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

M.J. KROPFF
Department of Theoretical Production Ecology, Agricultural University Wageningen, P.O.B. 430, 6700 AK Wageningen, The Netherlands

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Abstract

The impact of SO_2 on the ionic balance of plants and its implications for intracellular pH regulation was studied 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 removed by metabolic processes.

On the basis of 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). 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, calculated 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.

Introduction

In previous communications data were presented from three open-air exposure experiments, in which the effects of SO_2 (63–164 $\mu g\ SO_2\ m^{-3}$) on growth and production of faba beans were analysed (Kropff *et al.*, 1989a; b). The observed reductions in crop yield ranged from 7 to 17% mainly caused by chronic injury (macroscopic leaf damage appearing after long exposures) (Kropff, 1990). Subtle injury (direct effects on photosynthesis and respiration) were less important (Kropff, 1989; 1990; Kropff and Goudriaan, 1989).

Because sulphur is an essential nutrient for plant growth, a mechanistic understanding of chronic SO_2 injury requires insight in the metabolism of SO_2. Plants growing on soils which

cannot meet the sulphur requirements for plant growth , may use SO_2 as an additional source of sulphur (Olsen, 1957; Thomas *et al.*, 1943; 1944). 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).

Excess sulphur taken up by plants exposed to SO_2, is mainly stored as sulphate (Cowling and Bristow, 1979; Faller *et al.*, 1980, Maas, 1987) resulting in net H^+ production in the cells. When sulphate is reduced to sulphide during protein synthesis, the H^+ ions are neutralized by OH^- production. However, the increase in organic sulphur upon SO_2 exposure is quantitatively not important (Cowling and Bristow, 1979; Grill *et al.*, 1979; Maas, 1987).

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 (Cowling and Bristow, 1979); Eaton *et al.*, 1971; Jäger and Klein, 1976; Priebe *et al.*, 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 (Fischer, 1967, cited by Jäger and Klein, 1980; Grill, 1971; Jäger and Klein, 1977; Klein and Jäger, 1976; Thomas *et al.*, 1944).

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 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 from sufficient to neutralize the excess OH^- or H^+ produced (Davies, 1973; Raven, 1986; Raven and Smith, 1976; Smith and Raven, 1979; de Wit *et al.*, 1963). These buffering mechanisms are closely related to the uptake and assimilation of nutrient ons. 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, a conceptual model for the impact of SO_2 on regulation of intracellular pH was developed and evaluated using data on the ionic composition of the field-exposed *Vicia faba* plants and data from literature to find explanations for chronic SO_2 injury.

Materials and methods

Experiments

In an open-air exposure system 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 (Kropff *et al.*, 1989a). 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 by Kropff *et al.* (1989a).

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 ($55 \times 22 \times 25$ 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. Because the roots were not sampled, the concentrations of the plants mentioned in the text refer to the concentration in the aboveground parts of the plants.

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

Regulation of intracellular pH is closely related to the ionic balance of plants. Four groups of ions play an important role in the ionic balance of plants: 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; Houba *et al.*, 1971; de Wit *et al.*, 1963).

The organic anion content in the plants is a result of the operation of a biochemical pH stat mechanism (Davies, 1973). When H^+ or OH^- ions 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 of phosphoenolpyruvate to oxaloacetate, which in turn can be reduced to the strong acid malate. The production of H^+ ions activates malic enzyme, which results in the decarboxylation of malate to pyruvate (one carboxyl group), thereby neutralizing one carboxyl group to maintain intracellular pH.

Intracellular assimilation of nitrate and sulphate 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 and assimilation of neutral nitrogen sources (*e.g.* N_2 fixation) involves neither H^+ production nor OH^- production.

Assimilation of nitrogen and sulphur nutrients may be located either in the roots or in the shoots (Van Beusichem *et al.*, 1988a). 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 mechanisms for root and shoot-generated H^+ are different, the removal of H^+ and OH^- produced in the shoots and roots are discussed separately in this paper (following van Beusichem *et al.*, 1988a). 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. 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 and 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. The detailed analysis of van Beusichem *et al.* (1988a) showed that 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. 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 (Allen and Raven, 1987; Ben Zioni *et al.* 1971; van Beusichem *et al.*, 1988a).

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:

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

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

$$H_2SO_3 + \tfrac{1}{2}O_2 \rightarrow 2H^+ + SO_4^{2-}$$

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 and 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 Figure 1, which shows the metabolism of sulphur in a shoot cell. To simplify the figure, cations are omitted. Inorganic cations, 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 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 sulphur taken up as sulphate is reduced. When all sulphate reduction is located in the shoots and the rate of sulphate accumulation as a result 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.

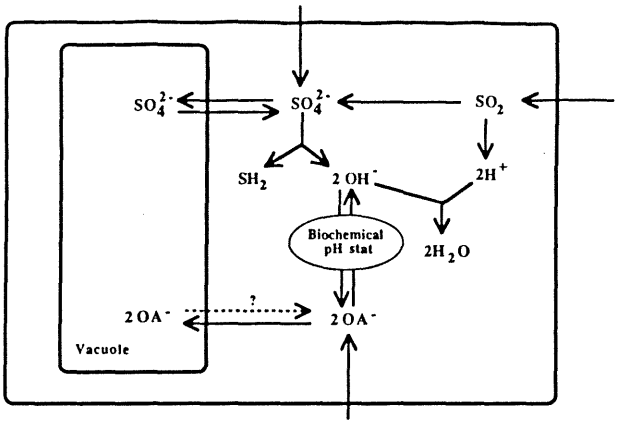

Fig. 1. Regulation of intracellular pH during SO_2 uptake by shoots of dinitrogen fixing plants. OA^- represents organic anions.

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.

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. Similiar effects on leaf area and dry matter production were ob-

served in two preceding experiments conducted in 1985 and 1986 at the same location (Kropff *et al.*, 1989a,b). 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 (Kropff, 1990)

SO_2 effects on ionic composition

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.

The contents of total-S and sulphate-S in the leaves, stems and pods of fumigated and control plants are presented in Figure 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, because it was shown in an earlier paper (Kropff, 1990) that the simulated uptake of sulphur from the air by the fumigated plants was close to the observed difference in sulphur content of the fumigated and control plants (Fig. 3). These findings confirm the conclusions of Cowling and Bristow (1979), Faller *et al.* (1970) and Maas (1987), who concluded that excess sulphur is mainly stored as sulphate. The data in this study yield 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 (Hållgren *et al.*, 1982; Wilson *et al.*, 1978).

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. 4). The difference in total inorganic anion content between the fumigated and the control plants was fully accounted for by sulphate ac-

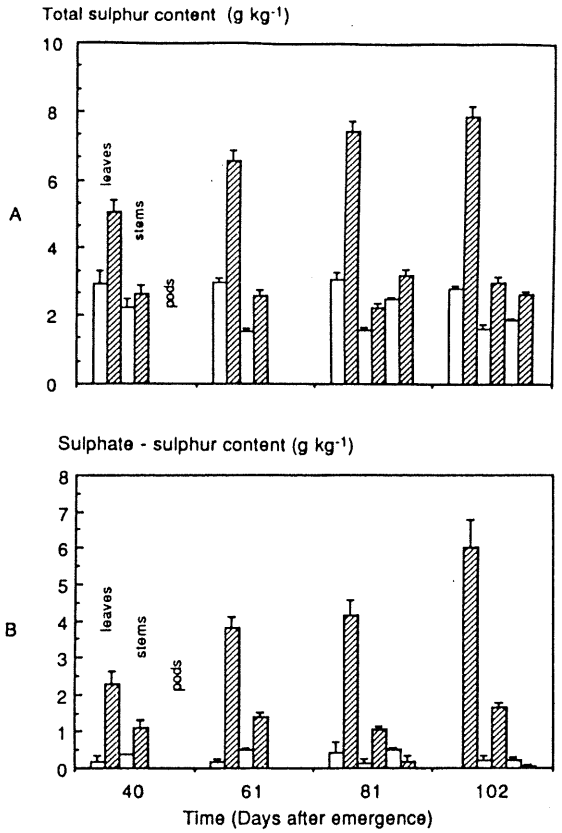

Fig. 2. Accumulation of total sulphur (**A**) and sulphate (**B**) in leaves, stems and pods of control (open columns) and fumigated plants (shaded columns) (mean and SE of mean).

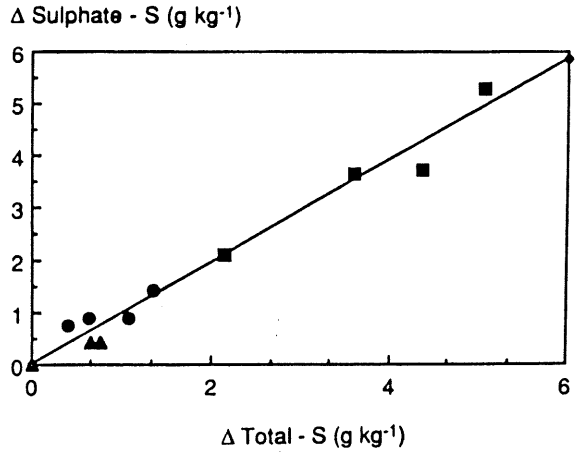

Fig. 3. Relation between accumulated total sulphur and sulphate-sulphur in the fumigated plants (corrected for SO_4^{2-}-sulphur and total sulphur contents of the control plants) in the leaves (■), stems (●) and pods (▲) during the growing period ($y = 0.002 + 0.97x$; $r^2 = 0.96$).

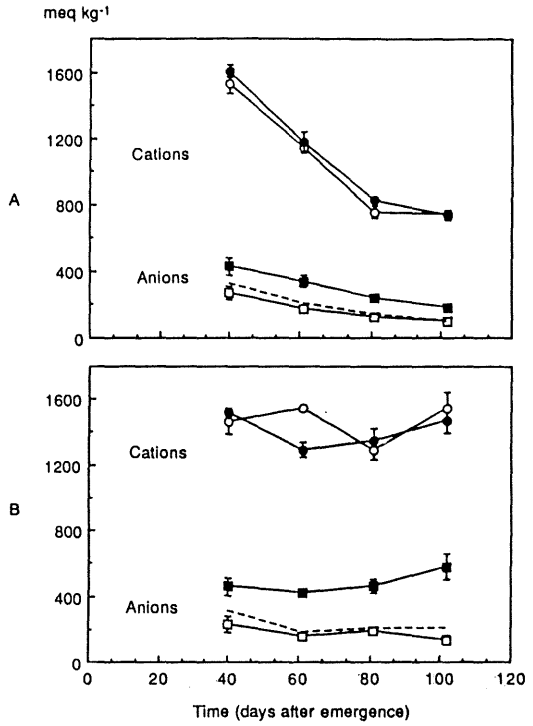

Fig. 4. Total inorganic cation (C) and inorganic anion (A) content of broad beans (**A.** for whole plants; **B.** for leaves), fumigated with SO_2 (C: ●; A: ■), or exposed to ambient SO_2 (C: ○; A: □) in the field. The inorganic anion contents of the fumigated plants, corrected for sulphate accumulation, are indicated by the broken line.

cumulation in the fumigated plants (Fig. 4A, broken line). The accumulation of sulphate in plants exposed to SO_2 was also responsible for a more than two-fold increase of the total foliar inorganic anion content (Fig. 4B). Because the difference between C and A equals the amount of organic anions (Arnon, 1939; Houba *et al.*, 1971; de Wit *et al.*, 1963), the amount of accumulated sulphate is equivalent to the decrease of organic anions in the plant. Chemical analysis of organic anion content in the dried material of the leaves showed a large difference in malate and citrate content of 320 and 395 meq per kg at 60 and 100 days after emergence respectively. Although this confirms the calculated (C-A) decrease in organic anion content, further experimentation is needed to analyse the effects quantitatively, because only a part of the organic anions can be determined in dried material. The decrease in organic anion content by SO_2 may explain the reduced chemical buffering capacity in plants exposed to SO_2 (Bytnerowycz *et al.*, 1987; Darral and Jäger; Grill, 1971; Jäger and Klein, 1977), because the chemical buffering capacity largely depends on organic anion content (Jäger and 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. 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. 5B). In contrast to these results, in an earlier field experiment in 1985 a significantly lower Ca content in the leaves of the fumigated plants was observed (Kropff *et al.*, 1989b). This may be explained by the stronger injurious effect of SO_2 on leaf area in 1985 as a result of the higher SO_2 concentration. Eaton *et al.* (1971) also demonstrated that the total amount of cations in tomatoes (*Lycopersium esculentum* Mill.) was un-

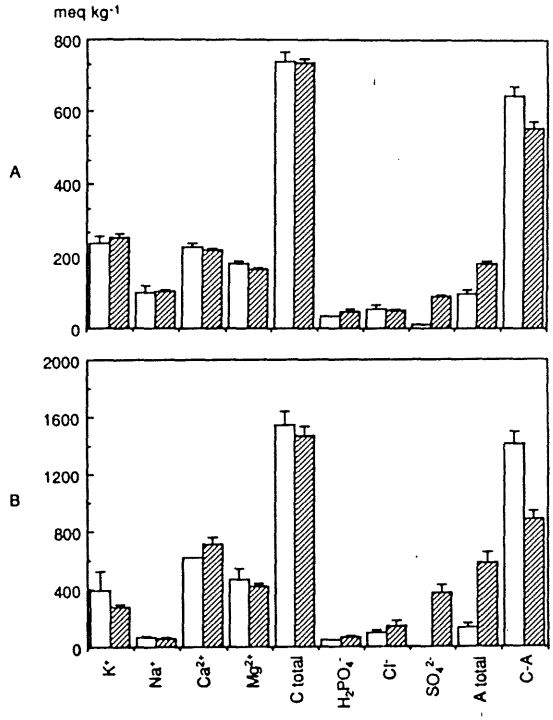

Fig. 5. Inorganic cation and inorganic anion composition of whole plants (**A**) and leaves (**B**), fumigated with SO_2 (shaded columns), or exposed to ambient SO_2 (controls, open columns) in the field, after a growing period of 102 days.

affected by SO_2, although they observed a small change in cation composition (more K and less Ca and Mg). Klein and 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 and Klein (1977) observed no effects on K, Ca, P and N in *Pisum sativum* due to SO_2 fumigations.

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). We observed a strong decrease in malate and citrate content in charge in the same order of magnitude as the decrease in C-A.

It is not likely indeed 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 nitrogeneous 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 (Jäger and Klein, 1980; Jäger *et al.*, 1985; Priebe *et al.*, 1978), while others did not find an effect (Cowling and Bristow, 1979; Pierre and 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 Anbazhagan *et al.*, (1988) and Jäger and Klein (1980), but no changes in proline content were observed by Brunold *et al.*, (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 quantitively unimportant. The only possibility left then is the mechanism proposed in this paper (Fig. 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 strong decrease in malate and citrate content was in the same order of magnitude. 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 (Kropff, 1990). The amount of protons produced in the leaves was $((10/32) \times (2=)$ 625 mol H^+ ha^{-1}, which equals 52 mol H^+ m^{-3} leaf, assuming (a) that all SO_2 is metabolized to sulphate resulting in the production of 2 mol H^+ ions per mol SO_2 (b) a 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 and 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 and 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 and 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 (Fischer, 1967, cited by Jäger and Klein, 1980; Grill, 1971; Jäger and Klein, 1977; Klein and Jäger, 1976; Thomas *et al.*, 1944).

However, our results indicate that chronic effects cannot be simply related to an exhaustion of metabolic buffering compounds when expressed as total organic anion content of the tissue: chronic injury and a strong leaf abscission was observed at the end of the growing season in the fumigated broad bean when the C-A content of the leaves of the fumigated plants was only reduced to about 900 meq kg^{-1} in the leaves (Fig. 4B), which would represent a high metabolic buffering capacity. However, the metabolic buffering capacity may not be related to the total organic anion content, because metabolic buffering takes place in the protoplasm (pH dependent enzymatic processes), whereas most organic anions are located in the vacuole (Smith and Raven, 1979). When chronic effects are related to the regulation of intracellular pH, our findings can only be understood when not enough organic anions are transported from the vacuole to the cytosol for buffering of protons by the biochemical pH stat at the end of the growing season. The metabolic buffering capacity of leaves then 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 (involving the production of OH^-) 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 (Baker *et al.*, 1986; Davies, 1980; Whitmore and Mansfield, 1983).

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 and 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 and 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 (Flores and Galston, 1984; Priebe *et al.*, 1978; Young and Galston, 1983) 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 and Klein (1976), Klein and 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 and Jäger (1976) and Jäger and Klein (1976) who studied the effects of varying nutrient supply are consistent with the mechanism proposed in this paper. 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 (Kropff *et al*, 1989a,b) 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 and Jäger (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 and Mansfield, 1978; Irving and Miller, 1984), especially when soil nitrogen is high (Taylor and 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 (quan-

titative) explanation of chronic SO_2 effects and their interactions with of airborne N and S containing pollutants on crops, forests and (semi) natural vegetation.

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