Modelling short-term effects of sulphur dioxide.
1. A model for the flux of SO₂ into leaves and effects on leaf photosynthesis

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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 feed back 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*) feed back 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 appears to be an important factor determining the susceptibility of plants to air pollutants. Thin leaves should 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.

Additional keywords: stomatal behaviour, sulphur metabolism, pollution.

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 and 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 (in the ppm range) or during long-term exposures to more realistic concentrations ($< 400 \mu g\ SO_2\ m^{-3}$). However, long-term exposures may result in decreased plant growth at concentrations as low as 40-400 $\mu g\ SO_2\ m^{-3}$ without causing visible injury (Bell et al., 1979; Ashenden and 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 and Van Laar, 1982).

Such models enable the integration of existing knowledge into ecophysiological processes that determine crop growth, including the effects of air pollutants on physiological and biochemical processes. Since air pollutants can influence physiological processes only upon entrance in cellular solutions, submodels are needed to calculate air pollutant fluxes into the leaf, for the metabolism of pollutants in cellular solutions and for 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,b) 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 paper, it is unlikely that short-term effects of SO_2 on photosynthesis are due to changes in cellular pH.

In a previous paper, the short-term effects of relatively low SO_2 concentrations on photosynthesis and stomatal behaviour were analysed quantitatively for broad bean leaves (*Vica faba* L.) (Kropff, 1987). It was shown that exposure to SO_2 increased 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 paper, a model for the effect of SO_2 on gas exchange of leaves will be derived on the basis of reported effects at the biochemical level. The model simulates the uptake of SO_2 by leaves, the balance of toxic SO_2-metabolites in the leaf, their direct

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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 (Kropff, 1989), species characteristics which determine SO_2 effects on photosynthesis of single leaves will be quantified from analysis of experimental data.

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. 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 conductance for diffusion of SO_2 into the leaf. Stomatal regulation is described based on the observation that the internal CO_2 concentration in the stomatal cavities tends to be constant at a given ambient CO_2 concentration (Kropff, 1987). The leaf
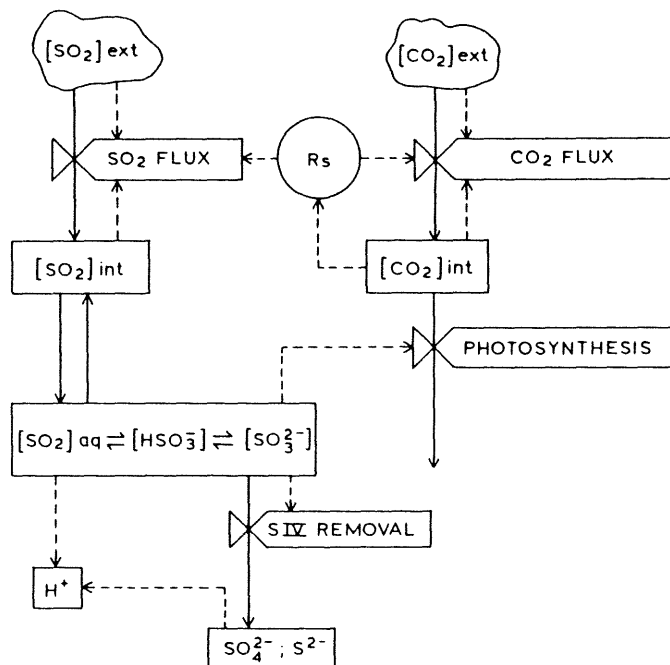
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* S(IV) = $[SO_2]_{aq}$ + $[HSO_3^-]$ + $[SO_3^{2-}]$

Fig. 1. A relational diagram of the model for fluxes of SO_2 and CO_2 into the leaf and the effects of S(IV) (= $[SO_2]_{aq}$ + $[HSO_3^-]$ + $[SO_3^{2-}]$) 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.

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 external CO_2 concentration (Morison, 1987). The effect of possible direct effects on stomata (like the opening of stomata during SO_2 exposure which has been observed by several workers (cf. Mansfield and 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 paper. Toxic S(IV) compounds are oxidized to sulphate (Asada and Kiso, 1973), which is less toxic than sulphite (Marques and 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 and 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 (Kropff, 1987). The model operates with time steps of 1 minute.

Model description

SO₂ 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 and 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 and Van Laar, 1978; Wong et al., 1979; Louwerse, 1980; Bell, 1982; Farquhar and 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 Faba bean leaves was not influenced by SO_2 after a 2-hour fumigation period at 0-800 μg SO_2 m^{-3} (Kropff, 1987). 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

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* RBP = ribulose-1,5-biphosphate

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 and Mansfield, 1971). Black and 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 (e.g. Olszyk and Tibbits, 1981). Black and 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 have been discussed by Black (1982) and Mansfield and 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 (Kropff, 1987), 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):

$$r_s = \frac{C_a - C_i}{P} - r_b \tag{1}$$

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 (g CO_2 m^{-2} s^{-1}), and C_a and C_i are the ambient and internal CO_2 concentrations (g 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 (Kropff, 1987). 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:

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

This results in:

$$r_{s,s} = 1.21 \, r_s \quad ; \quad r_{b,s} = 1.13 \, r_b \tag{2}$$

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

$$F = \frac{S_a - S_i}{r_{s,s} + r_{b,s}} \qquad (3)$$

in which S_a and S_i represent the ambient and internal (in the gas phase!) concentrations of SO_2 (mmol SO_2 m^{-3}) respectively and F is the rate of SO_2 uptake (mmol SO_2 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 concentation (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):

$$[SO_2]_g = \frac{[SO_2]_{aq}}{H} \qquad (4)$$

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 is cells involve the production or consumption of protons. Smith and 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 and Kondo (1985) and Smith and 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 a leaf thickness of 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 and Heber (1986), resulting in a proton production of 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 and Heber, 1986), the decrease will be not more than 0.05 pH unit per hour. Thus, even when metabolic buffering processes are not con-

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sidered, no effects of SO_2 on cellular pH are to be expected.

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

$$[S(IV)] = [SO_2]_{aq} + [HSO_3^-] + [SO_3^{2-}] \tag{5}$$

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

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

$$HSO_3^- \rightleftarrows SO_3^{2-} + H^+$$
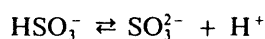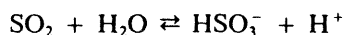
In equilibrium, the concentrations will be related according to:

$$K_1 = \frac{[HSO_3^-][H^+]}{[SO_2]_{aq}} \tag{6}$$

$$K_2 = \frac{[SO_3^{2-}][H^+]}{[HSO_3^-]} \tag{7}$$

The equilibrium constant of the first reaction is about 0.0148 mol l^{-1} (K_1) and of the second reaction 7×10^{-8} ml 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 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 Eqns 5, 6 and 7:

$$[SO_2]_{aq} = \frac{[S(IV)]}{1 + \dfrac{K_1}{[H^+]}\left(1 + \dfrac{K_2}{[H^+]}\right)} \tag{8}$$

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 and Kiso, 1973; Kondo et al., 1980). Sulphate may be transported into the vacuole or reduced to sulphide, which can be released as H_2S (Hållgren and Frederiksson, 1982; Rennenberg and 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 MM 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 et al. (1987) and Miller and Xerikos (1979) show that S(IV) oxidation 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 20-50 minutes.

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

$$\frac{dS(IV)}{dt} = \frac{F}{d} - \frac{S(IV)}{\tau_2} \tag{9}$$

where τ_2 is the time coefficient for S(IV) removal (s), F is the rate of SO_2 uptake (in mmol SO_2 m^{-2} s^{-1}), and d is the thickness of the leaf (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 and 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 and Hållgren (1980) who reported non-competitive inhibition while Khan and Malhotra (1982) observed competitive inhibition and confirmed the conclusions of Ziegler (1975). Parry and 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, Kropff (1987) demonstrated 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 cellular pH. However, they used protoplasts suspended in solutions containing very high sulphite concentrations. Earlier in this paper, using data from Pfanz and Heber (1986) and Darrall (1986), a pH decrease of less than 0.05 pH unit after 1 hour fumigation with 1350 μg SO_2 m^{-3} was calculated.

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According to data from Pfanz and Heber (1986) and Sakaki and 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 hypothesis of Pflanz 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 and Mansfield, 1982; McLaughlin et al., 1982; Teh and Swanson, 1982; Lorenc-Plucinska, 1986). However, it is not likely that photosynthesis is affected on a short-term by a feed back 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 and Swanson, 1982; Lorenc-Plucinska, 1986), whereas photosynthesis recovers very rapidly (Kropff, 1987). The observed suppression of SO_2 effects at elevated CO_2 concentrations (Black, 1982; Carlson, 1983; Kropff, 1987) also contradicts with such an explanation.

Sakaki and 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* chloroplasts 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-} 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 and Kondo (1985) who used isolated *Vicia faba* protoplasts and chloroplasts in sulphite solutions. Their data were used to construct Fig. 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 *Vicia faba* (Fig. 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 betwen 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:

$$P = P_0 (1 - k \text{ S(IV)}) \qquad ; \qquad \text{S(IV)} < 200 \text{ nmol mg}^{-1} \text{ chl} \qquad (10)$$

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. 2 (0.0025 per nmol S(IV) (mg^{-1} chl)). Sakaki and Kondo (1985) reported that

Relative photosynthesis (%)

100

80

60

40

20

0

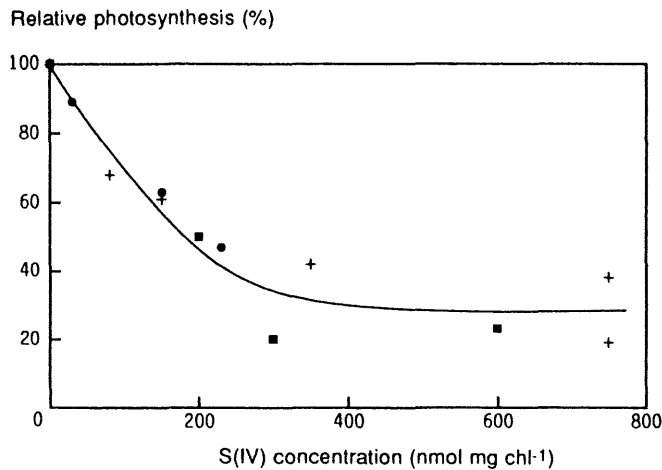200   400   600   800

S(IV) concentration (nmol mg chl-1)

Fig. 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 and Kondo (1985) (*Vicia faba* cv. Otafuku (+)). Curve was fitted by hand.

the volume of the protoplasts they used was 0.33 ml (mg^{-1} chl), so that the k value on a volume basis was 0.825 (mmol S(IV) l^{-1})$^{-1}$ (= 1 × 10^{-5} per μg S(IV) l^{-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:

$$P = P_{\text{max}} (1 - e^{-\varepsilon I / P_{\text{max}}})$$ (11)

where P_{max} (g 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; Kropff, 1987), so that the effect of S(IV) on photosynthesis can be described with an adapted version of Eqn 10:

$$P_{\text{max,s}} = P_{\text{max,0}} (1 - kS(IV))$$ (12)

where $P_{\text{max,0}}$ is the maximum rate of photosynthesis for control leaves and $P_{\text{max,s}}$ is the maximum rate of photosynthesis during exposure to SO$_2$. Since the rate of sulphite oxidation in leaves is light dependent (Rothermel and 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.

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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 *Vicia faba* L. data and the effect parameter was estimated from data from Alscher et al. (1987) and Sakaki and Kondo (1985). Parameter values are listed in Table 1.

Table 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)	0.001 g CO_2 m^{-2} s^{-1}
k	0.825 [mmol S(IV) l^{-1} leaf]$^{-1}$
CO_2 internal / CO_2 ambient	0.75
d leaf thickness	0.4 mm
T_2 time coefficient for S(IV) oxidation	2400 seconds

The effects 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 1, it can be calculated that the leaf resistance for CO_2 equals 155 s m^{-1}. Fig. 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).

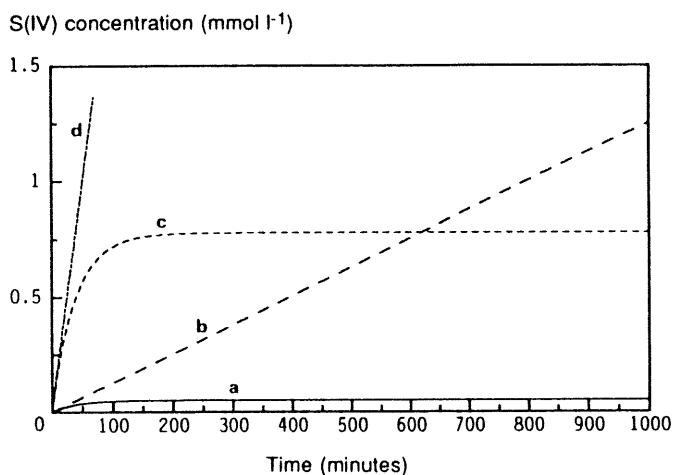S(IV) concentration (mmol l^{-1})

Fig. 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 1.
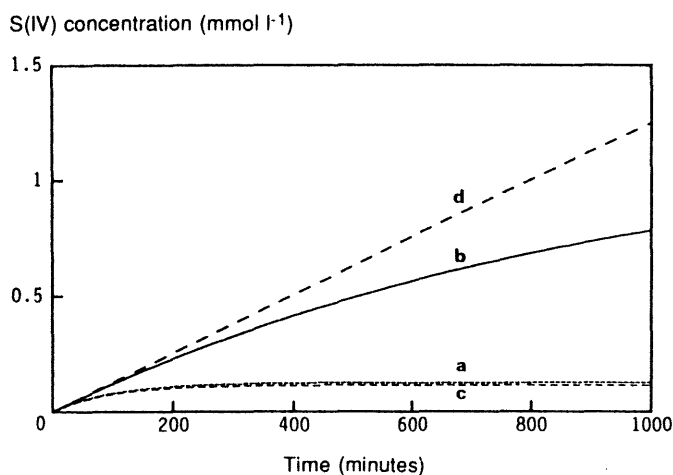
S(IV) concentration (mmol l⁻¹)

Fig. 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 1. The time constant for S(IV) oxidation (τ_2) was 100 minutes.

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).

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 also shows that a low leaf resistance leads to much higher S(IV) concentrations than for normal values for the leaf resistance, even when oxidation of S(IV) is included in the model. These results show 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. 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 exposures was subsequently analysed. Fig. 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 620-100 min) a quick equilibrium in photosynthetic rate and S(IV) concentration is reached, which is the general pattern in short-term
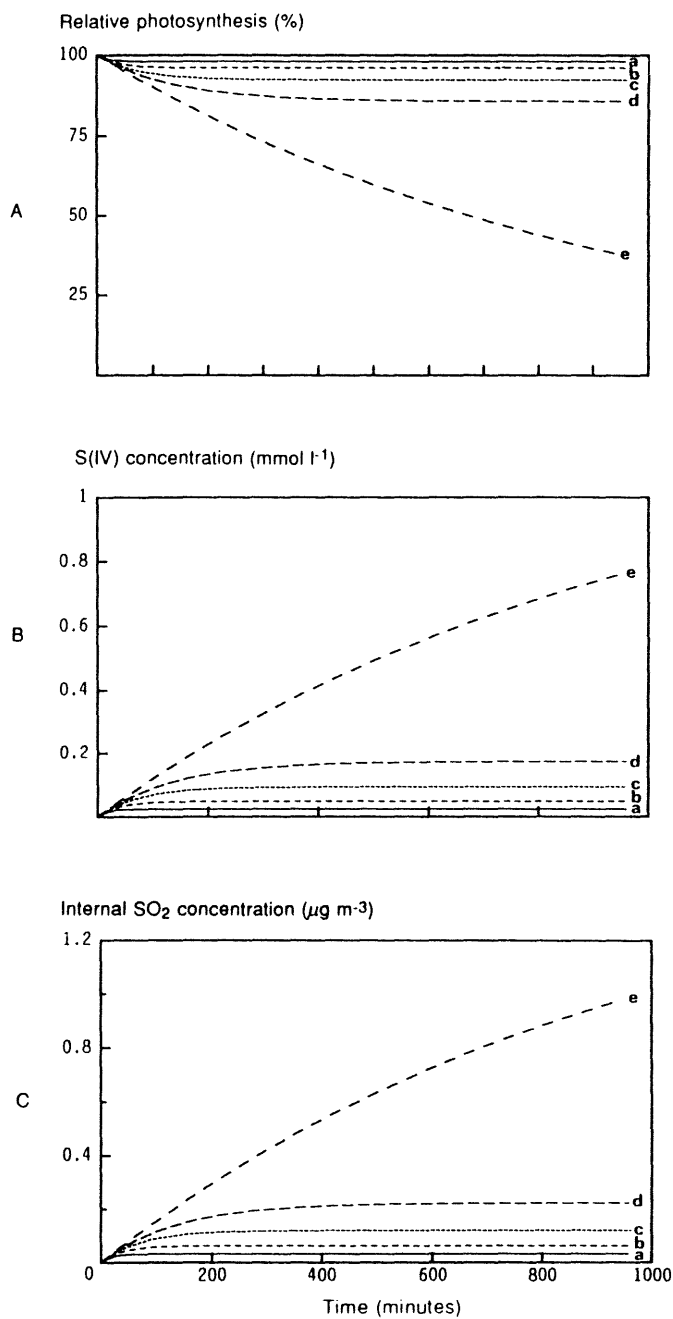
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Relative photosynthesis (%)

S(IV) concentration (mmol l⁻¹)

Internal SO₂ concentration (µg m⁻³)

Time (minutes)

Fig. 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: T_2 = 20 (a), 40 (b), 80 (c), 160 (d) or ∞ (e) minutes. Model parameters are listed in Table 1.

fumigation experiments (Black, 1982; Darrall, 1986; Kropff, 1987). The time follow-
ing the start of fumigation in which equilibrium rates of photosynthesis are reached,
is strongly dependent upon the rate of S(IV) oxidation (Fig. 5A). The same pattern
is simulated for the sulphite concentration in the leaf (Fig. 5B). Without oxidation the
rate of photosynthesis gradually decreases to very low values since sulphite accumulates
in the leaf (Fig. 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. 5C), since the internal SO_2 concen-
tration 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. 5C). Slow SO_2 uptake, rapid S(IV)
oxidation and the feedback mechanism of stomatal resistance, results in very low inter-
nal SO_2 concentrations in the stomatal cavities ($< 0.2 \mu g$ 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 and Unsworth, 1979; Carlson, 1983).

These results clearly show 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.

The effect of leaf morphology on the sensitivity to SO_2 was analysed by changing
the leaf thickness in the model. Fig. 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 den-
sity per unit leaf area remains unchanged. This may explain the strong differences in
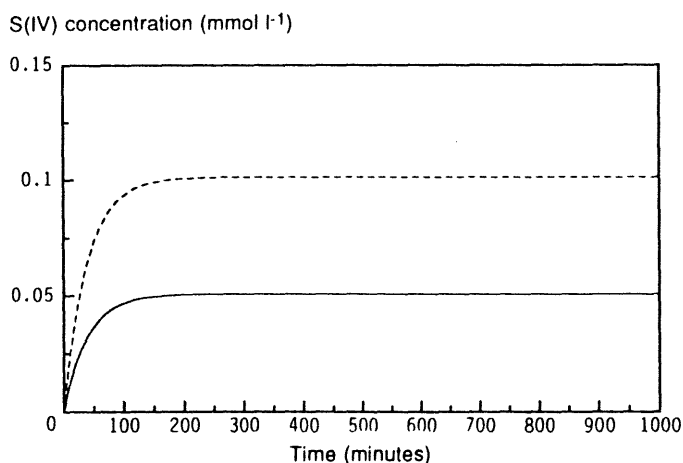sensitivity between plants grown in glasshouses or outdoors (Darrall, 1986). Thus, the

S(IV) concentration (mmol l⁻¹)

Fig. 6. The effect of leaf thickness (d = 0.4 mm (——) and d = 0.2 mm (------)) on S(IV) con-
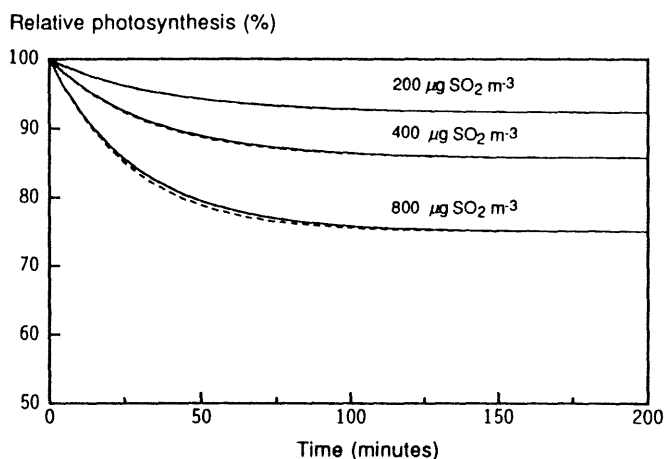centration in the leaf. Model parameters are listed in Table 1.

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Relative photosynthesis (%)

Fig. 7. The effect of a delay in stomatal closure when photosynthesis is reduced at 200, 400 and 800 μg SO_2 m^{-3}. Solid lines represent standard simulation, broken lines indicate the effect of the delay.

parameter 'flux density' or 'uptake rate' proposed by Black and 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 plant species sensitivity.

The influence of delayed stomatal closure on the time course of reductions of photosynthesis is shown in Fig. 7. A time coefficient of 10 minutes was introduced into the model (Kropff, 1987) and its effect was analysed at 3 different SO_2 concentrations. The reduction in photosynthesis is slightly increased during the first 100 minutes as a result of a larger uptake, since stomata remain open for a longer time.

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 (Kropff, 1989). To show the behaviour of the model, the time course of photosynthesis as (Bennet and Hill, 1973), was simulated using the means of biochemical parameters estimated for the two data sets. The results in Fig. 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, together with 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 a subsequent paper (Kropff, 1989).

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
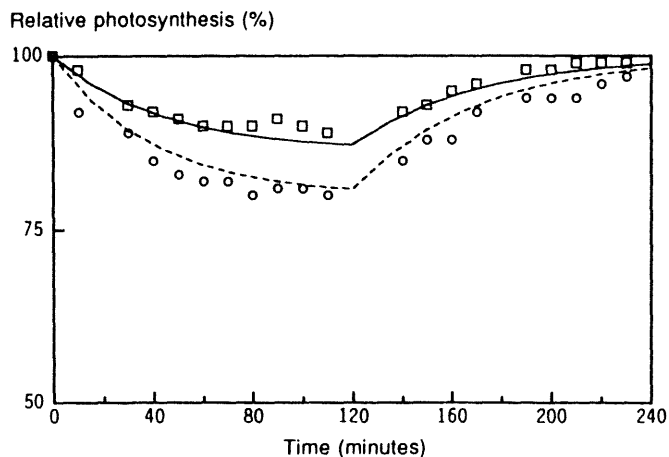
Relative photosynthesis (%)

Fig. 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 and Hill (1973). (k = 0.000 01, d = 0.4 mm, T_2 = 51 min. (Kropff, 1989)) at 675 μg SO_2 m^{-3} (□ observed; —— simulated) and 1080 μg SO_2 m^{-3} (o observed; ------ simulated).

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.

Acknowledgements

Samenvatting

Simulatie van de korte termijn effecten van zwaveldioxide. 1. Een model voor de flux van SO_2 in het blad en effecten op de fotosynthese van bladeren

Een model wordt gepresenteerd waarmee de flux van SO_2 vanuit de lucht in het blad en de effecten van SO_2-metabolieten op de bladfotosynthese en stomataire geleidbaarheid kan worden gesimuleerd. De S(IV)-balans in het blad wordt bepaald door de SO_2 opnamesnelheid, en de snelheid van S(IV)-verwerking door met name oxidatie tot sulfaat. Toxische S(IV)-componenten reduceren de fotosynthese en veroorzaken daardoor stomataire sluiting. Andere in de literatuur beschreven mechanismen voor de effecten van SO_2, zoals effecten door een daling van de pH, spelen geen rol op de korte termijn. Het model bevat twee parameters die de biochemische karakteristieken beschrijven: de tijdconstante voor S(IV)-oxidatie en een parameter die de gevoeligheid van de fotosynthese voor S(IV) beschrijft.

De simulatieresultaten laten zien dat de plant extreem toxische concentraties in het

blad kan voorkomen door: (*i*) de snelle oxidatie van S(IV) tot sulfaat, (*ii*) de relatief hoge weerstand voor SO₂-opname en (*iii*) de stomataire sluiting die een gevolg is van een gereduceerde fotosynthese. S(IV)-concentraties in het blad en SO₂-concentraties in de stomataire holten zijn kleiner dan 1% van de concentratie die zou ontstaan als deze mechanismen niet zouden werken. Bladdikte blijkt de gevoeligheid van planten voor SO₂ in sterke mate te bepalen. De effecten van SO₂ op de fotosynthese dienen te worden gerelateerd aan de opnamesnelheid per eenheid bladvolume, in plaats van bladoppervlak. Het model simuleert de reductie in fotosynthese gedurende een korte begassingsperiode en een herstelperiode nauwkeurig.

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