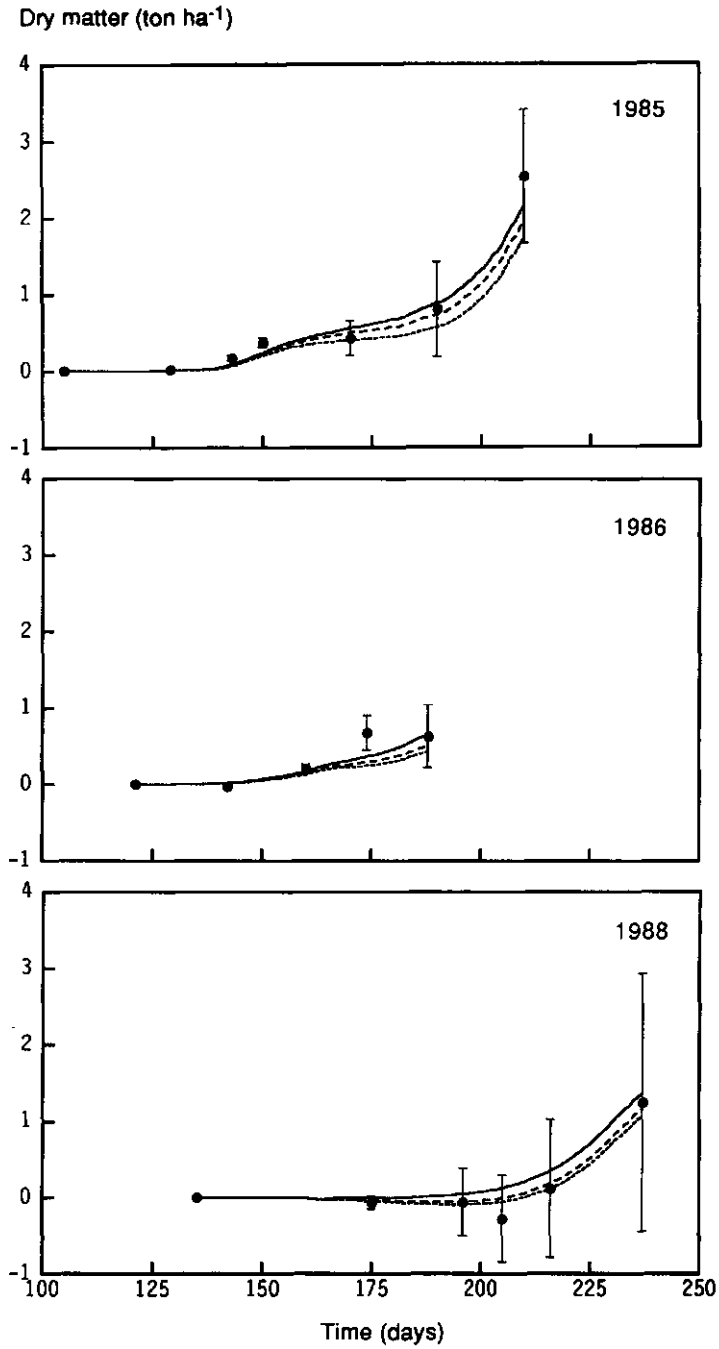


Fig. 9.4 Observed (●) and simulated difference in total dry matter production between the control and fumigated crop in 1985, 1986 and 1988. Three damage components were evaluated: (1) effect of differences in leaf area development and leaf fall (- - - -); (2) effect of (1) with an additional effect of SO_2 on photosynthesis (– – –), and (3) effect of (2) and an elevated maintenance respiration of the leaves (——).

The effect of reduced photosynthesis

Introduction of the submodel for uptake and effects of SO_2 on photosynthesis slightly improved the simulation results. An additional amount of the reduction (0.7 - 1.5%) in total dry matter production was explained from direct effects of SO_2 on photosynthesis (Table 9.2), 10% in 1985, 11% in 1986 and 6% in 1988. Comparable effects on pod production were simulated (Table 9.3). The effect of SO_2 on photosynthesis of leaf canopies at the concentrations applied here was small as was expected on basis of the findings in Chapter 6.

The submodel for SO_2 effects on leaf photosynthesis is based on observations from short-term fumigation experiments. In order to analyse the role of additional long-term effects on photosynthesis at concentrations field applied SO_2 concentrations (100/200 $\mu g\ m^{-3}$), field measurements on leaf and canopy photosynthesis were conducted in 1985, 1987 and 1988. In 1985, no effects on photosynthesis at light saturation were detected after a long exposure period (Table 9.4). Photosynthesis measurements of a single leaf number at high radiation levels during an 8 day fumigation period in 1987, showed no long-term effects on photosynthesis either (Table 9.5). Measurements on photosynthetic efficiency of whole canopies showed no difference between control and fumigated crops. Between 10 and 100 J $m^{-2}\ s^{-1}$ (PAR, Photosynthetically Active Radiation, absorbed by closed canopies with an LAI>6), the photosynthetic efficiency of the control crop was 12.3 g CO_2 MJ^{-1} (SE 0.7) and of the fumigated crop 12.0 g CO_2 MJ^{-1} (SE 0.5).

The effect of increased respiration

Dark respiration of leaves was measured in 1988 around the pod filling period. Although quantitative interpretation of dark respiration of leaf discs in terms of maintenance respiration is speculative, relative differences between treatments or varieties appear to be con-

Table 9.4 Net rate of CO_2 assimilation ($\mu g\ CO_2\ cm^{-2}\ h^{-1}$) of leaves of broad bean exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ SO_2\ m^{-3}$), of leaf numbers 10-14, 79 days after emergence. Radiation in J $m^{-2}\ s^{-1}$ (PAR) (n=14).

control		fumigated	
photosynthesis $\mu g\ CO_2\ cm^{-2}\ h^{-1}$	radiation J $m^{-2}\ s^{-1}$	photosynthesis $\mu g\ CO_2\ cm^{-2}\ h^{-1}$	radiation J $m^{-2}\ s^{-1}$
303	305	265	326

Where analysis of variance showed a significant difference between the treated plants and the control plants, the treated plant means are indicated as: * P<0.05, **P<0.01; *** P<0.001

sistent (de Visser *et al.*, in prep). A significant ($P<0.001$) increase of respiration (ca. 30%) in fumigated leaves was observed, which appeared to be independent of leaf age (characterized by leaf number) (Fig. 9.5). Similar results were obtained in 1985 when plants were transported from the field and photosynthesis of the youngest fully unfolded leaf was measured during two days. The above findings may be interpreted as an increased rate of leaf maintenance respiration, since the leaves were full grown. It seems reasonable to assume that the effect on respiration is independent of the length of the fumigation period, since all leaf layers showed the same pattern.

Introduction of an increased rate of maintenance respiration of the leaves throughout the growing season slightly improved the simulation of the depression of dry matter and pod production. The time course of observed and simulated yield loss (Fig. 9.4), however, indicates that an additional effect at the end of the growing season, rather than an increased maintenance respiration throughout the growing season, must have played a role. No consistent effects of SO_2 on respiration have been reported earlier. Both stimulating and inhibitory effects have been observed (*cf.* Black, 1984). More research on the interpretation of dark respiration in terms of maintenance respiration and effects of air pollutants is necessary.

Damage components

The analysis indicated that most of the observed reduction in total dry matter production can be explained by observed effects on leaf area development of the exposed plants, i.e. leaf fall and a reduced growth rate at the end of the growing season. Including direct ef-

Table 9.5 Net photosynthesis ($\mu g\ CO_2\ cm^{-2}\ h^{-1}$) of the fifth leaf number of *Vicia faba*, exposed to ambient or elevated SO_2 concentrations in 1987 (100 $\mu g\ SO_2\ m^{-3}$), in relation to the number of days after the start of fumigation. Plants had about 10 leaf layers.

		control		fumigated	
Days	*n*	photosynthesis $\mu g\ CO_2\ cm^{-2}\ h^{-1}$	radiation $J\ m^{-2}\ s^{-1}$	photosynthesis $\mu g\ CO_2\ cm^{-2}\ h^{-1}$	radiation $J\ m^{-2}\ s^{-1}$
0	20	255.6	232.6	259.5	239.7
2	6	276.6	202.0	284.4	208.1
7	16	237.6	246.1	231.8	247.1
8	4	269.2	267.3	254.7	223.2

Where analysis of variance showed a significant difference between the treated plants and the control plants, the treated plant means are indicated as: * $P<0.05$, ** $P<0.01$; *** $P<0.001$

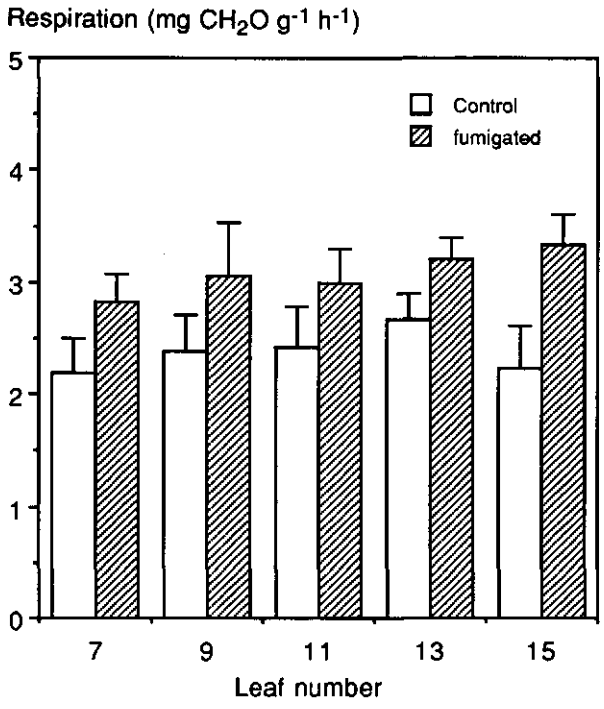


Fig. 9.5 Rates of dark respiration measured 3 hours after incubation for 5 leaf numbers of control (open columns) and fumigated (shaded columns) plants from the 1988 field experiment at the onset of the pod filling period.

fects of SO_2 on photosynthesis and leaf respiration improved the simulation results slightly. These small effects of SO_2 on photosynthesis and respiration agree with Baker *et al.* (1986, 1987) and McLeod *et al.* (1988). They observed no or small effects on total dry matter growth and leaf area in open-air fumigation experiments (50-300 μg SO_2 m^{-3}) with barley in spring and summer, indicating that there were no strong depressing effects on photosynthesis or stimulating effects on maintenance respiration. The leaf injury, observed in our experiments is a well known effect of long term exposures to low SO_2 concentrations (Guderian, 1977; Pierre & Queiroz, 1981,1982). Prediction of the effect of continuous exposure of crops and forests to low SO_2 concentrations requires quantitative insight in processes determining chronic injury.

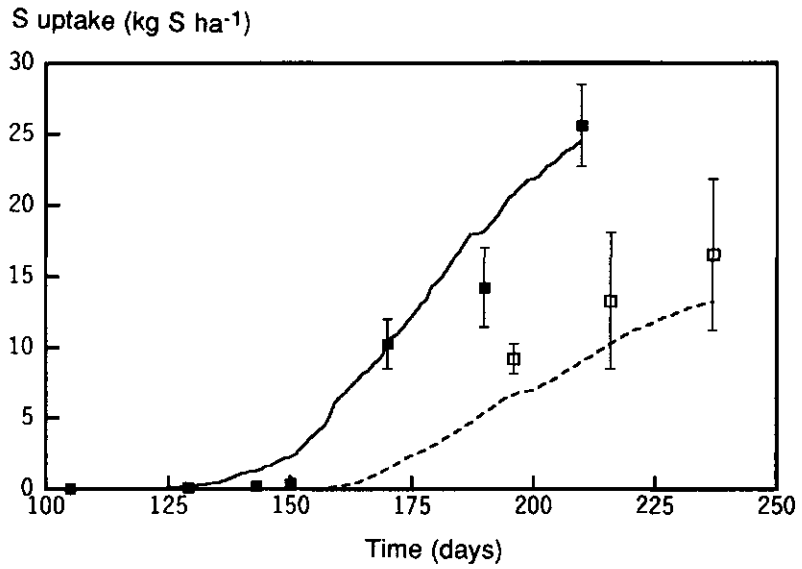


Fig. 9.6 Observed and simulated extra S uptake by the fumigated crop in 1985 (■, observed; ——, simulated) and 1986 (□, observed; – – –, simulated).

Simulation of SO_2 uptake by the canopy

The average deposition velocity (uptake by the crop) was about 5 mm s^{-1}, which is close to measured values of 8 mm s^{-1} in the daytime and 4 mm s^{-1} during the night for a wheat crop of which 3.5 mm s^{-1} was caused by deposition to leaf surfaces and soil (Unsworth *et al.*, 1985). The measured and simulated uptake of total sulphur by the canopy in 1985 and 1988 (total sulphur was not measured in 1986) are presented in Fig. 9.6. The observed sulphur uptake is defined here as the difference in sulphur content between the fumigated and control crop. The uptake of S by the 1985 crop was accurately simulated with the model, but the uptake of total S in 1988 was somewhat underestimated. The simulated underestimation of S uptake for the 1988 experiment may have been caused by the higher humidity in 1988, because a strong relationship exists between VPD (vapour pressure deficit) in the air and the ratio of the internal CO_2 concentration and the ambient CO_2 concentration (Morison, 1987). The simulation procedure for SO_2 uptake by leaf canopies can be improved by introduction of the VPD effect on stomatal behaviour.

Chapter 10

Long-term effects of SO_2 on plants, SO_2 metabolism and regulation of intracellular pH

Abstract The impact of SO_2 on the ionic balance of plants and its implications for intracellular pH regulation was studied in order to find explanations for long-term effects of SO_2. When sulphur taken up as SO_2 by the shoots of plants is not assimilated in organic compounds, but stored as sulphate, an equivalent amount of H^+ is produced. These H^+ ions are not buffered chemically, but are removed by metabolic processes.

On basis of existing knowledge on metabolic buffering mechanisms a conceptual model is proposed for the removal of shoot-generated H^+ by (*i*) OH^- ions, produced in the leaves when sulphate and nitrate are assimilated in organic compounds and/or by (*ii*) OH^- ions produced by decarboxylation of organic anions (a biochemical pH stat mechanism). These organic anions are produced by operation of the biochemical pH stat, to remove OH^- from cellular solutions. These OH^- ions appear in leaves or roots when sulphate and nitrate are reduced, and in the roots when differences in the uptake of inorganic cations and inorganic anions are counterbalanced by H^+ extrusion from the roots. The form in which nitrogen is supplied largely determines the potential of the plant to neutralize H^+ in the leaves during SO_2 uptake by the proposed mechanisms.

In field experiments with N_2 fixing *Vicia faba* L. crops, the increase of sulphate in the shoots of SO_2 exposed plants was equivalent in charge to the decrease of organic anion content, measured as the difference between inorganic cation content (C) and inorganic anion content (A) indicating that H^+ ions produced in the leaves following SO_2 uptake were partly removed by OH^- from sulphate reduction and partly by decarboxylation of organic anions.

The appearance of chronic SO_2 injury (leaf damage) in the field experiment at the end of the growing period is discussed in relation to the impact of SO_2 on the processes involved in regulation of intracellular pH. It is proposed that the metabolic buffering capacity of leaf cells is related to the rates of sulphate and nitrate reduction and the import rate of organic anions, rather than to the organic anion content in the vacuoles of the leaf cells.

10.1 Introduction

In Chapters 7 and 8, data were presented from three open-air exposure experiments, in which the effects of SO_2 (62-165 $\mu g\ m^{-3}$) on growth and production of *Vicia faba* L. crops were analysed (Chapters 7 and 8). The observed yield loss by SO_2 ranged from 7 to 17%. The backgrounds of these yield losses were analysed using mechanistic simulation models for crop growth (Chapter 9). This analysis showed that the observed yield loss was mainly caused by chronic injury (macroscopic leaf damage appearing after long exposures). This injury was observed at the end of the growing period and resulted in an accelerated abscission of the oldest leaves. Subtle injury (direct effects on photosynthesis and respiration) were less important (Chapter 9). This study revealed that quantification of chronic SO_2 injury in crops, forests and other types of vegetation requires insight in its biochemical/physiological backgrounds. Although chronic SO_2 injury, often interpreted as an accelerated leaf senescence is a well known phenomenon when plants are exposed to SO_2 (*cf.* Bell & Clough, 1973; Guderian, 1977; Linzon, 1978; Pierre & Queiroz, 1981, 1982), no attempts have been made to quantitatively explain these effects from the underlying mechanisms.

Because sulphur is an essential nutrient for plant growth, a mechanistic understanding of chronic SO_2 injury can only be achieved from insight in the metabolism of SO_2. Uptake of gaseous SO_2 by plants can either stimulate or reduce plant growth depending on the nutritional status of the plants (Bleasdale, 1952; Reinert *et al.*, 1969; Tingey, Heck & Reinert, 1971; Faller *et al.*, 1970; Cowling, Jones & Lockyer, 1973; Jäger & Klein, 1976; Klein & Jäger, 1976). Plants growing on soils which cannot meet the sulphur requirements for plant growth, may use SO_2 as an additional source of sulphur (Thomas *et al.*, 1943, 1944; Olsen, 1957). However, when cumulative SO_2 uptake exceeds a certain threshold, chronic injury and severe effects on plant growth may be observed (Linzon, 1978). This threshold varies considerably among species and varieties and depends upon the growing conditions (Rennenberg, 1984).

When SO_2 dissolves in the aqueous phase in the leaves, sulphite and bisulphite are formed, thereby producing H^+. Sulphite and bisulphite are quickly oxidized to sulphate (Miller & Xerikos, 1979; Alscher *et al.*, 1987). When the airborne sulphur is stored as sulphate, SO_2 uptake results in net H^+ production in the cells. However, when sulphate is subsequently reduced to sulfide during protein synthesis, the H^+ ions are neutralized by OH^- production. This may explain the stimulatory effect of SO_2 at low sulphur supplies and the inhibitory effect when plants are adequately supplied with sulphur. Because the ratio of protein sulphur to protein nitrogen is relatively constant for a given species (Dijkshoorn & van Wijk, 1967), it is reasonable to assume that the amount of sulphur necessary for protein synthesis depends upon its nitrogen assimilation. Excess sulphur taken up by plants exposed to SO_2, is mainly stored as sulphate (Faller et al., 1980, Maas, 1987, Cowling & Bristow, 1979). Organic sulphur also increases (accumulation of glutathione), but is quantitatively less important (Maas, 1987; Grill & Estenbauer, 1973; Grill et al., 1979; Cowling & Bristow, 1979).

It is unlikely that sulphate accumulation in plants exposed to SO_2 is responsible for chronic injury symptoms, because the sulphate content of healthy green leaves may be higher than in damaged leaves (Eaton, Olmstead & Taylor, 1971; Cowling & Bristow, 1979; Jäger & Klein, 1976; Priebe, Klein & Jäger, 1978). The most likely explanation for chronic SO_2 injury is a disturbance of intracellular pH regulation. Several workers indeed reported that the pH of leaf homogenates only shifted towards greater acidity when plants were lethally damaged after long-term SO_2 exposures (Thomas *et al.*, 1944; Fischer, 1967, cited by Jäger & Klein, 1980; Grill, 1971; Klein & Jäger, 1976; Jäger & Klein, 1977).

Model calculations showed that the cellular buffering capacity, based upon dissociation or association of weak acids, is insufficient to prevent cellular acidification during long-term SO_2 exposures at low concentrations (< 50 $\mu g\ m^{-3}$) (Laisk *et al.*, 1987a, b). However, it is well known that plants are able to maintain intracellular pH in spite of the large quantities of H^+ or OH^- produced in the cells during the assimilation of essential nutrient ions like NO_3^-, NH_4^+ and SO_4^{2-}, although their buffer capacity is far insufficient to neutralize the excess OH^- or H^+ produced (de Wit, Dijkshoorn & Noggle, 1963; Davies, 1973; Raven & Smith, 1976; Smith & Raven, 1979; Raven, 1986). These buffering mechanisms are closely related to the uptake and assimilation of nutrient ions.The role of these buffering mechanisms in the removal of H^+ ions produced following SO_2 uptake has not been studied to date.

In this study the impact of SO_2 on the uptake and assimilation of nutrient ions and implications for regulation of intracellular pH was studied in order to elucidate the chronic SO_2 injury observed in field experiments (Chapters 7, 8 and 9). A conceptual model for the neutralization of H^+ following SO_2 uptake is proposed and evaluated using data on the ionic composition of the field-exposed *Vicia faba* plants. The findings are discussed in relation to the observed chronic injurious effects in the experiments. The potential of the model to explain chronic effects of N and S containing air pollutants in relation to uptake, assimilation and distribution of nutrient ions is indicated.

10.2 Materials and methods

Experimental procedure

In an open-air exposure system, developed by Mooi & van der Zalm (1986), a broad bean crop (*Vicia faba* L., cv. Minica) was exposed to a mean concentration of 74 $\mu g\ SO_2\ m^{-3}$ in 1988. A control plot was located at 250 m distance from the system, exposed to background concentrations (9 $\mu g\ SO_2\ m^{-3}$). Details on frequency distribution of SO_2 concentrations during the growing season, O_3 concentrations and weather conditions are given in Chapter 7.

To avoid confounding effects of differences in environmental conditions between the plots, the broad bean crop was grown at a density of 20 plants per m^2 in plastic containers (55x22x25 cm) filled with a commercial potting mixture. The soil was adequately fertilized with P, K and trace elements. Nitrogen content of the soil (including fertilizer nitrogen) was 50 kg NO_3^- - N ha^{-1} and 68 kg N - NH_4^+ ha^{-1}. The soil was inoculated with Rhizobium. Water was supplied by a drip-irrigation system. The plants emerged on 15 May and growth was analysed by frequent harvesting up to 25 August. After collecting the plants in the field, they were divided into leaves, stems, and pods. Subsamples were analysed for contents of NO_3^-, SO_4^{2-}, $H_2PO_4^-$, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+} and total sulphur in the Chemical Laboratory of the Centre for Agrobiological Research. All results represent mean values of analyses of three individual plants.

Conceptual model for the effects of SO_2 on the regulation of intracellular pH

The analysis of SO_2 effects on regulation of intracellular pH in relation to the uptake and assimilation of nutrient ions, will be discussed first.

Ionic balance and regulation of intracellular pH. Four groups of ions which play an important role in the ionic balance of plants are inorganic cations, inorganic anions, organic anions and H^+ and OH^-. Organic cations (mainly amines and basic amino acids) are quantitatively unimportant in plants (van Beusichem, 1984). The main inorganic cations are K^+, Na^+, Ca^{2+} and Mg^{2+} and the main inorganic anions are Cl^-, $H_2PO_4^-$, NO_3^- and SO_4^{2-} (de Wit *et al.*, 1963).

The total content of inorganic cations (C) exceeds the total inorganic anion content (A) in plants, and the difference between the two (C-A) is stoichiometrically related to the organic anion (dissociated carboxylic acid) content, when expressed in charge equivalents (Arnon, 1939; Ulrich, 1941; de Wit *et al.*, 1963; Dijkshoorn, Lathwell & de Wit, 1968; Houba, van Egmond & Wittlich, 1971; van Egmond, 1975).

The organic anion content in the plants is a result of the operation of a so-called biochemical pH stat mechanism proposed by Davies (1973, 1986). When H^+ or OH^- are produced in the cellular solution, intracellular pH is maintained by carboxylation or decarboxylation of organic anions. The most important pH stat, which operates in the pH range between 6 and 8, involves malate. The appearance of OH^- in cells stimulates carboxylation by activating phosphoenolpyruvate carboxylase which converts phosphoenolpyruvate (one carboxyl group) to oxaloacetate (two carboxyl groups), which in turn can be reduced to the strong acid malate (which also has two carboxyl groups). The organic anion can be stored in the vacuole or transformed into other organic anions in the mitochondria via the TCA cycle. The production of H^+ ions activates malic enzyme, which results in the decarboxylation of malate to pyruvate (one carboxyl group), thus neutralizing one carboxyl group to maintain intracellular pH.

The form in which nitrogen is taken up by plants largely determines the organic anion

content, because intracellular assimilation of nitrate involves the production of OH^- ions (Dijkshoorn, 1962), inducing the formation of organic anions. Assimilation of NH_4^+ into organic compounds, however, results in H^+ production. Assimilation of neutral nitrogen sources (*e.g.* N_2 fixation) involves neither H^+ production nor OH^- production. This explains the generally low organic anion content in NH_4^+ grown plants (de Wit *et al.*, 1963). Like nitrate reduction, the assimilation of SO_4^{2-} also involves a net OH^- production. Another process determining the organic anion content is the operation of the biochemical pH stat in the roots, which removes OH^- ions appearing when H^+ is extruded to maintain electroneutrality.

Assimilation of nitrogen and sulphur nutrients may be located either in the roots or in the shoots. When assimilation takes place in the roots, H^+ or OH^- can be removed by the biochemical pH stat or by extrusion from the roots to the medium (a biophysical pH stat mechanism). This biophysical pH stat is coupled to the maintenance of electroneutrality in the plant and its medium. However, removal of these ions in shoot cells involves the operation of a biochemical pH stat. Because pH regulation differs between root and shoot-generated H^+, the removal of H^+ and OH^- produced in the shoots and roots are discussed separately in this paper (following van Beusichem, Kirkby & Baas, 1988), instead of calculating H^+ or OH^- produced in the plant as a whole as is done in the studies of Raven and co-workers (Raven & Smith, 1976; Smith & Raven, 1979; Raven, 1985, 1986, 1988). This is of course especially important in the analysis of effects of air pollutants which are taken up by the shoots. The processes of nutrient uptake, assimilation and distribution will be discussed in relation to the form in which nitrogen is supplied.

In dinitrogen fixing plants, neutral nitrogen is assimilated in the roots. H^+ is extruded from the roots into the medium to counterbalance the difference between inorganic cation and inorganic anion uptake:

$$H^+ \text{ extrusion} = (K+Na+Mg+Ca)_a - (P+Cl+S)_a$$

where *a* indicates the absorbed amount of inorganic cations and anions, which are expressed in charge equivalents. The intracellular OH^- production resulting from H^+ extrusion is removed by the formation of organic anions, which are mostly transported to the shoots (van Beusichem, 1983). SO_4^{2-} reduction is the only process producing OH^- ions in the shoots (Israel & Jackson, 1982; van Beusichem, 1981).

In NH_4^+ fed plants, the acidifying effect on the medium is stronger, because the uptake of NH_4^+ has to be counterbalanced:

$$H^+ \text{ extrusion} = (N+K+Na+Mg+Ca)_a - (P+Cl+S)_a$$

The detailed analysis of van Beusichem *et al.* (1988a) showed that total H^+ extrusion indeed counterbalanced the H^+ production in NH_4^+ assimilation as well as the difference in the uptake of inorganic cations (excluding NH_4^+) and inorganic anions, to maintain

electroneutrality. Because most nitrogen assimilation takes place in the roots and neutral amino acids are transported to the leaves (van Beusichem *et al.*, 1988a), most H^+ ions produced in the roots are extruded directly to the medium. The OH^- ions, produced in the cellular solution as a result of the H^+ extrusion counterbalancing the difference in uptake of non nitrogen containing inorganic cations and anions, was neutralized by the biochemical pH stat, producing organic anions in the roots.

When large amounts of NO_3^- are taken up, the uptake of inorganic anions exceeds the uptake of inorganic cations, necessitating a net OH^- extrusion to maintain electroneutrality.

$$OH^- \text{ extrusion} = (N+Cl+P+S)_a - (K+Na+Mg+Ca)_a$$

The assimilation of NO_3^- takes place partly in the leaves and partly in the roots, where OH^- ions are partly removed by extrusion and partly by the biochemical pH stat, producing organic anions (van Beusichem *et al.*, 1988a; Allen & Raven, 1987).

Regulation of intracellular pH during SO_2 uptake. The effects of SO_2 on the regulation of intracellular pH will be discussed for dinitrogen-fixing plants first.

When SO_2 dissolves in the aqueous phase in the leaf, sulphite and bisulphite are formed, producing H^+. The relative concentrations of the different compounds and degree of dissociation depends on intracellular pH:

$$SO_2 + H_2O \rightleftharpoons H^+ + HSO_3^- \rightleftharpoons 2H^+ + SO_3^{2-}$$

Sulphite and bisulphite formed in the leaf are quickly oxidized to sulphate (Alscher *et al.*, 1987; Miller & Xerikos, 1979).

$$H_2SO_3 + {}^1/_2\, O_2 \longrightarrow 2\, H^+ + SO_4^{2-}$$

When sulphate is reduced to sulphide, 2 OH^- ions are formed. Reduced sulphur can be utilized in protein synthesis, or emitted as gaseous H_2S, which may account for 10% of total SO_2 uptake (Hållgren & Frederiksson, 1982; Sekiya *et al.*, 1982). When SO_2-sulphur cannot be reduced, but is stored as sulphate, uptake of SO_2 ultimately results in net H^+ production in the cells.

The possible mechanisms for regulation of intracellular pH during SO_2 uptake in relation to the uptake and assimilation of nutrient ions is illustrated in Fig. 10.1. Inorganic cations (C^+), inorganic anions (*e.g.* SO_4^{2-}) and organic anions (OA^-) are transported to the shoot. Organic anions are mainly stored in the vacuoles. Sulphate content is the result of its uptake from the soil and SO_2 uptake by the leaves. When sulphate is reduced, OH^- ions are produced, which can be used for organic anion synthesis in the biochemical pH stat, or to neutralize H^+ produced following SO_2 uptake. Excess sulphate will be stored in the vacuoles (Maas, 1987; Rennenberg, 1984). The decrease of organic anions will be

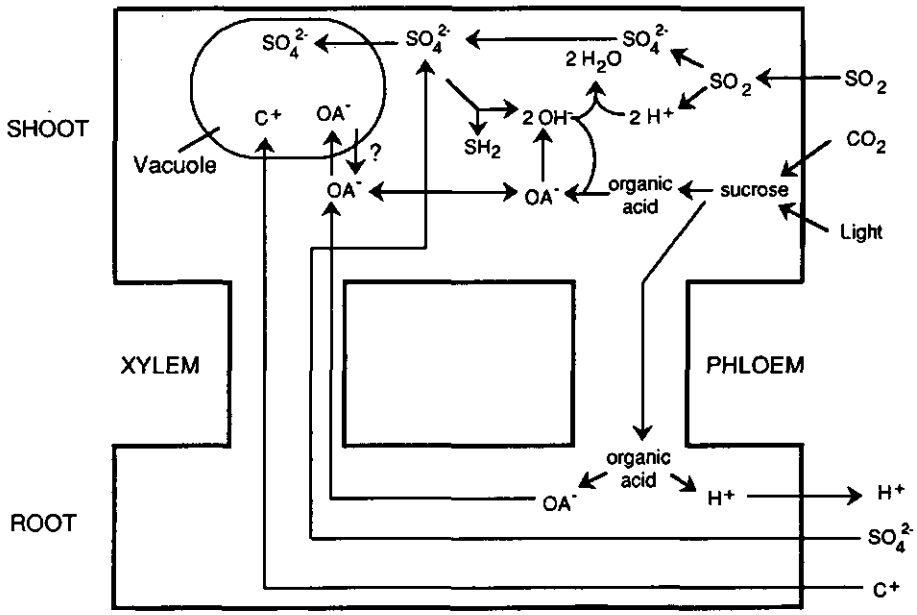


Fig. 10.1 Regulation of intracellular pH during SO_2 uptake by shoots of dinitrogen fixing plants. OA^- represents organic anions and C^+ indicates inorganic cations.

equivalent in charge to the amount of accumulated sulphate, when SO_2 has no effect on the uptake or distribution of other nutrient ions. This implicates that the amount of sulphate stored in the vacuoles, is a measure for the cumulative net H^+ production in the shoots following SO_2 uptake. When all sulphate reduction is located in the shoots and the rate of SO_2 uptake exceeds the rate of sulphate reduction, the only possibility for neutralization of the extra H^+ produced during SO_2 uptake is decarboxylation of imported organic anions, which have been produced in the roots following H^+ extrusion.

In NH_4^+ fed plants the situation will be almost the same as in dinitrogen-fixing plants, because organic anion production in the roots is also related to the difference between inorganic cation (excluding NH_4^+) and inorganic anion uptake.

In NO_3^- fed plants the situation is completely different, because large amounts of OH^- are produced in the shoot when NO_3^- is assimilated. Therefore, NO_3^--grown plants will have a much higher capacity for H^+ neutralization in the shoots, and should be less susceptible to chronic SO_2 damage.

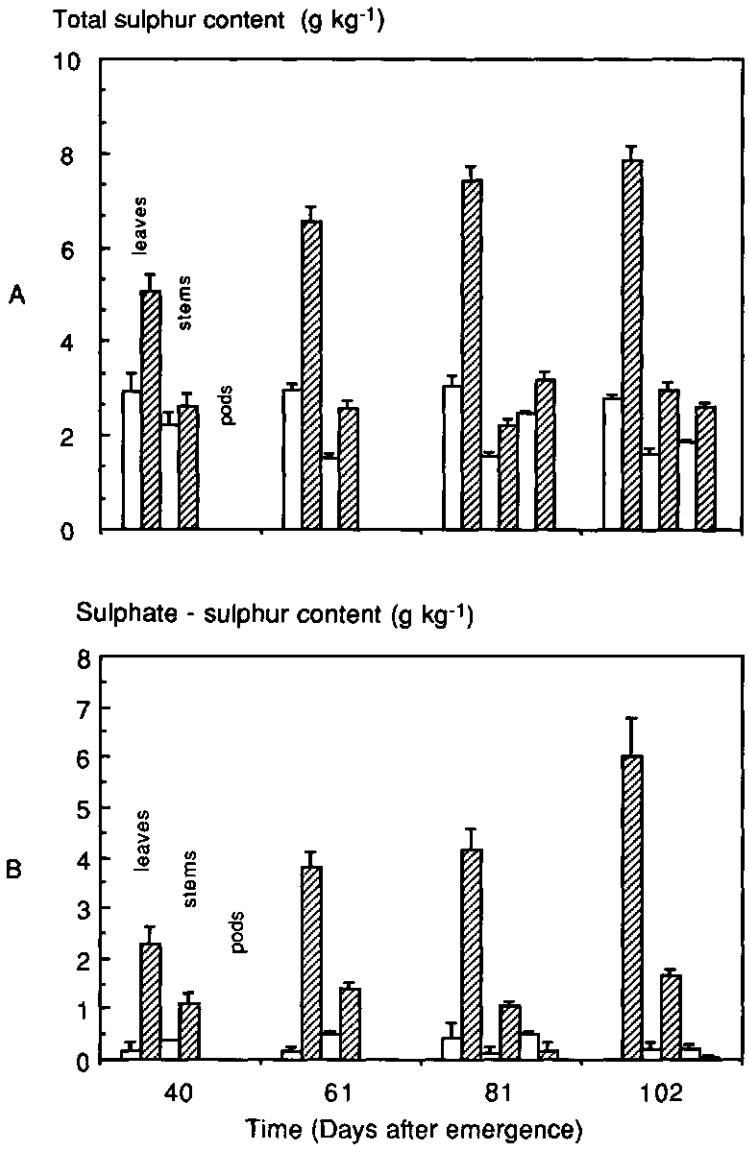


Fig. 10.2 Accumulation of total sulphur (A) and sulphate (B) in leaves, stems and pods of control (open columns) and fumigated plants (shaded columns) (mean and SE of mean).

10.3 Results and discussion

Plant growth and crop production

Growth of fumigated plants was reduced during pod filling at the end of the growing period. Total dry matter production was reduced by 9% at final harvest. Leaf injury was observed in the fumigated plants at the end of the growing period. The injury consisted of brown/red spots which started at leaf edges and proceeded from the bottom of the canopy upwards, followed by abscission of the oldest leaves. Similar effects on leaf area and dry matter production were observed in two preceding experiments conducted in 1985 and 1986 at the same location (Chapters 7 and 8). Simulation analysis demonstrated that SO_2 effects on total dry matter production in all three experiments were mainly caused by the reduction of the green leaf area in the fumigated plots at the end of the growing period (Chapter 9)

Nitrate was not detected in any of the samples. Nitrogen fixation in the nodules appeared to be the most important nitrogen source, because about 450 kg N ha^{-1} was taken up by the plants (1985 experiment at the same conditions), whereas only 50 kg nitrate-N ha^{-1} and 68 kg ammonium-N ha^{-1} was available from the potting mixture and fertilizer. It is reasonable to assume that NO_3^- and NH_4^+ were used in the beginning of the growing period, followed by a switch to nitrogen fixation (van Beusichem & Nelemans, 1989).

SO_2 effects on ionic composition

The contents of total-S and sulphate-S in the leaves, stems and pods of fumigated and control plants are presented in Fig. 10.2. The strongest increase of total-S and sulphate-S was observed in the leaves. The fate of the airborne sulphur was analysed by relating the difference in accumulated sulphate-S to the difference in total-S between control and fumigated plants. The near 1:1 relationship indicates that only a very small amount of SO_2 was converted into organic sulphur (Fig. 10.3). These findings confirm the conclusions of Faller *et al.* (1970); Cowling & Bristow (1979) and Maas (1987), who concluded that excess sulphur is mainly stored as sulphate. The data in this study yields no information on detoxification of SO_2 by reduction of sulphite or sulphate to sulphide emitted as H_2S. This emission may account for 10% of S uptake (Wilson, Bressan & Filner, 1978; Hållgren *et al.*, 1982).

Total inorganic cation content of the plants was not affected by SO_2, whereas the total inorganic anion content was elevated in the fumigated plants during the entire growing period (Fig. 10.4). The difference in total inorganic anion content between the fumigated and the control plants was fully accounted for by sulphate accumulation in the fumigated plants (Fig. 10.4A). The accumulation of sulphate in plants exposed to SO_2

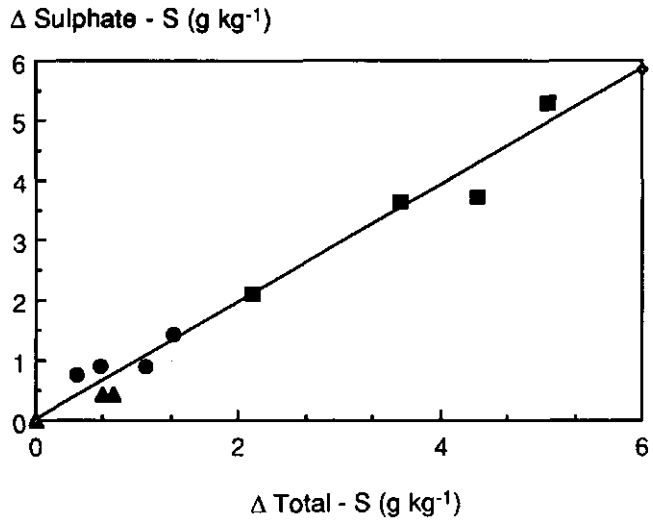


Fig. 10.3 Relation between accumulated total sulphur and sulphate-sulphur in the fumigated plants (corrected for SO_4^{2-} - sulphur and total sulphur contents of the control plants) in the leaves (■), stems (●) and pods (▲) during the growing period ($y = 0.002 + 0.97x$; $r^2 = 0.96$).

was also responsible for a more than two-fold increase of the total foliar inorganic anion content (Fig. 10.4B). Because the difference between C and A equals the amount of organic anions, the amount of accumulated sulphate is equivalent to the decrease of organic anions in the plant. The decrease in organic anion content by SO_2 may explain the reduced chemical buffering capacity in plants exposed to SO_2 (Grill, 1971; Jäger & Klein, 1977; Darral & Jäger, 1984; Bytnerowycz *et al.*, 1987), because the chemical buffering capacity largely depends on organic anion content (Jäger & Klein, 1976).

The effect of SO_2 on cation and anion composition of the plants at final harvest (102 days after emergence) is illustrated in Fig. 10.5. The cation composition of the plants was unaffected by SO_2 while the anion composition was strongly affected, due to the much higher SO_4^{2-} content in fumigated plants (up to a factor 9). These effects were even more pronounced in the leaves (Fig. 10.5B). Eaton *et al.* (1971) also demonstrated that the total amount of cations in tomatoes (*Lycopersium esculentum* Mill.) was unaffected by SO_2, although they observed a small change in cation composition (more K and less Ca and Mg). Klein & Jäger (1976) did not find an effect of SO_2 on K and Ca content in *Pisum sativum* grown at optimal nutrition either, while Jäger & Klein (1977) observed no effects on K, Ca, P and N in *Pisum sativum* due to SO_2 fumigations.

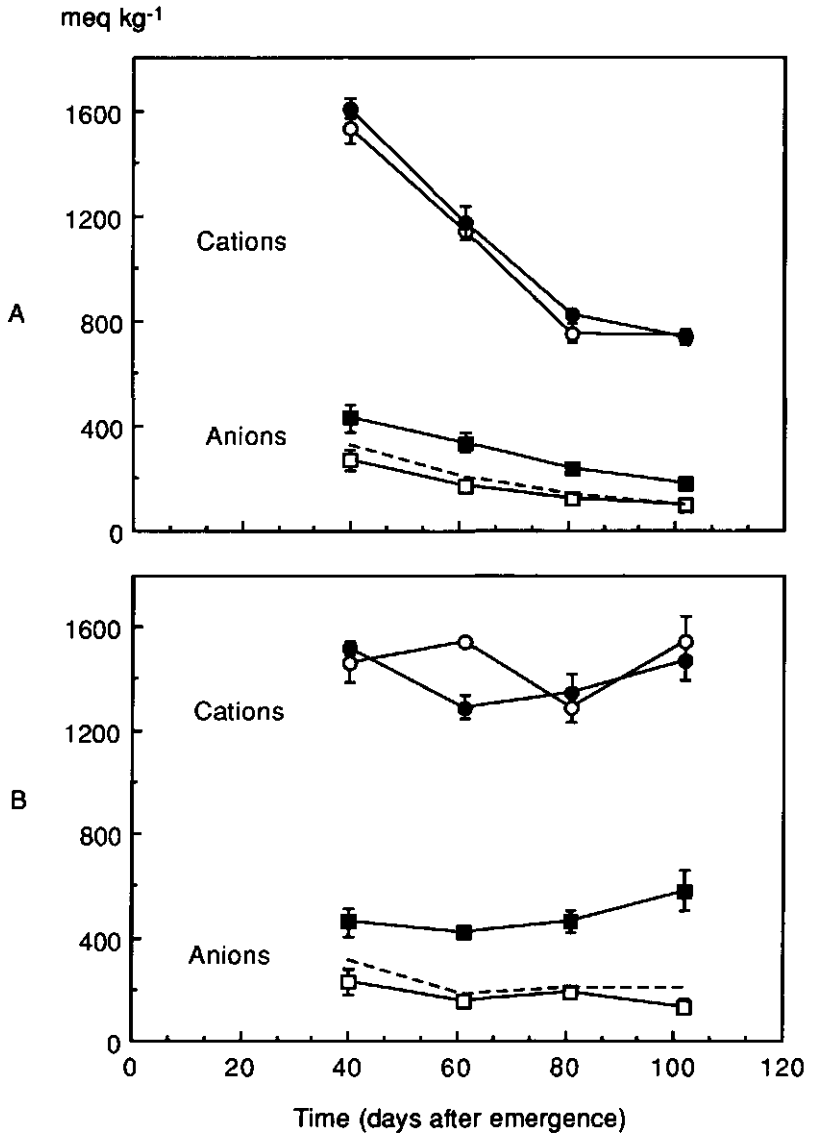


Fig. 10.4 Total inorganic cation (C) and inorganic anion (A) content of broad beans (A. for whole plants; B. for leaves), fumigated with SO_2 (C: ● ; A: ■), or exposed to ambient SO_2 (C: ○ ; A: □) in the field. The inorganic anion contents of the fumigated plants, corrected for sulphate accumulation, are indicated by the broken line.

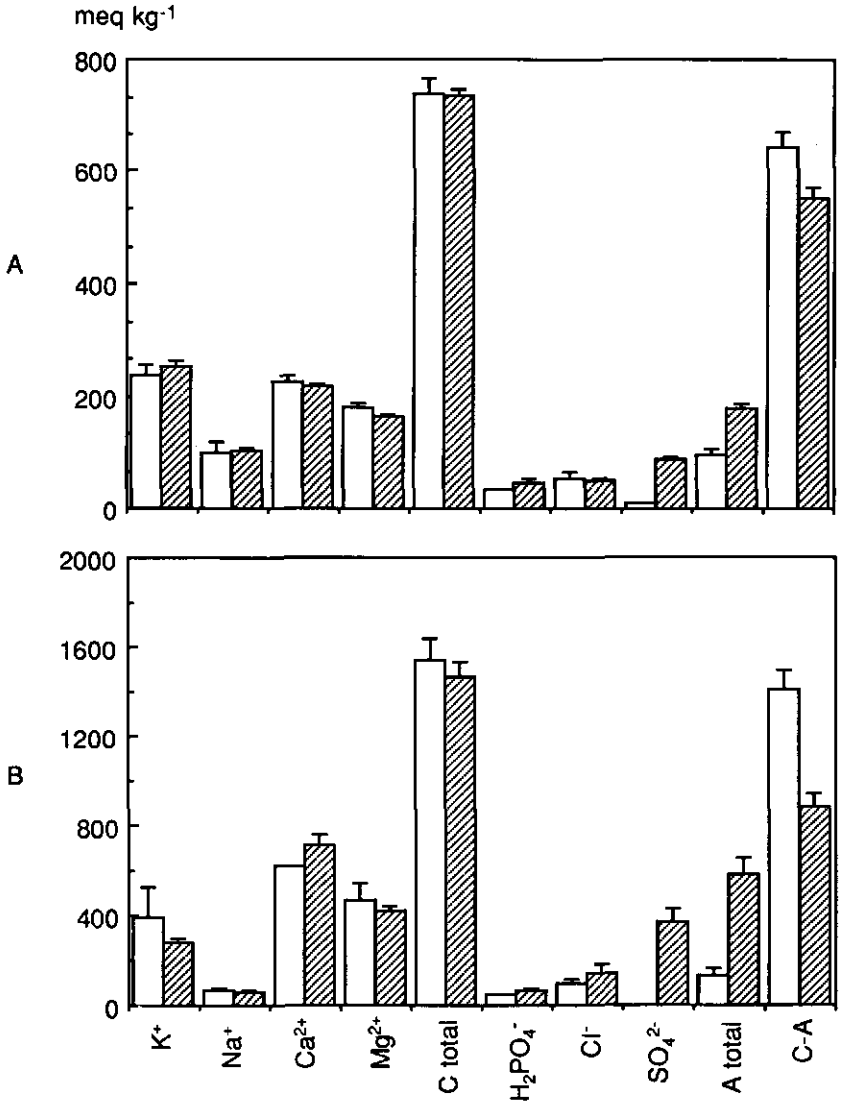


Fig. 10.5 Inorganic cation and inorganic anion composition of whole plants (A) and leaves (B), fumigated with SO_2 (shaded columns), or exposed to ambient SO_2 (controls, open columns) in the field, after a growing period of 102 days.

Uptake of SO_2 and regulation of intracellular pH

The experimental data showed no effects on inorganic cation and inorganic anion contents, except for the increased sulphate content. This indicates either a decrease in organic anion content or an increase in organic cation content (*e.g.* polyamines). It is not likely that the H^+ ions are removed by organic cation production. Raven (1986) demonstrated that possible biochemical pH stat mechanisms other than those involving organic anion synthesis or breakdown are not quantitatively important. The only possibility in the short term would be the production of primary (proline) and secondary nitrogenous metabolites (*e.g.* glycine, polyamines) which might operate as a biochemical pH stat. Because these compounds are also involved in other processes such as osmoregulation, long-term accumulation is not likely to be an effective defense mechanism (Raven, 1986). Some workers observed a slight increase in the content of polyamines following SO_2 exposure (Priebe *et al.*, 1978; Jäger & Klein, 1980; Jäger, Bender & Grünhage, 1985), while others did not (Cowling & Bristow, 1979; Pierre & Queiroz, 1982). Priebe *et al.* (1978) calculated that the increase in polyamines accounted for only 10% of the H^+ production as a result of SO_2 exposure. Increased proline concentrations in SO_2 stressed plants were reported by Jäger & Klein (1980) and Anbazhagan, Krishnamurthy & Bhagwat (1988), but no changes in proline content were observed by Brunold, Landolt & Lavanchy (1983). In view of the reported effects on these components it can be concluded that the neutralization of H^+ ions produced in connection with SO_2 uptake by synthesis of these components is quantitatively unimportant. The only possibility left then is the mechanism proposed in this Chapter (Fig. 10.1).

The C-A content of the leaves (where H^+ neutralization takes place), decreased in charge equivalent to the extra amount of SO_4^{2-} produced following SO_2 uptake. The inorganic cation content of plants is not influenced and the negative charge of the organic anions is taken over by SO_4^{2-}. The results indicate that H^+ generated in shoots exposed to SO_2, is neutralized by OH^- produced when SO_4^{2-} is reduced in the leaf or by OH^- released by operation of the biochemical pH stat by decarboxylation of organic anions (e.g. malate) available in the cytosol of leaf cells. The ratio of sulphate-S / organic-S in leaves of fumigated plants changed from 1.1, 40 days after emergence, to 2.1 at final harvest. Because the plants acquired their nitrogen mainly from N fixation, the main source for foliar OH^- production was SO_4^{2-} reduction, which neutralized not more than 50% of the H^+ produced when all sulphate reduction occurred in the shoots. Because nitrate reduction probably contributed to the OH^- production in the leaves in the beginning of the growing period and because the dynamics of ion uptake and assimilation were not analysed, the relative contribution of the different mechanisms to the removal of H^+ cannot be distinguished. More insight in the mechanism can be obtained from detailed analyses of the dynamics of nutrient ion uptake, assimilation and distribution in relation to organic anion metabolism (*e.g.* van Beusichem *et al.*, 1988a).

The significance of these biochemical buffering mechanisms can be illustrated by the following calculations. In the open-air fumigation experiments with *Vicia faba* crops, at

least 10 kg S ha^{-1} was taken up as SO_2 without growth reduction or visible injury (Chapter 9). The amount of protons produced in the leaves was ((10/32) x 2=) 625 mol H^+ ha^{-1}, which equals 52 mol H^+ m^{-3} leaf, assuming (a) all SO_2 is metabolized to sulphate resulting in the production of 2 mol H^+ ions per mol SO_2 (b) leaf thickness 0.4 mm, and (c) an average Leaf Area Index over the growing period of 3. When a buffering capacity of 20 mol H^+ m^{-3} pH unit $^{-1}$ is assumed (Smith & Raven, 1979), intracellular pH should have been reduced by 2.6 pH units over the period. Since small changes in cellular pH (0.4 units) already lead to serious deterioration of physiological processes in chloroplasts (Sakaki & Kondo, 1985), this calculation illustrates that metabolic processes rather than physico-chemical buffering processes are involved in the removal of excess H^+ generated during SO_2 uptake by leaves.

Chronic SO_2 injury and regulation of intracellular pH

The most likely cause of chronic effects is acidification of the cytosol, when the metabolic buffering capacity is exhausted. The rate of pH decrease will depend on the physico-chemical buffering capacity, which is strongly reduced by SO_2 (Jäger & Klein, 1976). Indeed, it has been demonstrated that the pH of leaf homogenates only shifted towards greater acidity when plants were lethally damaged, whereas no pH changes were measured when the leaves were still green (Thomas *et al.*, 1944; Fischer, 1967, cited by Jäger & Klein, 1980; Grill, 1971; Klein & Jäger, 1976; Jäger & Klein, 1977).

Chronic effects cannot be simply related to an exhaustion of metabolic buffering compounds (organic anions). In the fumigated broad bean, chronic injury and a strong leaf abscission was observed between the last two harvests. The C-A content of the leaves of the fumigated plants was reduced, but organic anion content was still about 900 meq kg^{-1} in the leaves (Fig. 10.4B), representing a potentially high metabolic buffering capacity. However, most organic anions are located in the vacuole (Smith & Raven, 1979) and are not directly available for metabolic buffering in the cytosol. Our results suggest that the metabolic buffering capacity of leaves is not related to the organic anion content of the leaves, but is related to the dynamics of SO_4^{2-} and NO_3^- reduction in the leaf and to import of organic anions in the cytosol. Analogously, van Beusichem *et al.* (1988b) concluded that nitrate reductase activity in the shoots (stimulated by organic anions) was coupled to the import and not to the level of organic anions, whereas import was very small compared to the level of organic anions. This may also explain that chronic injury appears in older leaves at the bottom of the canopy, because these leaves are metabolically less active. The extreme sensitivity of plants grown at low light levels and low temperatures may also be understood from this mechanism (Davies, 1980; Jones & Mansfield, 1982; Whitmore & Mansfield, 1983; Colvill *et al.*, 1985; Baker *et al.*, 1986, 1987).

Chronic effects may also be caused indirectly by disturbance of pH regulation when nitrogenous components are produced to maintain intracellular pH. This phenomenon was demonstrated by Coleman & Richards (1956) in plants exposed to low K^+. They showed that the accumulation of putrescine (a polyvalent cation which may be produced for maintenance of intracellular pH in K^+ deficient plants) was responsible for the leaf injury observed in K^+ deficient plants. Bernard & Larher (1971, cited by Priebe *et al.*, 1978) also observed severe symptoms of toxicity when polyamines were applied in high concentrations. It is known that polyamines increase in response to Mg^{2+} deficiency, low external pH, high levels of NH_4^+, SO_2 exposure and osmotic shocks (Young & Galston, 1983; Flores & Galston, 1984; Priebe *et al.*, 1978). Priebe *et al.* (1978) found a twofold putrescine content in SO_2 fumigated plants (*Pisum sativum*) grown on NO_3^- and a 7 times higher putrescine content in fumigated plants grown on 50% NH_4^+ as compared to the control. The putrescine accumulation was strongly correlated to the SO_2 induced growth effects, indicating that disturbance of pH regulation is likely to be the cause of chronic effects. It is not yet clear, however, whether acidification per se or the accumulation of pH stabilizing components are responsible for chronic injury.

It is generally accepted that mass movements of ions require pumps and biophysical pH stats and that biochemical pH stats are concerned only with the fine control of cytosolic pH (Davies, 1986). Indeed, quantitative analyses showed that H^+ produced during NH_4^+ assimilation is removed by extrusion of H^+ to the rooting medium (van Beusichem *et al.*, 1988a). However, H^+ production during NH_4^+ assimilation is located in the roots (van Beusichem *et al.*, 1988a). It is unlikely that H^+ ions, produced in the leaves following SO_2 uptake are transported through the phloem (Raven, 1986, 1988). If the removal of H^+ produced in shoots would be realistic, differences in the susceptibility between NH_4^+ fed plants and NO_3^- fed plants would not be expected. However, Jäger & Klein (1976), Klein & Jäger (1976) and Priebe *et al.* (1978) demonstrated that NH_4^+ fed plants are much more susceptible to chronic SO_2 effects than NO_3^- fed plants.

The reported experimental data of Klein & Jäger (1976) and Jäger & Klein (1976) who studied the effects of varying nutrient supply are consistent with the mechanism proposed in this Chapter. They fumigated peas (*Pisum sativum*) with 400 μg SO_2 m^{-3} for 18 days. Plants grown at optimal nutrient supply with NO_3^- nitrogen were not affected by SO_2, whereas plants grown with ammonium instead of nitrate, showed a strong decrease of growth and severe visible leaf injury. Plants grown at 50% NH_4^+, and low K, or a low medium pH were also reduced in growth. In all these treatments the organic anion production as reflected in its content was reduced resulting in reduced chemical (and metabolic) buffering capacities. These data clearly demonstrate the importance of nutrient supply, especially the form in which nitrogen is applied. This may explain the strong differences in effects of SO_2 between experiments. In the field experiments with broad bean (Chapters 7 and 8) the main source of OH^- in the leaves was sulphate reduction, because the plants fixed dinitrogen. The absence of chronic injury in other experiments (Baker *et al.*, 1986) may be explained by a higher H^+ neutralizing capacity as a result of the application of nitrate containing fertilizers.

Sulphate accumulation is a measure for the cumulative amount of H^+ produced during SO_2 uptake. Several authors have demonstrated that the ratio between inorganic sulphur to organic sulphur can be used as an indicator for SO_2 damage (Gasch *et al.*, 1988; Legge *et al.*, 1988). Gasch *et al.* (1988) concluded that damaging effects on trees may be expected when the ratio exceeds 1. However, the results of Klein & Jager (1976) show that the ratio inorganic-S / organic-S is not sufficient as diagnostic tool, because this ratio in plants grown on NO_3^- may be very high (7.5) without chronic effects.

Chronic SO_2 effects and interactions with NO_2 or NH_3

Uptake of NO_2 also yields in H^+ when it enters leaf solutions. The ultimate effect on pH is neutral when the nitrogen is incorporated in organic compounds. The extreme sensitivity of plants exposed to combinations of SO_2 and NO_2 (Ashenden & Mansfield, 1978; Irving & Miller, 1984; Klarer, Reinert & Huang, 1984), especially when soil nitrogen is high (Taylor & Bell, 1988), may be understood from the same mechanism as proposed for SO_2. When plants are adequately supplied with sulphur and nitrogen, sulphate, nitrate and nitrite will accumulate in the shoot following exposure to NO_2 and SO_2 and an equivalent amount of H^+ ions must be buffered. An extra damaging factor may be the accumulation of toxic nitrite. The mechanism of interaction between SO_2 and NO_2 may be studied by fumigating plants growing on nutrient solutions of different compositions.

When NH_3 is taken up by leaves from the atmosphere and dissolves, OH^- and NH_4^+ ions are formed. Because its assimilation involves H^+ production, no effects on long term pH regulation are expected when NH_3 nitrogen is used for the synthesis of organic compounds. When both NO_2 (or SO_2) and NH_3 are taken up but are not assimilated, the effects on cellular pH regulation will be small because the acidifying effects of NO_2 will be compensated by the alkaline effect of NH_3. Indeed, Zeevaart (1976) found that NH_3 could decrease the damaging effect of NO_2 damage on plants.

The model presented in this paper may provide a framework for further research on (quantitative) explanation of chronic SO_2 effects and their interactions with of airborne N and S containing pollutants on crops, forests and (semi) natural vegetation.

Chapter 11

General discussion

Much experimental work has been expended over many years in analysing the effects of air pollutants, and SO_2 in particular, on plants. Much less work has been done on quantitative interpretation of experimental data with mechanistic models which can integrate and explain the various observed phenomena (Kercher & King, 1985). Such models may help to quantify the impact of air pollutants on plant communities in industrialized countries, and may be used to estimate the effectivity of emission control measures. The plant communities affected by air pollutants, range from relatively simple systems like agricultural crops growing at potential production situations to extremely complex systems like forests and (semi)natural vegetation. Because experimental assessment of the impact of air pollutants at the system level for complex systems like forests is not feasible, mechanistic models may be helpful to integrate effects observed at the process level for estimation of effects at the system level.

In this thesis, the mechanisms behind SO_2 effects on plant growth and crop production were quantitatively analysed by combining model development with experimental work at different levels of biological organization. To permit such an analysis, this study was restricted to the impact of elevated concentrations of a single air pollutant (SO_2) on broad bean (*Vicia faba* L.) crops growing under field conditions and adequately supplied with water and nutrients.

Physiological effects of SO_2

Crop growth at potential production situations is mainly determined by the processes photosynthesis, respiration and translocation. Effects of SO_2 on all these processes have been reported earlier (reviewed by Hällgren, 1978, 1984; Black, 1982; Darrall, 1989; Chapter 3). Because SO_2 uptake results in the release of H^+ ions, effects on intracellular pH may also occur. Generally, a distinction is made between short-term and long-term effects. Because these expressions may be misleading short-term effects have to be defined as effects which can already be observed after short exposure periods (hours). Short-term effects may also play a role after long-term exposures. In contrast, long term-effects have to be defined as effects which can be observed only after long exposures (days or weeks). Therefore long-term effects are based on different mechanisms. Chronic

leaf injury, possibly caused by effects on intracellular pH regulation, is an example of these long-term effects. Both types of effects have been studied in this thesis.

Photosynthesis and stomatal behaviour Photosynthesis is only affected by short-term exposures to SO_2 at high radiation levels (Chapter 2). Generally, the level of inhibition increases rapidly in the first hour after the onset of fumigation, followed by stabilization (Chapters 2-5). Separation of stomatal and non-stomatal factors in photosynthetic depression by SO_2 revealed that stomatal behaviour was not affected by SO_2 (Chapter 2). The biochemical mechanisms behind observed SO_2 effects on photosynthesis have been analysed and discussed in Chapters 2 and 3. Short-term effects of SO_2 on photosynthesis could be quantitatively explained by an inhibitory effect of SO_2 on CO_2 binding to RBP carboxylase/oxygenase (Chapters 2 and 3). Other reported effects, like effects on light reactions of photosynthesis, effects of SO_2 on cellular pH, and effects on translocation of sugars are not responsible for the observed reductions in photosynthesis during short exposures to SO_2 (Chapter 3).

The analysis of mechanisms in Chapters 2 and 3 resulted in a model for the flux of atmospheric SO_2 into leaves and the effects of SO_2 metabolites (S(IV) compounds) on leaf photosynthesis and stomatal resistance (Chapter 3). The model contains only two parameters which describe the biochemical characteristics determining the sensitivity of leaf photosynthesis: a time coefficient for S(IV) oxidation and a parameter describing the sensitivity of photosynthesis for S(IV). The pattern of rapid photosynthetic reduction and rapid recovery following fumigation was accurately described with the model. A summary version of a model for SO_2 flux into leaves and effects on photosynthesis was used to estimate values for the two model parameters by combining non-linear regression of two equations on gas exchange data. Parameter estimates agreed very well with experimentally determined values (Chapter 4). Differences in photosynthetic sensitivity of plants could be explained by differences in the time coefficient for sulphite oxidation and leaf thickness. A similar analysis of gas exchange data was presented by Schut (1985), who developed several models for short-term effects of ozone, based on various hypotheses to elucidate possible mechanisms behind ozone effects.

It is well known that depressing SO_2 effects on plant growth are most severe during winter periods, when temperature and irradiance are low and humidity is high. The influence of temperature and humidity on physiological SO_2 effects was studied by gas exchange measurements (Chapter 5). Data analysis with the model following the procedure described in Chapter 4, revealed that S(IV) oxidation proceeds at a slower rate at lower temperatures, resulting in a stronger accumulation of S(IV) and stronger reduction of photosynthesis. Model results were confirmed by experimental analyses of the S(IV) concentration in leaves after the fumigation period, which showed that more S(IV) accumulated in leaves exposed at a lower temperature. Although it is well known that the rate of S(IV) oxidation in aqueous solutions is very sensitive to temperature, its

implications for effects of SO_2 on photosynthesis were not recognized before. This work demonstrated that mechanistic physiological models may be used to identify and quantify environmental effects on processes at the underlying biochemical level by utilizing easy to perform gas exchange measurements.

Effects of SO_2 on photosynthesis after long-term exposures were analysed in field experiments (Chapter 9). No effects of SO_2 on photosynthesis were detected in leaves of the broad bean plants exposed to 100-200 μg SO_2 m^{-3} in the field. Similar results were found when photosynthesis of the same leaf numbers in broad bean plants was followed during exposure to SO_2 for two months, in SO_2 concentrations ranging from 0-300 μg m^{-3} (Smeets, Kropff & Kooyman, unpublished results). No detectable effects of these concentrations were expected on basis of the mechanism responsible for short-term effects (competition between S(IV) compounds and CO_2, Chapters 2 and 3). It was concluded that long-term effects of SO_2 on photosynthesis are not important with respect to crop loss in broad bean. Other data from long-term fumigations at low SO_2 concentrations are scarce and do not present a clear picture (Darrall, 1989).

Respiration Dark respiration increased as a result of short-term SO_2 fumigation, but not significantly (Chapter 2). Contradictory reports on SO_2 effects on dark respiration after short exposures have been published (Black, 1984). A significant effect of SO_2 on dark respiration of leaves was observed in a field experiment in 1988 (30% increase), halfway through the growing period, following a long fumigation period (Chapter 9). Because data on effects of long-term exposures on respiration are scarce (Black, 1984), more detailed analyses of these effects on rates of respiration are needed.

Translocation Although the rate of translocation is very sensitive to SO_2 (Noyes, 1980; Jones and Mansfield, 1982; McLaughlin *et al.*, 1982; Teh & Swanson, 1982; Lorenc-Plucinska, 1986), no strong effects on dry matter allocation patterns to above-ground parts were observed in the three field experiments (Chapter 9). Dry matter allocation to the roots was not studied. Because dry matter allocation to the roots can be inhibited by SO_2 (Darrall, 1989), the effects on total dry matter production of the crop, including the roots, may have been stronger. More insight in SO_2 effects on translocation may be obtained by analysis of dynamic dry matter allocation patterns and the dynamics of translocation over the day. Challa (1976) demonstrated that starch depletion in leaves always occurred at the same moment of the day (after a fixed dark period), in spite of differences in sugar production. The question to be answered is whether the SO_2 exposed plant is capable of translocating its sugars produced in the daytime, within the 24 hours available, to avoid accumulation of sugars in the leaves. In answering this question, the dynamics of SO_2 effects on translocation have to be taken into account. Phloem loading may be reduced in the daytime when SO_2 is taken up at a high rate, but will hardly be affected in the dark period when SO_2 uptake will be much slower due to stomatal closure.

Regulation of intracellular pH The backgrounds of chronic effects have hardly received any attention in literature. A conceptual model for chronic effects of SO_2 was presented in Chapter 10. Many phenomena observed after long-term exposures of plants to SO_2 can be clarified by the concept based on the hypothesis that chronic effects are due to effects of SO_2 on regulation of intracellular pH. When excess sulphur is stored as sulphate and is not assimilated into organic compounds, the plant has to buffer an amount of H^+ ions in charge equivalent to the amount of sulphate. It is well known that mechanisms for regulation of intracellular pH in plants are based upon metabolic reactions, which are closely related to the uptake and assimilation of nutrient ions. The form in which nitrogen is supplied strongly affects the metabolic buffering capacity of the cytosol in leaf cells. Nitrate-grown plants produce large amounts of OH^- in the cytosol, when nitrate and relatively small amounts of sulphate are reduced and assimilated into organic compounds, whereas sulphate reduction and breakdown of organic anions are the only sources of OH^- production in NH_4^+ grown plants and N_2 fixing plants. It was clearly demonstrated by Klein & Jäger (1976) that nitrate grown plants are much less sensitive to chronic SO_2 effects than NH_4^+ grown plants. This theory may explain why chronic injury was observed in field experiments with the N_2 fixing broad bean, whereas other workers observed no chronic effects in field crops (barley, fertilized with nitrate) exposed to even higher concentrations of SO_2. The concepts developed in Chapter 10 have to be worked out in more detail by careful research at the subcellular level. It has to be confirmed that chronic effects are due to disturbances of regulation of intracellular pH (directly or indirectly by accumulation of diamines) and that the organic anions stored in the vacuoles are not available to buffer H^+ in the cytosol. Quantification of the processes involved in metabolic buffering of pH at the subcellular level, is an essential requirement for the development of mechanistic models for the long-term effects of N and S containing air pollutants.

Mechanisms of SO_2 effects on plant growth

In the general introduction a distinction was made between acute, chronic and subtle injurious effects of SO_2 on plants (Linzon, 1978). Acute injury appears as visible damage on foliage and may be observed after short exposures to excessively high SO_2 concentrations. Chronic injury was defined as visible damage after long exposures to variable SO_2 concentrations and subtle injury was defined as growth reduction without visible damage. Several workers concluded that completely different mechanisms are the basis of the various types of effects, because no relationship was found between the sensitivity of species and cultivars to different types of injury (reviewed by Bell, 1985; Garsed, 1985). In this study, chronic and subtle injury have been explained on the basis

of different mechanisms. Photosynthetic reduction was explained by competition between SO_2, O_2 and CO_2 for the carboxylating enzyme Rubisco (Chapters 2 and 3), and chronic effects have been related to the long term effects of SO_2 exposure on regulation of intracellular pH (Chapter 10). Acute injury, which was not observed in our experiments (Chapters 7 and 8), is assumed to be related to the damaging effect on cellular structures in the leaf by high S(IV) concentrations or by oxygen radicals, which may be produced when S(IV) is quickly oxidized to sulphate (Tingey & Olszyk, 1985).

The results from this study demonstrated that chronic injury may play a significant role in the effects of SO_2 on crop growth and production. Effects on photosynthesis and respiration were responsible for a only a small part of the observed growth reductions in broad bean (Chapter 9). This method for analysis of damage components is very useful in determining research priorities at the physiological level (*cf.* Rabbinge *et al.*, 1985; van der Werf, 1988; Chapter 9). The detailed analyses of SO_2 effects on physiological processes demonstrated that SO_2 effects on photosynthesis are not expected to cause substantial reductions in crop growth at ambient concentrations and high temperatures in Western Europe (Chapter 6). However, the results presented in Chapter 5 indicate that effects at lower temperatures in winter, spring and autumn when SO_2 concentrations are generally higher than the annual mean, may substantially reduce photosynthesis, because the rate of S(IV) removal is slowed down. This may explain the reported growth reductions without visible damage in cereals exposed to SO_2 in winter periods (Baker *et al.*, 1986). Quantification of these effects at winter conditions should be based on experimental analyses of S(IV) oxidation in leaves in relation to leaf age, temperature and radiation level. The procedure described in Chapter 4 for short-term exposures can be used to analyse changing biochemical characteristics after long-term exposures, by repeated fumigation of leaves for short periods with a higher concentration, during which gas exchange is measured. Parameter values can be derived by fitting the model to the data from the short-term exposures.

SO_2 effects on plant growth and crop production

The effects of SO_2 on plant growth observed in field experiments (Chapters 7 and 8), demonstrated that severe effects (mainly due to chronic injury) of SO_2 on crop yield may occur at SO_2 concentrations which are observed in large areas of Western Europe and North America (Fowler & Cape, 1982). The effects of SO_2 on seed yield (Chapter 7) were in the range of effects observed in other field studies with leguminous crop species (soybean and snapbean). The methodology in these studies ranged from linear gradient sources to plots differing in distance from smelters emitting large amounts of SO_2 (McLaughlin & Taylor, 1985)

In open-air exposure studies as presented here, the influence of other air pollutants may play an important role. Several authors have observed interactions between O_3 and SO_2 (review by Kohut, 1985). Additive, less than additive and more than additive effects

have been observed. However, dose response relationships derived from experiments conducted in the field generally indicated no interactive effects of the pollutants SO_2 and O_3 (Kohut, 1985). Experiments with broad bean in growth chambers showed no interactive effects of SO_2 and O_3 after 2 months at concentrations ranging from 0-300 μg m^{-3} SO_2 in combination with 0, 100 or 200 μg O_3 m^{-3} (Smeets, Kropff & Kooyman, unpubl. results). Future research on interaction effects of air pollutants should be directed towards the analysis of mechanisms, because the results of reported experiments are contradictory (Wellburn, 1988).

In the control plot in 1986, a strong infection with the fungal pathogen *B. fabae* was observed, whereas plants in the fumigated plot were hardly infected. Similarly, in 1985 and 1986, only slight infections have been observed. Similar findings were published by Heggestad *et al.* (1986), McLeod *et al.* (1988) and McLeod (1988). These results indicate that air pollutant-disease interactions may have considerable importance for disease control in agriculture.

Methodology Only a few systems for open-air exposure of vegetation without any form of plant enclosure, in which a reasonably uniform distribution of gas over the treated area is obtained, have been developed in the past decades (Greenwood *et al.*, 1982; McLeod *et al.*, 1985; Chapter 7). Most experimental work on the effects of air pollutants on plants has been conducted in laboratory or greenhouse growth chambers and open-top chambers, in which the microclimate strongly differs from the field, resulting in plants with a different physiological and morphological status (*e.g.* photosynthetic capacity of leaves and leaf thickness). Extrapolation of effects observed in these facilities to field situations, may result in over or underestimation, because climatic factors strongly modify effects of air pollutants (Black, 1982; Unsworth & Mansfield, 1980).

An often overlooked reason for incorrect estimates of effects on plants in field situations is the role of interplant competition. Air pollutant effects are often studied on more or less isolated plants instead of plants growing at a realistic density (Roberts, 1984). However, these effects on growth of isolated plants are not representative for those on a closed crop canopy. Morphological growth components are extremely important for the growth of isolated plants but hardly influence the growth rate of a closed leaf canopy (LAI > 4) (de Wit *et al.*, 1978). This may result in severe overestimation of air pollutant effects on crops, because both direct effects on morphological development and indirect effects on morphology by impairment of physiological processes, largely determine the effects observed on growth of isolated plants. Effects on growth of closed canopies, however, are mainly determined by effects on physiological processes like photosynthesis and respiration. Morphological aspects are only important during early growth and maturation and should be studied for these growth phases explicitly.

Another confounding aspect is the influence of low radiation on the sensitivity of plants (Davies, 1980). Because radiation exponentially decreases with the LAI inside a

closed canopy, the radiation level at which leaves are exposed to air pollutants differs with canopy height. This effect is not studied when isolated plants are used.

The available area in growth chambers generally limits the setup of experiments. When various treatments or species are located in the same chambers and plants are grown to maturity, interplant competition can hardly be avoided. The bias, arising from competition between plants from different treatments (or species) in such experiments in growth chambers may be considerable (Schapendonk & Spitters, 1984) and should be avoided in studies on air pollutant effects. When the effects of air pollutants on crops are studied in open-air exposure systems, open-top chambers or other types of growth chambers, the plots have to be well bordered to avoid competition effects from adjacent plots. In the experiments discussed in this thesis (Chapter 7), much attention was paid to this aspect. Plants were periodically harvested from net subplots surrounded by two or three border rows, to avoid effects of surrounding plots. The bias in evaluation of treatment effects that may arise from the use of poorly bordered plots was reviewed by Spitters (1979).

In contrast to studies on air pollutant effects on agricultural crops, morphological effects are important when results are used to estimate air pollutant effects on mixed cultures (vegetation). Slight changes in physiological *and* morphological characteristics of a species, may strongly affect the competitive strength of the plants (Spitters & Aerts, 1983; Kropff, 1988a, b). Thus, for an understanding of the impact of air-pollutants on growth of plants in mixed vegetation, SO_2 effects on both morphological and physiological processes have to be quantified.

Modelling responses of crops and vegetation to air pollutants

To reach meaningful conclusions with respect to effects of air pollutant effects on agricultural crops or vegetation, a quantitative understanding of mechanisms behind these effects is essential, since experimentation at the system level at all relevant conditions is not a realistic possibility. Mechanistic models may be used for integration of insight at the process level and to extrapolate effects observed at the process level to the system level. However, such models need to be evaluated with data on pollutant effects at the system level as much as possible, because our insight in the behavior of biological systems is insufficient to allow extrapolation of effects observed at the single process level to the system level (de Wit, 1982). Therefore we started with the development of models for the effects of SO_2 on agricultural crops, which permit experimentation at the system level. The main advantage of process models come when they are used as an integrated part of research with experimental studies (Luxmoore, 1988). Experimentation at the system level can be restricted to carefully chosen situations, and experimentation at the process level may become very efficient, because model development involves the generation of well defined questions. The physiological studies, described in this thesis, demonstrated the advantages of combining experimental research with modelling (Chapters 2-6).

The relatively few models that have been developed for SO_2 effects on plant growth and crop production have not been evaluated with independent experimental data sets (Kercher & King, 1985; Krupa & Kickert, 1987; Luxmoore, 1988). This may be due to the lack of suitable data sets at the system level. Most studies on SO_2 effects on plant growth deal with effects on more-or-less isolated plants grown in enclosure systems, instead of plants growing in the field at crop densities. To overcome these limitations of the existing data sets, we conducted three field experiments in which plants were sampled in such a way that information was obtained from plants grown at a fixed crop density, without confounding border effects.

When models contain more than two integration levels, validation of submodels which only span two integration levels is required (Chapters 3-6), especially for the development of models for the impact of air pollutants on complex systems, with which experimentation at the system level is not feasible. In addition to the experimental validation of submodels, estimates of parameters and their range may be obtained by fitting the model to observations on the system itself to avoid unrealistic predictions as much as possible. The method developed by Klepper (1989) may provide a powerful tool when the models become more complex and cannot be analytically solved.

The consequences of atmospheric pollutant deposition to forest and natural vegetation by direct as well as indirect effects on plant growth due to altered soil conditions, may be studied by combining physiological growth models for the impact of air pollutants taken up by the shoots (Chapter 9) with models for the impact of atmospheric deposition on soil processes. Several models for the effects of acidic deposition on soils (*e.g.* acidification, nutrient availability, mineral weathering of aluminum) have been developed and evaluated (van Grinsven, 1988). The most striking symptoms of forest decline are premature loss and yellowing of needles or leaves. The conceptual model, presented in Chapter 10, may serve as the link between models for physiological effects of air pollutants taken up by the shoots and models for the effects of air pollutant deposition on soil processes which determine nutrient availability and uptake. This requires experimental research on the uptake of nutrient ions by the roots in dependence of soil conditions and the metabolism of N and S compounds in the plants in relation to the regulation of intracellular pH.

Sulphur dioxide and other air pollutants may affect composition of vegetation by shifting the competitive relations between plants (Shugart & McLaughlin, 1985). When physiological responses of competing species are quantified, the effects on plant growth in communities may be modelled with existing models for species communities. These models are based upon the same principles as the models for monocultures. Competition is simulated mechanistically by distribution of the growth determining and limiting resources over the different competing species. Such models have recently been developed and evaluated with experiments on competition between crops and weeds and between trees of different age classes (Spitters & Aerts, 1983; van Gerwen, Spitters & Mohren,

1987; Kropff, 1988a, b; Spitters, 1989). This approach was recently applied to the single plant level, to permit the analysis of growth of individual plants in a canopy situation (Stokkers, Goudriaan, Kropff and den Dulk, unpublished results)

Although acute effects of air pollutants on plants in the vicinity of emission sources have been strongly reduced, the need to reduce the impact of ambient concentrations on ecosystems is great. Measures for emission reductions have to be based on thorough insight in the effects of air pollution on crops, forests and natural vegetation. This thesis has attempted to provide a basis for the development of quantitative models for the effects of atmospheric pollution on crops, forest and vegetation to estimate the impact of air pollution control measures on plant communities, where experimentation is impossible.

Summary

In industrialized areas sulphur dioxide is one of the major air pollutants that can damage vegetation. Three categories of SO_2 effects on plants that may be distinguished are acute, chronic and subtle injury. Acute injury results from short exposures (less than one day) to high SO_2 concentrations and appears as irreversible visible damage in leaf foliage (necrosis). Chronic injury (chlorotic or necrotic damage) is the result of long term exposure (days to years) to variable concentrations. Generally, older leaves are damaged followed by early abscission. Subtle effects are defined as changes in physiological and biochemical processes, causing growth reductions (or stimulation) without visible injury. Widespread concern that agricultural crops and vegetation are affected by SO_2 at sublethal concentrations is based on evidence that subtle injury may cause crop losses and influence vegetation composition. This study aimed to quantitatively explain effects of SO_2 on growth and production of crops from insight in the foliar uptake of SO_2, and the interference of its metabolites with physiological processes. To permit such an analysis, experiments at different levels of biological organization were conducted with broad bean (*Vicia faba* L., cv. Minica). This experimental work was directly coupled to the development of mechanistic models relating phenomena observed at the different levels of organization.

The major processes determining the growth rate of closed crop canopies are photosynthesis and respiration. Effects of short-term SO_2 exposures on these processes were analysed by gas exchange measurements (Chapter 2). Photosynthesis was only affected by SO_2 at high radiation levels. The level of inhibition increased rapidly after the onset of fumigation followed by a stationary state after about one hour. The relative contributions of stomatal and non-stomatal factors to photosynthetic inhibition were distinguished. This analysis showed that mesophyll resistance to CO_2 diffusion increased primarily as a result of SO_2 exposure, causing a rapid reduction in photosynthesis after commenced fumigation. Stomatal resistance was affected indirectly as a result of the decrease in net photosynthesis. Gas-exchange measurements were interpreted in biochemical terms. Effects on photosynthesis could be quantitatively explained by an inhibitory effect of SO_2 on CO_2 binding to RBP carboxylase / oxygenase. Other reported effects, *i.e.* effects on light reactions of photosynthesis, SO_2 effects on cellular pH and translocation of sugars, cannot be responsible for the observed reductions in photosynthesis by SO_2 during short exposures.

On basis of this analysis, a model was developed for the flux of atmospheric SO_2 into leaves and the effects of SO_2 metabolites (S(IV)[1] compounds) on leaf photosynthesis and stomatal resistance (Chapter 3). The model contains two parameters describing biochemical characteristics, a time coefficient for S(IV) oxidation and a parameter describing the sensitivity of photosynthesis for S(IV). The pattern of rapid photosynthetic reduction by SO_2 and rapid recovery following fumigation was accurately described with the model. A summary version of a model for SO_2 flux into leaves and effects on photosynthesis was used to estimate values for the two model parameters, by combined non-linear regression of two equations (Chapter 4). Parameter estimates agreed very well with experimentally determined values. Model results indicated that differences in photosynthetic sensitivity of plants are mainly due to differences in the rate of S(IV) oxidation and leaf thickness.

This approach was useful in explaining effects of temperature and humidity on photosynthetic depression by SO_2 (Chapter 5). Lowering the temperature by 10 °C increased photosynthetic depression by a factor 2.7. Model analysis of the data indicated that this was due to stronger accumulation of S(IV) at the lower temperatures as a result of slower S(IV) oxidation rates. Model results were confirmed by measured S(IV) concentrations. These findings may explain reported growth reductions in crops exposed to SO_2 under winter conditions, when both VPD and temperatures are low.

The effect of short-term exposure of SO_2 on canopy photosynthesis of a broad bean crop was measured using mobile equipment in the field (Chapter 6). Canopy photosynthesis was only affected at high radiation levels and was reduced by up to 6% by fumigation with 800 $\mu g\ m^{-3}$ SO_2. A model for canopy photosynthesis was extended with the submodels for SO_2 effects on leaf photosynthesis. Model performance was evaluated with experimentally obtained data. Diurnal course of canopy photosynthesis and SO_2 effects were accurately simulated by the model.

The effects of SO_2 on growth and production of broad bean crops were determined under field conditions using a newly developed open-air exposure system (Chapters 7 and 8). The treated plots were exposed to a mean SO_2 concentration of 165 $\mu g\ m^{-3}$ in 1985, 62 $\mu g\ m^{-3}$ in 1986 and 74 $\mu g\ m^{-3}$ in 1988. The background concentration was about 10 μg $SO_2\ m^{-3}$. In 1985 and 1988, the growth rate of the crop was depressed at the end of the pod filling period. Total dry matter production was reduced by 17% in 1985 and 9% in 1988, and seed yield was reduced by 23% in 1985 and 10% in 1988. This reduction was mainly due to a reduction of pod dry weight. In 1986, dry matter growth was not analysed up to the end of the growing season due to a severe infection of *Botrytis fabae* (Chocolate spot disease) in the control plot during the pod-filling period. Slight *B. fabae* infections in the control plots were also observed in 1985 and 1988. Because hardly any infections were observed in the exposed plots, our experiments indicated a suppressing effect of SO_2 on the disease. In none of the experiments significant reductions of dry matter growth have been observed in the vegetative and early reproductive phases. Plant height, number

[1] $S(IV) = [SO_2]_{aq} + [HSO_3^-] + [SO_3^{2-}]$

of internodes and number of pods were not affected by SO_2. A slight reduction in specific leaf area was observed in the SO_2 exposed plants at the end of the growing season. Leaf area development was strongly affected during the pod-filling period in 1985 and 1988 as a result of leaf injury and defoliation in the fumigated plots. N and Mg content of the different organs was unaffected by SO_2. The S content was strongly elevated in the leaves and pods of fumigated plants, while the Ca content of the leaves was reduced by SO_2. chlorophyll content of different leaf numbers was unaffected by SO_2.

In Chapter 9, the observed effects of SO_2 on growth and production of broad bean were interpreted by a mechanistic simulation model. It consisted of an elementary model for crop growth, extended with submodels for the microclimate and the submodel for foliar uptake of SO_2 and effects on leaf photosynthesis (described in Chapters 3 and 6). Direct effects of SO_2 on leaf photosynthesis explained about 10 % of the observed yield loss. A 30% increase in maintenance respiration of leaves, observed in the 1988 experiment, explained another 10% of the observed yield reduction. The major part of the observed reduction in total dry matter production was explained by chronic SO_2 injury. The effect consisted of dry matter loss through leaf abscission and a reduced growth rate due to less absorbed radiation by the canopy. Total SO_2-sulphur uptake by the fumigated crops, an important component of dry deposition of SO_2, was accurately simulated by the model.

In view of the importance of chronic effects, their mechanistic backgrounds were studied in Chapter 10 by determining the impact of SO_2 on regulation of intracellular pH. When SO_2 is not assimilated in organic compounds, but is stored as sulphate, an equivalent amount of H^+ has to be buffered. These H^+ ions are removed by metabolic processes. A conceptual model was developed to describe the processes involved. H^+ is either neutralized by OH^- ions produced in the leaves when sulphate and nitrate are assimilated in organic compounds or by OH^- ions produced by decarboxylation of organic anions (a biochemical pH stat mechanism). These organic anions are produced by a biochemical pH stat, removing OH^- from cellular solutions when sulphate and nitrate are reduced, or when differences in the uptake of non-nitrogen containing inorganic cations and inorganic anions are counterbalanced by H^+ extrusion from the roots. Nitrate-grown plants produce large amounts of OH^- in leaves as a result of nitrate reduction and therefore have a much higher capacity for H^+ removal than N_2 fixing plants like the broad bean used in this study or ammonium-grown plants. The increase of sulphate in the shoots of the SO_2 exposed broad bean (Chapters 7 and 8) was equivalent in charge to the decrease of the organic anion content which was measured as the difference between inorganic cation content (C) and inorganic anion content (A). It is proposed that the metabolic buffering capacity of leaf cells is related to the reduction of sulphate and nitrate and the import of organic anions, rather than to the organic anion content in the vacuoles of the leaf cells. The appearance of chronic SO_2 injury (leaf damage) in the field experiment at the end of the growing period is discussed in relation to the impact of SO_2 on the processes involved in regulation of intracellular pH. Further research at the cellular level is required for a

quantitative understanding of these chronic effects.

In Chapter 11 the results of this study are discussed in view of the development of mechanistic models for estimation of the impact of air pollutants on crops, forests and (semi) natural vegetation.

Samenvatting

In geindustrialiseerde gebieden is zwaveldioxide (SO_2) een van de belangrijkste luchtverontreinigingscomponenten die schade kunnen veroorzaken aan gewassen en vegetaties. Drie soorten SO_2 effecten kunnen worden onderscheiden: acute schade, chronische schade en effecten op de groei van planten zonder dat er zichtbare schade optreedt. Acute schade wordt veroorzaakt door korte blootstelling van planten aan hoge concentraties en wordt gekarakteriseerd door necrotische vlekken op de (meestal jongste volgroeide) bladeren. Chronische schade is het gevolg van langdurige blootstelling aan relatief lage concentraties en wordt hier gedefiniëerd als zichtbare necrotische schade aan de oudere bladeren. Vaak wordt gesproken van een versnelde veroudering.

De laatste decennia is er veel experimenteel onderzoek verricht naar de effecten van SO_2 op planten. Weinig aandacht is er besteed aan de kwantitatieve interpretatie van de waargenomen effecten met mechanistische modellen. Met dergelijke modellen kunnen de effecten op gewassen en vegetaties worden gekwantificeerd op grond van kennis over de effecten op procesnivo. Na uitgebreide validatie kunnen deze modellen worden gebruikt om de effectiviteit van maatregelen voor emissiereductie te voorspellen. Vooral voor complexe systemen als bossen en vegetaties, waarvoor de invloed van luchtverontreiniging experimenteel niet is vast te stellen, kunnen deze modellen gebruikt worden om effecten die op procesnivo zijn waargenomen te integreren teneinde de invloed van luchtverontreiniging op het systeem te kunnen kwantificeren. Het in dit proefschrift beschreven onderzoek was gericht op het verkrijgen van kwantitatief inzicht in de manier waarop SO_2 de groei van planten beïnvloedt. Teneinde het vereiste experimentele en modelmatige onderzoek op de verschillende integratienivo's (van subcellulair nivo tot gewas of vegetatienivo) mogelijk te maken is het onderzoek naar de invloed van SO_2 uitgevoerd met een landbouwgewas (tuinbonen), groeiend onder potentiële productieomstandigheden. Het experimentele werk op de verschillende integratienivo's was direct gekoppeld aan de ontwikkeling van modellen waarmee de waargenomen verschijnselen op de verschillende nivo's met elkaar in verband gebracht kunnen worden.

De belangrijkste fysiologische processen die de groeisnelheid van gewassen bepalen zijn de fotosynthese en de respiratie. Effecten van korte-termijn blootstellingen aan SO_2 op deze processen zijn geanalyseerd door de gasuitwisseling van bladeren te meten (Hoofdstuk 2). Fotosynthese werd alleen beïnvloed door SO_2 bij hoge lichtnivo's. Na de start van de begassing daalde de fotosynthese snel tot er een stationair nivo bereikt werd (binnen een uur). Door het verloop van de fotosynthese en de interne CO_2 concentratie te

volgen tijdens de korte begassingsperiodes, kon worden vastgesteld dat de opgenomen SO_2 een direct effect heeft op de fotosynthese en dat de huidmondjes sluiten in reactie op de daling van de fotosynthese. Door de resultaten van de gaswisselingsmetingen op biochemisch nivo te interpreteren werd vastgesteld dat de daling van de fotosynthese wordt veroorzaakt door een competitive remming door SO_2 van CO_2 binding aan het CO_2 bindend enzym. Andere effecten die in de literatuur worden genoemd, zoals een pH daling en effecten op de translocatie van suikers kunnen niet verantwoordelijk zijn voor de gevonden reducties in fotosynthese tijdens korte blootstellingen van bladeren aan SO_2. Op grond van deze analyse werd een model ontwikkeld voor de opname van SO_2 door bladeren, het metabolisme van SO_2 en de effecten van SO_2 metabolieten (S(IV)[1]) op de fotosynthese en stomataire geleidbaarheid (Hoofdstuk 3). Het model bevat slechts 2 parameters die de biochemische processen karakteriseren: een tijdscoëfficiënt voor de oxidatie van S(IV) en een parameter die de gevoeligheid van de fotosynthese voor S(IV) beschrijft. Het patroon van een snelle daling in fotosynthese na de start van de begassing, gevolgd door stabilisatie en een snel herstel na het beëindigen van de begassing, werd door het model nauwkeurig beschreven. Door het model analytisch op te lossen en te fitten op gegevens van vele gaswisselingsexperimenten, werden waarden voor de 2 parameters geschat (Hoofdstuk 4). Deze waarden kwamen goed overeen met de parameterwaarden die uit literatuurgegevens konden worden afgeleid. De analyse in Hoofdstuk 4 geeft aan dat verschillen in gevoeligheid tussen planten vooral veroorzaakt worden door variatie in de tijdscoëfficiënt voor S(IV) oxidatie en waarschijnlijk ook door verschillen in bladdikte.

Deze benadering (het schatten van biochemische karakteristieken uit de resultaten van eenvoudig uit te voeren gaswisselingsmetingen) bleek zeer waardevol bij de verwerking en interpretatie van gegevens uit experimenten waarin de invloed van temperatuur en luchtvochtigheid op de gevoeligheid van de fotosynthese voor SO_2 werd bestudeerd (Hoofdstuk 5). Een toename van de luchtvochtigheid bleek een opening van de huidmondjes te veroorzaken, waardoor de opnamesnelheid van SO_2 toenam, wat leidde tot een sterkere remming van de fotosynthese. Het verlagen van de temperatuur met 10 °C leidde tot een toename van de fotosynthese reductie met een factor 2,7. Uit de modelanalyse bleek dat dit volledig was te verklaren door de sterkere accumulatie van S(IV) bij de lagere temperatuur, die wordt veroorzaakt door een trager verlopende oxidatiesnelheid van S(IV). Deze theoretische interpretatie werd bevestigd door metingen van de S(IV) concentratie. Deze resultaten kunnen mogelijk de door diverse onderzoekers gevonden groeireducties in gewassen in de winter en het vroege voorjaar verklaren, aangezien de temperatuur dan laag is en de vochtigheid hoog.

Het effect van korte SO_2 blootstellingen op de fotosynthese van een gewas tuinbonen werd gemeten met mobiele apparatuur in het veld (Hoofdstuk 6). De gewasfotosynthese werd slechts gereduceerd bij een hoge lichtintensiteit (tot 6% reductie bij 800 $\mu g\ m^{-3}\ SO_2$). Een model voor de invloed van SO_2 op de fotosynthese van gewassen werd opgesteld, door bestaande modellen voor de fotosynthese van gewassen uit te

[1] $S(IV) = [SO_2]_{aq} + [HSO_3^-] + [SO_3^{2-}]$

breiden met de submodellen voor de opname en effecten van SO_2 op de bladfotosynthese. Het verloop van de fotosynthese tijdens en na begassing met SO_2 werd bevredigend gesimuleerd met het model.

De invloed van SO_2 op de groei en productie van een veldgewas tuinbonen werd geanalyseerd met een nieuw ontwikkeld veldbegassingssysteem (Hoofdstukken 7 en 8). De behandelde veldjes werden blootgesteld aan 165 $\mu g\ m^{-3}$ SO_2 in 1985, 62 $\mu g\ m^{-3}$ SO_2 in 1986 en 74 $\mu g\ m^{-3}$ SO_2 in 1988 (seizoensgemiddelde). De achtergrondsconcentratie was ongeveer 10 $\mu g\ m^{-3}$ SO_2. In 1985 en 1988 werd de groeisnelheid van de gewassen gereduceerd tegen het eind van de groeiperiode. De reductie in totale droge stof bedroeg 17% in 1985 en 9% in 1988. De zaadopbrengst werd gereduceerd met 23% in 1985 en 10% in 1988. Het meest in het oog springende effect was de chronische schade die werd waargenomen. Aan het eind van de peulvullingsfase was het bladoppervlak sterk gereduceerd in de begaste veldjes door bladschade aan de oudste bladeren, gevolgd door bladval. In 1986 werd de groei van de gewassen niet gevolgd tot aan de afrijping, omdat het controle veld sterk geïnfecteerd was met *Botrytis*. In 1985 en 1988 werden ook lichte infecties met *Botrytis* in de controle veldjes waargenomen. Omdat er nauwelijks sprake was van aantastingen in de begaste veldjes, wijzen deze gegevens op een remmende invloed van SO_2 op de *Botrytis* aantasting. Groeireducties in de vegetatieve periode en vroege generatieve periode werden niet waargenomen. Planthoogte, aantal internodiën en aantal peulen werden niet beïnvloed. Een lichte reductie in het specifiek bladoppervlak werd waargenomen in begaste planten aan het eind van het groeiseizoen. Het gehalte aan N en Mg werd niet beïnvloed door SO_2. Het zwavelgehalte was echter sterk verhoogd in de begaste gewassen, terwijl het Ca gehalte in de bladeren van de begaste planten verlaagd was. Het chlorofyl gehalte werd niet beïnvloed.

In Hoofdstuk 9 worden de waargenomen effecten van SO_2 op de gewasgroei geïnterpreteerd met een mechanistisch simulatiemodel, bestaande uit een gewasgroeimodel uitgebreid met de submodellen voor de opname en effecten van SO_2 op de bladfotosynthese. Directe effecten van SO_2 op de fotosynthese verklaarden slechts 10% van de waargenomen groeireducties. Een verhoging van de onderhoudsrespiratie van de bladeren met 30% (waargenomen in 1988) verklaarde ook ongeveer 10% van de waargenomen opbrengstverliezen. Het grootste deel van de groeireductie bleek veroorzaakt door chronische schade (zichtbare schade aan de oudste bladeren), bestaande uit twee componenten: (*i*) het verlies aan gewicht door bladval en (*ii*) een gereduceerde lichtabsorptie door een afgenomen bladoppervlak, waardoor de gewasfotosynthese werd gereduceerd. De opname van zwavel door de gewassen, een belangrijk onderdeel van de droge depositie van SO_2, werd goed gesimuleerd met het model.

Omdat met name de chronische effecten verantwoordelijk bleken voor de gevonden opbrengstverliezen, is getracht hiervoor een fysiologische verklaring te vinden in Hoofdstuk 10, door de invloed van SO_2 op de regulatie van de intracellulaire pH te bestuderen. Verschillende onderzoekers hebben een relatie gevonden tussen bladschade en de intracellulaire pH. Als SO_2 niet wordt geassimileerd in organische verbindingen hoopt zich sulfaat op, waarbij een equivalente hoeveelheid H^+ ionen gebufferd moet worden. Deze H^+ ionen worden gebufferd door metabolische processen. Een conceptueel model voor de betrokken processen is opgesteld op grond van bestaande inzichten. H^+ wordt of geneutraliseerd door OH^- ionen, die gevormd worden bij de assimilatie van sulfaat en nitraat in organische verbindingen, of door de decarboxylatie van organische anionen via het zogenaamde pH stat mechanisme. Deze organische anionen zijn gevormd door hetzelfde pH stat mechanisme om OH^- ionen weg te werken die vrijkomen in de cellen van spruit en wortel bij de assimilatie van sulfaat en nitraat in organische verbindingen, of in de wortel als er H^+ wordt uitgescheiden om electroneutraliteit te handhaven bij een ongelijke opname van cationen en anionen. Planten die gevoed worden met nitraat, produceren veel OH^- ionen in de bladeren tijdens de assimilatie en hebben dus een veel grotere metabolische buffercapaciteit dan planten die met ammonium gevoed worden, of planten die atmosferische stikstof binden (zoals tuinbonen). Het is door anderen inderdaad aangetoond dat planten die met ammonium gevoed worden veel gevoeliger zijn voor chronische SO_2 schade dan planten die met nitraat gevoed worden.

De hoeveelheid geaccumuleerd sulfaat in de begaste planten uit de veldbegassingsexperimenten (Hoofdstukken 7 en 8) was equivalent aan de afname in organische anionen. Het organisch anion gehalte werd bepaald via het verschil in anorganische cationen en anionen. De hoeveelheid organische anionen in de bladeren (een vermeende maat voor de metabolische buffercapaciteit van de plant) was nog zeer hoog in beschadigde bladeren. Hieruit werd de conclusie getrokken dat als de waargenomen chronische effecten inderdaad het gevolg zijn van effecten op de regulatie van de cellulaire pH op lange termijn, de metabolische buffercapaciteit gekoppeld moet zijn aan de import snelheid van organische anionen in het cytoplasma en aan de snelheid van sulfaat en nitraat assimilatie en niet aan de hoeveelheid organische anionen in de cel (vooral in de vacuole). Mechanismen voor de waargenomen chronische effecten in de veldexperimenten worden bediscussiëerd in relatie tot de regulatie van intracellulaire pH. Nieuw onderzoek op subcellulair nivo is nodig om de vele vragen met betrekking tot chronische effecten van SO_2 te kunnen beantwoorden. Het conceptuele model biedt de mogelijkheid om de relatie tussen effecten van diverse N en S houdende luchtverontreinigingscomponenten na opname via de bladeren te koppelen aan de opname van nutrient ionen door de wortel. Hierdoor wordt het wellicht mogelijk modellen voor de groei van gewassen, bossen en vegetaties te koppelen aan modellen die effecten van luchtverontreiniging op bodemprocessen simuleren.

In Hoofdstuk 11 worden de resultaten van het onderzoek in een breder kader geplaatst en wordt er uitgebreid ingegaan op methodologische problemen bij experimenten naar de invloed van luchtverontreiniging op gewassen en vegetaties. Konkurrentie tussen

planten kan een belangrijke oorzaak zijn van de vaak tegenstrijdige onderzoeksresultaten m.b.t. de effecten van luchtverontreiniging op de groei van planten. Om inzicht te krijgen in de effecten van luchtverontreiniging op meersoortige vegetaties dienen konkurrentie-aspecten expliciet in het onderzoek te worden bestudeerd. Tenslotte worden perspectieven geschetst voor de ontwikkeling van mechanistische kwantitatieve modellen voor de invloed van luchtverontreiniging op gewassen, bossen en andere vegetaties.

References

Agrawal, , M., Nandi, P.K. & Rao, D.N., 1985. Effects of sulphur dioxide fumigation on soil systems and growth behaviour of *Vicia faba* plants. Plant and Soil, 86: 69-78.

Allen, S. & Raven, J.A., 1987. Intracellular pH regulation in *Ricinus communis* grown with ammonium or nitrate as N source: the role of long distance transport. Journal of Experimental Botany 38: 580-596.

Alscher, R., Bower, J.L. & Zipfel, W., 1987. The basis for different sensitivities of photosynthesis to SO_2 in two cultivars of pea. Journal of Experimental Botany 38: 99-108.

Alvey, N., Galwey, N. & Lane, P., 1982. An introduction to Genstat. Academic press, London, New York, 152 pp.

Anbazhagan, M., Krishnamurthy, R. & Bhagwat, K.A., 1988. Proline: an enigmatic indicator of air pollution tolerance in rice cultivars. Journal of Plant Physiology 133: 122-123.

Anonymus, 1988. Luchtkwaliteit. Jaarverslag 1986. Rapport nr. 228703002, Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven. (in Dutch)

Arnon, D.I., 1939. Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. Soil Science 48: 295-307.

Asada, K. & Kiso, K., 1973. Initiation of aerobic oxidation of sulphite by illuminated spinach chloroplasts. European Journal of Biochemistry 33: 253-257.

Ashenden, T.W. & Mansfield, T.A., 1977. Influence of wind speed on the sensitivity of ryegrass to SO_2. Journal of Experimental Botany 28: 729-735.

Ashenden, T.W. & Mansfield T.A., 1978. Extreme pollution sensitivity of grasses when SO_2 and NO_2 are present in the atmosphere together. Nature 273: 142-143.

Baker, C.K., Unsworth, M.H. & Greenwood, P., 1982. Leaf injury on wheat plants exposed in the field in winter to SO_2. Nature 299: 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.

Baker, C.K., Fullwood, A.E. & Colls, J.J., 1987. Tillering and leaf area of winter barley exposed to sulphur dioxide in the field. New Phytologist 107: 373-385.

Barton, J.R., McLaughlin, S.B. & McConathy, R.K., 1980. The effects of SO_2 on components of leaf resistance to gas exchange. Environmental Pollution (series A) 21: 255-265.

Bell, C.J., 1982. A model of stomatal control. Photosynthetica 16: 486-495.

Bell, J.N.B., 1985. SO_2 effects on the productivity of grass species. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.), Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 209-226.

Bell, J.N.B. & Clough, W.S., 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.
Ben Zioni, A., Vaadia, Y. & Lips, S.H., 1971. Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiologia Plantarum 24: 288-290.
Bennett, J.H. & Hill, A.C., 1973. Inhibition of apparent photosynthesis by air pollutants. Journal of Environmental Quality 2: 526-530.
Beusichem, M.L. van, 1981. Nutrient absorption by pea plants during dinitrogen fixation. 1. Comparision with nitrate nutrition. Netherlands Journal of Agricultural Science 30: 317-33.
Beusichem, M.L. van, 1983. Xylary charge distribution and nitrogen transport in *Pisum sativum* L. during dinitrogen fixation or nitrate nutrition. Zeitschrift für Pflanzenfysiologie 109: 449-458.
Beusichem, M.L. van, 1984. Non-ionic nitrogen nutrition of plants. Ph.D. thesis, Agricultural University, Wageningen, 141 pp.
Beusichem, M.L. van, Kirkby, E.A. & Baas, R., 1988a. Influence of nitrate and ammonium nutrition on the uptake, assimilation and distribution of nutrients in *Ricinus communis*. Plant Physiology 86: 914-921.
Beusichem, M.L. van, Nelemans, J.A. & Bienfait, H.F., 1988b. Interrelationships between trans-plasma membrane electron/proton transfer stoichiometry, organic acid metabolism, and nitrate reduction in Dwarf Bean (*Phaseolus vulgaris*). Plant Physiology 87: 269-273.
Beusichem, M.L. van & Nelemans, J.A., 1989. Cation-anion uptake balance, root nitrate reductase and nitrogenase activity in nodulated pea plants in relation to NO_3^- supply. *In:* Proceedings Internationnal Congress of Plant Physiology, New Delhi 1988, in press.
Black, C.R. & Black, V.J., 1979. The effects of low concentrations of sulphur dioxide on stomatal conductance and epidermal cell survival in field bean (*Vicia faba* L.). Journal of Experimental Botany 30: 291-298.
Black, V.J., 1982. Effects of sulphur dioxide on physiological processes in plants. *In*: Unsworth, M.H. & Ormrod, D.P. (Eds.), Effects of gaseous air pollution in Agriculture and Horticulture. Butterworths, London, pp. 67-91.
Black, V.J., 1984. The effect of air pollution on apparent respiration. *In*: Koziol, M.J. & Whatley, F.R. (Eds.), Gaseous air pollutants and plant metabolism. Butterworths, London, pp. 231-248.
Black, V.J. & Unsworth, M.H., 1979a. A system for measuring effects of sulphur dioxide on gas exchange of plants. Journal of Experimental Botany 30: 81-88.
Black, V.J. & Unsworth, M.H., 1979b. Effects of low concentrations of sulphur dioxide on net photosynthesis and dark respiration. Journal of Experimental Botany 30: 473-483.
Black, V.J. & Unsworth, M.H., 1979c. Resistance analysis of sulphur dioxide fluxes to *Vicia faba*. Nature 282: 68-69.
Black, V.J. & Unsworth, M.H., 1980. Stomatal responses to sulphur dioxide and vapor pressure deficit. Journal of Experimental Botany 31: 667-677.
Bleasdale, J.K.A., 1952. Atmospheric pollution and plant growth. Nature 169: 367-377.
Brisley, H.R., Davis, C.R. & Booth, J.A., 1959. Sulphur dioxide fumigation of cotton with special reference to its effect on yield. Agronomy Journal 51: 77-80.
Bruinsma, J., 1963. The quantitative analysis of chlorophylls a and b in plant extracts.

Photochemistry and Photobiology 2: 241-249.
Brunold, C., Landolt, W. & Lavanchy, P., 1983. SO_2 and assimilatory sulfate reduction in beech leaves. Physiologia Plantarum 59: 313-318.
Bytnerowycz, 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. Agriculture, Ecosystems and Environment 20: 37-47.
Cape, J.N., 1984. The importance of solution equilibria in studying the effects of sulphite in plants. Environmental Pollution (series A) (series A) 34: 259-274.
Carlson, R.W., 1983. The effect of SO_2 on photosynthesis and leaf resistance at varying concentrations of CO_2. Environmental Pollution (series A) 30: 309-321.
Challa, H., 1976. An analysis of the diurnal course of growth, carbon dioxide exchange and carbohydrate reserve content of cucumber. Ph.D. thesis, Agricultural University, Wageningen, 88 pp.
Clarke, A.G. & Radojevic, M., 1987. Oxidation of SO_2 in rainwater and its role in acid rain chemistry. Atmospheric Environment 21: 1115-1123.
Coleman, R.G. & Richards, F.J., 1956. Physiological studies in plant nutrition. XVIII. Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. Annals of Botany 20: 393-409.
Colvill, K.E., Horsman, D.C., Roose, M.L., Roberts, T.M. & Bradshaw, A.D., 1985. Field trials on the influence of air pollutants, and sulphur dioxide in particular, on the growth of ryegrass (*Lolium perenne* L.). Environmental Pollution (series A) 39: 235-266.
Cowling, D.W. & Bristow, A.W., 1979. Effects of SO_2 on sulphur and nitrogen fractions and on free amino acids in perennial ryegrass. Journal Sci. Food Agric. 30: 354-360.
Cowling, D.W. & Lockyer, D.R., 1978. The effect of SO_2 on *Lolium perenne* L. grown at different levels of sulphur and nitrogen nutrition. Journal of Experimental Botany 29: 257-265.
Cowling, D.W. & Koziol, M.J., 1982. Mineral nutrition and plant response to air pollutants. *In*: Unsworth, M.H. & Ormrod, D.P. (Eds.), Effects of gaseous air pollution in agriculture and horticulture. Butterworths, London, pp. 349-375.
Cowling, D.W., Jones, L.H.P. & Lockyer, D.R., 1973. Sulphur dioxide increased yield through correction of sulphur deficiency in ryegrass exposed to sulphur dioxide. Nature 243: 479-480.
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.
Darrall, N.M. & Jäger, H.-J., 1984. Biochemical diagnostic tests for the effect of air pollution on plants. *In*: Koziol, M.L. & Whatley, F.R. (Eds.), Gaseous air pollutants and plant metabolism. Butterworths, London, Wellington, pp. 333-349.
Davies, D.D., 1973. Metabolic control in higher plants *In*: Milborrow, B.V. (Ed.), Biosynthesis and its control in plants. Academic press, New York, pp. 1-20.
Davies, D.D., 1986. The fine control of cytosolic pH. Physiologia Plantarum 67: 702-706.
Davies, T., 1980. Grasses more sensitive to SO_2 pollution in conditions of low irradiance

and short days. Nature 284: 483-485.
Davis, C.R., 1972. Sulphur dioxide fumigation on soybeans: effect on yield. Journal of the Air Pollution Control Association 22: 964-966.
Dijkshoorn, W., 1962. Metabolic regulation of the alkaline effect of nitrate utilization in plants. Nature 194: 165-167.
Dijkshoorn, W. & van Wijk, A.L., 1967. The sulpur requirements of plants as evidenced by the sulphur - nitrogen ratio in the organic matter: a review of published data. Plant and Soil 26: 129-157.
Dijkshoorn, W., Lathwell, D.J. & de Wit, C.T., 1968. Temporal changes in carboxylate content of ryegrass with stepwise changes in nutrition. Plant and Soil 29: 369-390.
Eaton, F., Olmstead, W.R. & Taylor, O.C., 1971. Salt injury to plants with special reference to cations versus anions and ion activities. Plant and Soil 35: 533-547.
Edwards, G. & Walker, D.A., 1983. C_3, C_4: mechanisms, and cellular and environmental regulation of photosynthesis. Blackwell Scientific Publications, Oxford, London, 542 pp.
Egmond, F. van, 1975. The ionic balance of the sugarbeet plant. Ph.D. thesis, Agricultural University, Wageningen, 70 pp.
Faller, N., Herwig, K. & Kuhn, H., 1970. Die aufnahme von schwefeldioxid aus der luft. 1. Einfluss auf den pflanzlichen ertrag. Plant and Soil 33: 177-191.
Farquhar, G.D. & Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33: 317-345.
Flores, H.E. & Galston, A.W., 1984. Osmotic stress-induced polyamine accumulation in cereal leaves. Plant Physiology 75: 102-109.
Fowler, D. & Cape, J.N., 1982. Air pollutants in agriculture and horticulture. *In*: Unsworth, M.H. & Ormrod, D.P. (Eds.), Effects of gaseous air pollutants in agriculture and horticulture. Butterworth, London, pp. 1-26.
Furukawa, A., Natori, T. & Totsuka, T., 1980. The effect of SO_2 on net photosynthesis in sunflower leaf. Studies on the effect of air pollutants on plants and mechanisms of phytotoxicity. Research Reports from the N.I.E.S. 11: 1-8.
Gaastra, P., 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Mededelingen van de Landbouw Hogeschool te Wageningen 59-13.
Garsed, S.G., 1984. Uptake and distribution of pollutants in the plant and residence time of active species. *In*: Koziol, M.J. & Whatley, F.R. (Eds.), Gaseous air pollutants and plant metabolism. Butterworths, London, Wellington, pp. 83-103.
Garsed, S.G., 1985. SO_2 uptake and transport. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.). Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 75-95.
Garsed, S.G. & Read, D.J., 1977. Sulphur dioxide metabolism in soybean (*Glycine max*. var. biloxi). I. The effects of light and dark on the uptake and translocation of $^{35}SO_2$ products. New Phytologist 79: 583-92.
Gasch, G., Grünhage, L., Jäger, H.-J. & Wentzel, K.-F., 1988. Das verhältnis der schwefelfractionen in fichtennadeln als indikator für immissionsbelastungen durch schwefeldioxid. Angewandte Botanik 62: 73-84.
Gerwen, C.P. van, Spitters, C.J.T. & Mohren, G.M.J., 1987. Simulation of competition for light in even-aged stands of Douglas fir. Forest, Ecology and Management 18: 135-152.
Gezelius, K. & Hållgren, J.-E., 1980. Effects of SO_3^{2-} on the activity of ribulose bi-

phosphate carboxylase from seedlings of Pinus silvestris. Physiologia Plantarum 49: 354-358.
Goudriaan, J., 1977. Crop micrometeorology: a simulation study. Simulation Monographs, Pudoc, Wageningen, 249 pp.
Goudriaan, J., 1982. Potential production processes. *In*: Penning de Vries, F.W.T. & van Laar, H.H. (Eds.). Simulation of plant growth and crop production. Simulation Monographs, Pudoc, Wageningen, pp 98-113.
Goudriaan, J., 1986. A simple and fast numerical method for the calculation of daily totals of crop photosynthesis. Agricultural and Forest Meteorology 38: 251-255.
Goudriaan, J., 1988. The bare bones of leaf angle distribution in radiation models for canopy photosynthesis and energy exchange. Agricultural and Forest Meteorology 43: 155-169.
Goudriaan, J. & van Laar, H.H., 1978. Relations between leaf resistance, CO_2-concentration and CO_2-assimilation in maize, beans, lalang grass and sunflower. Photosynthetica 12: 241-249.
Grashoff, C., Klein Hulze, J.A. & Smid, H.G., 1987. Opbrengstvariabiliteit bij veldbonen en erwten. CABO publikatie 435.
Greenwood, P., Greenhalgh, A., Baker, C. & Unsworth, M.H., 1982. A computer-controlled system for exposing field crops to gaseous air pollutants. Atmospheric Environment 16: 2261-2266.
Grill, D., 1971. Die puffercapazitat gesunder und rauchgeschadigter fichtennadeln. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 78: 612-622.
Grill, D. & Esterbauer, H., 1973. Cystein und glutathion in gesunden und SO_2 geschadigten fichtennadeln. European Journal of Forest Pathology 3: 65-71.
Grill, D., Esterbauer, H. & Klotsch, U., 1979. Effect of sulfur dioxide on glutathione in leaves of plants. Environmental Pollution (series A) 19: 187-194.
Grinsven, H. van, 1988. Impact of atmospheric deposition on soils. Quantification of chemical and hydrologic processes. Ph.D. thesis, Agricultural University, Wageningen, 215 pp.
Guderian, R., 1977. Air Pollution. Springer Verlag, Berlin, Heidelberg, New York, 127 pp.
Hållgren, J.-E., 1978. Physiological and biochemical effects of sulphur dioxide on plants. *In*: Nriagu, I.O. (Ed.)., Sulphur in the environment. Part II: Ecological impacts, Wiley, New York, pp. 163-209.
Hållgren, J.-E., 1984. Photosynthetic gas exchange in leaves affected by air pollutants. *In*: Koziol, M.J. & Whatley, F.R. (Eds.), Gaseous air pollutants and plant metabolism, Butterworths, London, pp. 147-159.
Hållgren, J.-E. & Fredriksson, S., 1982. Emission of hydrogen sulfide from sulfur dioxide fumigated pine trees. Plant Physiology 70: 456-459.
Hållgren, J.-E. & Gezelius, K., 1982. Effects of SO_2 on photosynthesis and Ribulose biphosphate carboxylase in pine trees seedlings. Physiologia Plantarum 54: 153-161.
Hållgren, J.-E., Linder, S., Richter, A., Troeng, E. & Granat, L., 1982. Uptake of SO_2 in shoots of scots pine: field measurements of net flux of sulphur in relation to stomatal conductance. Plant, Cell and Environment 5: 75-83.
Heagle, A.S., Body, D.E. & Neely, G.E., 1974. Injury and yield responses of soybean to chronic doses of ozone and sulfur dioxide in the field. Phytopathology 64: 132-136.

Heggestad, H.E., Bennet, J.H., Lee, E.H. & Douglas, L.W., 1986. Effects of increasing doses of sulfur dioxide and ambient ozone on tomatoes: Plant growth, leaf injury, elemental composition, fruit yields, and quality. Phytopathology 76: 1338-1344.

Houba, V.F.G., van Egmond, F. & Wittich, E.M., 1971. Changes in production of organic nitrogen and carboxylates (C-A) in young sugarbeet plants grown in nutrient solutions of different nitrogen composition. Netherlands Journal of Agricultural Science 19: 39-47.

Hove, L.W.A. van, Tonk, W.J.M., Pieters, G.A., Adema, E.H. & Vredenberg, W.J., 1988. A leaf chamber for measuring the uptake of pollutant gases at low concentrations by leaves, transpiration and carbon dioxide assimilation. Atmospheric Environment 22: 2515-2523.

Hove, L.W.A. van, Adema, E.H., Vredenberg, W.J. & Pieters, G.A., 1989. A study of the adsorption of NH_3 and SO_2 on leaf surfaces. Atmospheric Environment (in press).

Hüber, K., Esterbauer, H., Jäger, H.-J. & Grill, D., 1987. Detection of sulphite in plants. Environmental Pollution (series A) 46: 127-136.

Ibusuki, T. & Takeuchi, K., 1987. Sulfur dioxide oxidation by oxygen catalyzed by mixtures of manganese (III) in aqueous solutions at environmental reaction conditions. Atmospheric Environment 21: 1555-1560.

Irving, P.M. & Miller, J.E., 1984. Synergistic effect on field-grown soybeans from combinations of sulfur dioxide and nitrogen dioxide. Canadian Journal of Botany 62: 840-846.

Israel D.W. & Jackson, W.A., 1982. Ion balance, uptake, and transport processes in N_2-fixing and nitrate- and urea-dependent soybean plants. Plant Physiology 69: 171-178.

Jäger, H.-J. & Klein, H., 1976. Modellversuche zum einfluss der nahrstoffversorgung auf die SO_2 empfindlichkeit von pflanzen. European Journal of Forest Pathology 6: 347-354.

Jäger, H.-J. & Klein, H., 1977. Biochemical and physiological detection of sulfur dioxide injury to pea plants (*Pisum sativum*). Journal of the Air Pollution Control Association 27: 464-466.

Jäger, H.-J. & Klein, H., 1980. Biochemical and physiological effects of SO_2 on plants. Angewandte Botanik 54: 337-348.

Jäger, H.-J., Bender, J. & Grünhage, L., 1985. Metabolic responses of plants differing in SO_2 sensitivity towards SO_2 fumigation. Environmental Pollution (Series A) 39: 317-335.

Jensen, K.F. & Noble, R.D., 1984. Impacts of ozone and sulphur dioxide on net photosynthesis of hybrid poplar cuttings. Canadian Journal of Forest Research 14: 385-388.

Jones, H.G., 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. Plant, Cell and Environment 8: 95-104.

Jones, T. & Mansfield, T.A., 1982. Studies on dry matter partitioning and distribution of ^{14}C-labelled assimilates in plants of *Phleum pratense* exposed to SO_2 pollution. Environmental Pollution (series A) 28: 199-207.

Kellog, W.W., Cadle, R.D., Allen, E.R., Lazarus, A.L. & Martell, E.A., 1972. The sulfur cycle. Science 175: 587-596.

Kercher, J.R., 1978. A model of leaf photosynthesis and the effects of simple gaseous sulfur compounds (H_2S and SO_2). URCL-52643. Laurence Livermore National

Laboratory, Livermore, California, 37 pp.
Kercher, J.R. & King, D.A., 1985. Modelling effects of SO_2 on the productivity and growth of plants. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.), Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 357-374.
Khan, A.A. & Malhotra, S.S., 1982. Ribulose biphosphate carboxylase and glycolate oxidase from Jack pine: effects of sulphur dioxide fumigation. Phytochemistry 21: 2607-2612.
Klarer, C.I., Reinert, R.A. & Huang, J.S. 1984. Effects of sulfur dioxide and nitrogen dioxide on vegetative growth of soybean. Phytopathology 74: 1104-1106.
Klein, H. & Jäger, H.-J., 1976. Einfluss der nahrstoffversorgung auf die SO_2-empfind lichkeit von erbsenpflanzen. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 83: 555-568.
Klepper, O., 1989. A model for the carbon flows in relation to macrobenthic food supply in the Oosterschelde estuary (SW Netherlands). Ph.D. thesis, Agricultural University, Wageningen, in press.
Kohut, R., 1985. The effects of SO_2 and O_3 on plants. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.), Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 296-312.
Kondo, N., Akiyama, Y., Fujiwara, M. & Sugahara, K., 1980. Sulfite oxidizing activities in plants. Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. Research Report of the National Institute for Environmental Studies 11: 127-136.
Koziol, M.J. & Whatley, F.R. (Eds.), 1984. Gaseous air pollutants and plant metabolism. Butterworths, London, Wellington.
Krause, G.H., 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiologia Plantarium 74: 566-574.
Kress, L.W., Miller, J.E. & Smith, H.J., 1986. Impact of ozone and sulphur dioxide on soybean yield. Environmental Pollution (Series A) 41: 105-123.
Kropff, M.J., 1987. Physiological effects of sulphur dioxide. 1. The effect of SO_2 on photosynthesis and stomatal regulation of *Vicia faba* L. Plant, Cell and Environment 10: 753-760.
Kropff, M.J., 1988a. Modelling the effects of weeds on crop production. Weed Research 28: 465-471.
Kropff, M.J., 1988b. Simulation of crop weed competition. *In:* Cavalloro, R. & El Titi, A. (Eds.), Weed control in vegetable production. Proceedings of the EC expert's group. Stuttgart, 1986. Balkema, Rotterdam.
Krupa, S. & Kickert, R.N., 1987. An analysis of numerical models of air pollutant exposure and vegetation. Environmental Pollution (series A) 44: 127-158.
Kyle, D.J. & Ohad, I., 1986. The mechanism of photoinhibition in higher plants and greenalgae. *In*: Staehelin, L.A. & Arntzen, C.J. (Eds.), Encyclopedia of Plant Physiology, New Series 19. Springer-Verlag, Berlin, pp. 468-475.
Laing, W.A., Ogren, W.L. & Hageman, R.H., 1974. Regulation of soybean net photosynthesis CO_2 fixation by the interaction of CO_2, O_2 and ribulose 1,5 biphosphate carboxylase. Plant Physiology 54: 678-685.
Laisk, A., Pfanz, H., Schramm, M.J. & Heber, U., 1988a. Sulphur-dioxide fluxes into different cellular compartments of leaves photosynthesizing in a polluted atmosphere.

I. Computer analysis. Planta 173: 230-240.
Laisk, A., Pfanz, H. & Heber, U., 1988b. Sulphur-dioxide fluxes into different cellular compartments of leaves photosynthesizing in a polluted atmosphere. II. Consequences of SO_2 uptake as revealed by computer analysis. Planta 173: 241-252.
Legge, A.H., Bogner, J.C. & Krupa, S.V., 1988. Foliar sulphur species in Pine: a new indicator of a forest ecosystem under air pollution stress. Environmental Pollution (Series A) 55: 15-27.
Linzon, S.N., 1978. Effects of airborne sulphur pollutants on plants. *In*: Nriagu, I.O. (Ed.), Sulphur in the environment, part II: Ecological impacts, Wiley, New York, pp. 109-162.
Lockyer, D.R., Cowling, D.W. & Jones, H.P., 1976. A system for exposing plants to atmospheres containing low concentrations of sulphur dioxide. Journal of Experimental Botany 27: 387-409.
Lorenc-Plucinska, G., 1986. Effect of sulphur dioxide on the partitioning of assimilates of Scots pine (*Pinus sylvestris* L.) seedlings of different susceptibility to this gas. European Journal of Plant Pathology 16: 266-273.
Losch, R. & Tenhunen, J.D., 1981. Stomatal responses to humidity - phenomenon and mechanism. *In*: Jason, P.G. & Mansfield, T.A. (Eds.). Stomatal physiology. Cambridge University Press, Cambridge, pp.137-161.
Louwerse, W., 1980. Effects of CO_2 concentration and irradiance on the stomatal behaviour of maize, barley and sunflower plants in the field. Plant, Cell and Environment 3: 391-398.
Louwerse, W. & Eikhoudt, J.W., 1975. A mobile laboratory for measuring photosynthesis, respiration and transpiration of field crops. Photosynthetica 9: 31-34.
Louwerse, W. & van Oorschot, J.L.P., 1969. An assembley for routine measurements of photosynthesis, respiration and transpiration of intact plants under controlled conditioning. Photosynthetica 3: 305-315.
Luxmoore, R.J., 1988. Assessing the mechanisms of crop loss from air pollutants with process models. *In*: Heck, W.W., Taylor, O.C. & Tingey, D.T. (Eds.), Assessment of crop loss from air pollutants. Elseviers Applied Science, London, New York, pp. 417-444.
Maas, F.A., 1987. Responses of plants to sulfur containing air pollutants (H_2S and SO_2). Ph.D. thesis, University Groningen, 110 pp.
Majernik, O. & Mansfield, T.A., 1971. Effects of SO_2 pollution on stomatal movements in *Vicia faba*. Phytopathologische Zeitschrift 71: 123-128.
Malhotra, S.S. & Khan, A.A.,1984. Biochemical and physiological impact of major pollutants. *In*: Treshow, M. (Ed.), Air Pollution and Plant Life, Wiley, New York, pp. 113-157.
Mansfield, T.A. & Freer-Smith, P.H., 1984. The role of stomata in resistance mechanisms. *In*: Koziol, M.J. & Whatley F.R. (Eds.), Gaseous air pollutants and plant metabolism, Butterworths, London, pp.131-146.
Marques, I.A. & Anderson, L.E., 1986. Effects of arsenite, sulfite, and sulfate on photosynthetic carbon metabolism in isolated pea (*Pisum sativum* L., cv. Little Marvel) chloroplasts. Plant Physiology 82: 488-493.
Martin, L.R., 1984. Kinetic studies of sulfite oxidation in aqueous solution. *In*: Calvert, J.G. (Ed.), SO_2, NO and NO_2 oxidation mechanisms: atmospheric considerations. Acid Precipitation 3, Butterworths Publishers, Boston-London, pp. 63-100.
McLaughlin, S.B., McConathy, R.K., Durick, D. & Mann, L.K., 1982. Effects of

chronic air pollution stress on photosynthesis, carbon allocation and growth of white pines. Forest Science 28: 60-71.
McLaughlin, S.B. & Taylor, G.E., 1981. Relative humidity: Important modifier of pollutant uptake by plants. Science 211: 167.
McLaughlin, S.B. & Taylor, G.E., 1985. SO_2 effects on dicot crops: Some issues, mechanisms, and indicators. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.). Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 227-250.
McLeod, A.R., 1988. Effects of open air fumigation with sulphur dioxide on the occurrence of fungal pathogens in winter cereals. Phytopathology 78: 88-94.
McLeod, A.R., Fackrell, J.E. & Alexander, K., 1985. Open-air fumigation of field crops: Criteria and design for a new experimental system. Atmospheric Environment 19: 1639-1649.
McLeod, A.R., Roberts, T.M., Alexander, K. & Cribb, D.M., 1988. Effects of open-air fumigation with sulphur dioxide on the growth and yield of winter barley. New Phytologist 109: 67-78.
Miller, J.E., Sprügel, D.G., Muller, R.N., Smith, H.J. & Xerikos, P.B., 1980. Open-air fumigation system for investigating sulphur dioxide effects on crops. Phytopatology 70: 1124-1128.
Miller, J.E. & Xerikos, P.B., 1979. Residence time of sulphite in SO_2 'sensitive' and 'tolerant' soybean cultivars. Environmental Pollution (series A) 18: 259-264.
Mohren, G.M.J., 1987. Simulation of forest growth, applied to douglas fir stands in the Netherlands. Ph.D. thesis, Agricultural University, Wageningen, 184 pp. ISBN 09-9002432-8.
Monteith, J.L., 1973. Principals of environmental physics. Edward Arnold, London, 241 pp.
Mooi, J. & van der Zalm, A.J.A., 1986. Research on the effects of higher than ambient concentrations of SO_2 and NO_2 on vegetation under semi-natural conditions. IPO-report No. R 317, 33 pp.
Mooney, H.A., Küppers, M., Koch, G., Gorham, J., Chu, C. & Winner, W.E., 1988. Compensating effects to growth of carbon partitioning changes in response to SO_2 induced photosynthetic reduction in radish. Oecologia 75: 502- 506.
Morison, J.I.L., 1987. Intercellular CO_2 concentration and stomatal response to CO_2. *In*: Zeiger, E. & Farquhar, G.D. (Eds.), Stomatal function. Stanford University press. Stanford, California. pp. 229-251.
Müller, R., Miller, J.E. & Sprügel, D.G., 1979. Photosynthetic response of field grown soybeans with sulphur dioxide. Journal of Applied Ecology 16: 567-576.
Noyes, R.D., 1980. The comparative effects of sulphur dioxide on photosynthesis and translocation in bean. Physiological Plant Pathology 16: 73-79.
Olsen, R.A., 1957. Absorption of sulfur dioxide from the atmosphere by cotton plants. Soil Science 84: 107-111.
Olszyk, D.M., Bytnerowicz, A., Kats, G., Dawson, P.J., Wolf, J. & Thompson, C.R., 1986. Effects of sulfur dioxide and ambient ozone on winter wheat and lettuce. Journal of Environmental Quality 15: 363-369.
Olszyk, D.M. & Tibbitts, T.W., 1981. Stomatal response and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposures. I. Influence of pollutant level and leaf maturity. Plant Physiology 67: 539-549

Öquist, G., Greer, D.H. & Ögren, E., 1987. Light stress at low temperature. *In*: Kyle, D.J., Osmond, C.B. & Arntzen, C.J. (Eds.). Photoinhibition. Elsevier Science Publishers, London, New York, pp. 67-87.

Pande, P.C. & Mansfield, T.A., 1985. Responses of winter barley to SO_2 and NO_2 alone and in combination. Environmental Pollution (series A) 39: 281-291.

Parry, M.A.J. & Gutteridge, S., 1984. The effect of SO_3^{2-} and SO_4^{2-} ions on the reactions of ribulose biphosphate carboxylase. Journal of Experimental Botany 35: 157-168.

Penning de Vries, F.W.T. & van Laar, H.H. (Eds.), 1982. Simulation of plant growth and crop production. Simulation Monographs, Pudoc, Wageningen, 308 pp.

Pfanz, H. & Heber, U., 1986. Buffer capacities of leaves, leaf cells, and leaf cell organelles in relation to fluxes of potentially acidic gases. Plant Physiology 81: 597-602.

Pfanz, H., Martinoia, E., Lange, O.L. & Heber, U., 1987. Flux of SO_2 into leaf cells and cellular acidification by SO_2. Plant Physiology 85: 928-933.

Pierre, M. & Queiroz, O., 1981. Enzymic and metabolic changes in bean leaves during continuous pollution by subnecrotic levels of SO_2. Environmental Pollution (Series A) 25: 41-51.

Pierre, M. & Queiroz, O., 1982. Modulation by leaf age and SO_2 concentration of the enzymic response to subnecrotic SO_2 pollution. Environmental Pollution (series A) 28: 209-212.

Posthumus, A.C., 1981. De invloed van luchtverontreiniging op planten. Natuur en Techniek 49: 488-507.

Powles, S.B., 1984. Photoinhibition of photosynthesis induced by visible light. Annual Review of Plant Physiology 35: 15-44.

Priebe, A., Klein, H. & Jäger, H.-J., 1978. Role of polyamines in SO_2-polluted pea plants. Journal of Experimental Botany 29: 1045-1050.

Rabbinge, R., Jorritsma, I.T.M. & Schans, J., 1985. Damage components of powdery mildew in winter wheat. Netherlands Journal of Plant Pathology 91: 235-247.

Rabbinge, R., Ward, S.A. & van Laar, H.H., 1989. Simulation and systems management in crop protection. Simulation Monographs, Pudoc, Wageningen.

Raven, J.A., 1985. pH regulation in plants. Science Progress (Oxford) 69: 495-509.

Raven, J.A., 1986. Biochemical disposal of excess H^+ in growing plants. New Phytologist 104:175-206.

Raven, J.A., 1988. Acquisition of nitrogen by the shoots of land plants: its occurrence and implications for acid-base regulation. The New Phytologist 109: 1-20.

Raven, J. A. & Smith, F.A., 1976. Nitrogen assimilation and transpiration in vascular land plants in relation to intracellular pH regulation. New Phytologist 76: 415-431.

Reinert, R.A., Tingey, D.T., Heck, W.W. & Wyecliff, C., 1969. Tobacco growth influenced by low concentration of sulfur dioxide and ozone. Agronomy abstracts 61: 34.

Rennenberg, H., 1984. The fate of excess sulfur in higher plants. Annual Review of Plant Physiology 35: 121-153.

Rennenberg, H. & Filner, P., 1982. Stimulation of H_2S emission from pumpkin leaves by inhibition of glutathion synthesis. Plant Physiology 26: 1045-1055.

Roberts, J.K.M., Ray, P.M., Wade-Jardetzky, N. & Jardetzky, O., 1981. Extent of intracellular pH changes during H^+ extrusion by maize root tip cells. Planta 152: 74-78.

Roberts, T.M., 1984. Long-term effects of sulphur dioxide on crops: an analysis of dose-response relations. Philosophical Transactions of the Royal Society of London B 305: 299-316.

Rothermel, B. & Alscher, R., 1985. A light-enhanced metabolism of sulfite in cells of *Cucumis sativus* L. cotyledons. Planta 166:105-110.

Sakaki, T. & Kondo, N., 1985. Inhibition of photosynthesis by sulfite in mesophyll protoplasts isolated from *Vicia faba* L. in relation to intracellular sulfite accumulation. Plant and Cell Physiology 26: 1045-1055.

Saunders, P.W.J., 1985. Regulations and research on SO_2 and its effects on plants in the European Communities. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.), Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 37-55.

Saxe, H., 1983. Long-term effects of low levels of SO_2 on bean plants (*Phaseolus vulgaris*). I. Immission-response pattern of net photosynthesis and transpiration during life-long, continuous measurements. Physiologia Plantarum 57: 101-107.

Schapendonk, A.H.C.M. & Spitters, C.J.T., 1984. Interplant competition as a biasing factor in evaluating pre-treatment effects in cucumber. Scientia Horticulturae 24: 115-122.

Schulte-Hostede, F.S., Darral, N.M., Blank, L.W. & Wellburn, A.R. (Eds.), 1988. Air pollution and plant metabolism. Elseviers Applied Science, London, New York.

Schulze, E.-D., 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. Annual Review of Plant Physiology 37: 247-274.

Schulze, E.-D., Turner, N.C., Gollan, T. & Shackel, K.A., 1987. Stomatal responses to air humidity and to soil drought. *In*: Zeiger, E. & Farquhar, G.D. (Eds.), Stomatal function. Stanford University press. Stanford, California, pp. 311-321.

Schut, H.E., 1985. Models for the physiological effects of short O_3 exposures on plants. Ecological Modelling 30: 175-207.

Sekiya, J., Schmidt, A., Rennenberg, H., Wilson, L.G. & Filner, P., 1982. Hydrogen sulfide emission by cucumber leaves in response to sulfate in light and dark. Phytochemistry 23: 2173-2178.

Shugart, H.H. & McLaughlin, S.B., 1985. Modelling SO_2 effects on forest growth and community dynamics. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.), Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 478-494.

Sij, J.W. & Swanson, C.A., 1974. Short-term kinetic studies on the inhibition of photosynthesis by sulphur dioxide. Journal of Environmental Quality 3: 103- 107.

Sisson, W.B., Booth, J.A. & Throneberry, G.O., 1981. Absorption of SO_2 by Pecan (*Carya illinoensis* (Wang K. Koch) and Alfalfa (*Medicago sativa* L.) and its effect on net photosynthesis. Journal of Experimental Botany 32: 523-534.

Smith, F.A. & Raven, J.A., 1979. Intracellular pH and its regulation. Annual Review of Plant Physiology 30: 289-311.

Spitters, C.J.T., 1979. Competition and its consequences for selection in barley breeding Ph.D. Thesis, Agricultural University, Wageningen, 268 pp.

Spitters, C.J.T., 1986. Separating the diffuse and direct component of global radiation and its implications for modelling canopy photosynthesis. Part I. Components of incoming radiation. Agricultural and Forest Meteorology 28: 217-229.

Spitters, C.J.T., 1989. Weeds: population dynamics, germination and competition. *In*: Rabbinge, R, Ward, S.A. & van Laar, H.H. (Eds.), Simulation and systems

management in crop protection. Simulation Monographs, Pudoc, Wageningen, in press.
Spitters, C.J.T. & Aerts, R., 1983. Simulation of competition for light and water in crop-weed association. Aspects of Applied Biology 4: 467-483.
Spitters, C.J.T., Toussaint, H.A.M. & Goudriaan, J., 1986. Separating the diffuse and direct component of global radiation and its implications for modelling canopy photosynthesis. Part II. Calculation of canopy photosynthesis. Agricultural and Forest Meteorology 28: 231-242.
Spitters, C.J.T., van Keulen, H. & van Kraalingen, D.W.G., 1989. SUCROS '87.:A simple and universal crop growth simulator. *In*: Rabbinge, R, Ward, S.A. & van Laar, H.H. (Eds.), Simulation and systems management in crop protection. Simulation Monographs, Pudoc, Wageningen, in press.
Sprügel, D.G., Miller, J.E., Muller, R.N., Smith, H.J. & Xerikos, P.B., 1980. Sulphur dioxide effects on the yield and seed quality in field grown soybeans. Phytopathology 70: 1129-1133.
Taylor, H.J. & Bell, J.N.B., 1988. Studies on the tolerance to SO_2 of grass populations in polluted areas. V. Investigations into the development of tolerance to SO_2 and NO_2 in combination and NO_2 alone. New Phytologist 110: 327-338.
Teh, K.H. & Swanson, C., 1982. Sulphur dioxide inhibition of translocation in bean plants. Plant Physiology 69: 88-92.
Temple, P.J., Cao Hong, Fa, & Taylor, O.C., 1985. Effects of SO_2 on stomatal conductance and growth of Phaseolus vulgaris. Environmental Pollution (series A) 37: 267-279.
Thomas, M.D., 1951. Gas damage to plants. Annual Review of Plant Physiology 2: 293-322.
Thomas, M.D., Hendricks, H.R., Collier, G.R. & Hill, G.R., 1943. The utilization of sulfate and sulphur dioxide for the nutrition of alfalfa. Plant Physiology 18: 345-371.
Thomas, M.D., Hendricks, H.R. & Hill, G.R., 1944. Some chemical reactions of sulfur dioxide after absorption by alfalfa and sugarbeets. Plant Physiology 19: 212-226.
Tingey, D.T. & Olszyk D.M., 1985. Intraspecies variability in metabolic responses to SO_2. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.). Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 178-208.
Tingey, D.T. & Reinert, R.A., 1971. The effect of ozone and sulphur oxides singly and in combination on plant growth. Environmental Pollution (Series A) 9: 117-125.
Tingey, O.T., Heck, W.W. & Reinert, R.A., 1971. Effect of low concentrations of ozone and sulfur dioxide on foliage, growth and yield of radish. Journal of the American Society of Horticultural Science 96: 36-71.
Ulrich, A., 1941. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. American Journal of Botany 28: 526-537.
Umbreit, W.W., Burris, R.H. & Stauffer, J.F., 1957. Manometric techniques. Burgess, Minneapolis. 326 pp.
Unsworth, M.H., Biscoe, P.V. & Black, V., 1976. Analysis of gas exchange between plants and polluted atmospheres. *In*: Mansfield, F.A. (Ed.), Effects of air pollutants on plants. Cambridge University Press, London, pp. 7-16.
Unsworth, M.H. & Mansfield, T.A., 1980. Critical aspects of chamber design for

fumigation experiments on grasses. Environmental Pollution (series A) 23: 115-120.
Unsworth, M.H. & Ormrod, D.P., 1982. Effects of gaseous air pollution in agriculture and horticulture. Butterworths, London, 532 pp.
Unsworth, M.H., Crawford, D.V., Gregson, S.K. & Rowlatt, S.M., 1985. Pathways for sulfur from the atmosphere to plant and soil. *In*: Winner, W.E., Mooney, H.A. & Goldstein, R.A. (Eds.). Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues. Stanford University press, Stanford, California, pp. 375-388.
Wellburn, A., 1988. Air pollution and acid rain: the biological impact. Longman Scientific & Technical, Harlow, 274 pp.
Werf, W. van der, 1988. Yellowing viruses in sugarbeet; epidemiology and damage. Ph.D. Thesis, Agricultural University Wageningen, 152 pp.
West, P.W. & Gaeke, G.C., 1956. Fixation of sulfur dioxide as sulfitomercurate II and subsequent colorimetric estimation. Analytical Chemistry 28: 1816-1819.
Whitmore, M.E. & Mansfield, T.A., 1983. Effects of long-term exposures to SO_2 and NO_2, on *Poa pratensis* and other grasses. Environmental Pollution (Series A) 31: 217-235.
Wilson, L.G., Bressan, R.A. & Filner, P., 1978. Light dependent emission of hydrogen sulfide from plants. Plant Physiology 61: 184-189.
Winner, W.E. & Mooney, H.A., 1980a. Ecology of SO_2 resistance. I. Effects of fumigations on gas exchange of decidious and evergreen shrubs. Oecologia (Berlin) 44: 290-295.
Winner, W.E. & Mooney, H.A., 1980b. Ecology of SO_2 resistance. II. Photosynthetic changes of shrubs in relation to SO_2 absorption and stomatal behavior. Oecologia (Berlin) 44: 296-302.
Winner, W.E., Mooney, H.A. & Goldstein, R.A., 1985. Sulfur dioxide and vegetation. Physiology, Ecology and Policy issues Stanford University press, Stanford, California, 593 pp.
Wit, C.T. de, 1968. Plant production. Miscellaneous Papers Landbouw Hogeschool, Wageningen 3: 25-50.
Wit, C.T. de, 1982. Simulation of living systems. *In*: Penning de Vries, F.W.T. & van Laar, H.H. (Eds.), Simulation of plant growth and crop production. Simulation Monographs, Pudoc, Wageningen, pp. 3-8.
Wit, C.T. de, Dijkshoorn, W. & Noggle, J.C., 1963. Ionic balance and growth of plants. Verslagen Landbouwkundig onderzoek 69-15, Wageningen.
Wit, C.T. de, *et al.*, 1978. Simulation of assimilation, respiration and transpiration of crops. Simulation Monographs, Pudoc, Wageningen. 140 pp.
Woledge, J., Bunce, J.A. & Tewson, V., 1989. The effect of air humidity on photosynthesis of ryegrass and white clover at three temperatures. Annals of Botany 63: 271-279.
Wong, S.C., Cowan, I.R. & Farquhar, G.D., 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282: 424-426.
Wright, E.A., 1987. Effects of sulphur dioxide and nitrogen dioxide, singly and in mixture, on the macroscopic growth of three birch clones. Environmental Pollution (Series A) 46: 209-221.
Young, N.D. & Galston, A.W., 1983. Putrescine and acid stress. Plant Physiology 71: 767-771.
Zeevaart, A.J., 1976 Some effects of fumigating plants for short periods with NO_2. Environmental Pollution (series A) 11: 97-108.

Ziegler, I., 1975. The effect of SO_2 pollution on plant metabolism. Residue Review 56: 79-105.

Curriculum vitae

Martin Kropff werd geboren op 4 april 1957 te Asperen. Na het behalen van het atheneum-B diploma aan het Christelijk Lyceum te Veenendaal studeerde hij Biologie (oecologisch gericht) aan de Rijksuniversiteit te Utrecht, waarvan hij in 1984 het diploma (met lof) behaalde. Het vakkenpakket bestond uit een hoofdvak biologie, bestaande uit de hoofdrichting oecofysiologie en nevenrichtingen didaktiek van de biologie en milieukunde, en de bijvakken toegepaste onderwijskunde en theoretische teeltkunde. Aansluitend op zijn studie kwam hij in dienst van de Landbouwhogeschool (thans Landbouwuniversiteit) bij de vakgroep Luchthygiëne- en verontreiniging met als taak het verrichten van onderzoek en het geven van onderwijs op het vakgebied 'effecten van luchtverontreiniging op planten'. Hiertoe werd hij gestationeerd op het Instituut voor Plantenziektenkundig Onderzoek. Sinds 1985 is hij werkzaam bij de vakgroep Theoretische Produktie-ecologie. Vanaf 1987 is hij tevens als projectcoördinator verbonden aan een samenwerkingsproject van het Instituut voor Plantenziektenkundig Onderzoek met de vakgroepen Luchthygiëne- en verontreiniging en Theoretische Produktie-ecologie van de Landbouwuniversiteit, dat ten dele wordt gefinancierd door het Nationaal Programma Verzuringsonderzoek.