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Physiological effects of sulphur dioxide. 1. The effect of SO_2 on photosynthesis and stomatal regulation of *Vicia faba* L.

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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 2 h 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.

Key-words: *Vicia faba*; Papilionaceae, broad bean; photosynthesis; stomatal behaviour.

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; Sprugel *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 is dependent on plant age, growing conditions like nutrient availability, water supply, irradiance and temperature) and microclimatic factors (Black, 1982; Hällgren, 1984).

The rate of photosynthesis at light saturation appears to be negatively correlated with the rate of uptake of SO_2 through the stomata (Black & Unsworth, 1979b; Winner & Mooney, 1980a,b; Black, 1982; Carlson, 1983). Photosynthetic light use efficiency is not influenced by SO_2 (Black & Unsworth, 1979b; Hällgren & Gezelius, 1982). As 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 have 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 & Hällgren (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 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.

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Abbreviations: RBP = ribulose-1,5-bisphosphate.

Materials and methods

Plant material and experimental system

Plants of *Vicia faba* (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 h. The soil-moisture level was maintained at field capacity. CO_2 assimilation measurements were started when the plants were flowering and had about 14 pairs of leaflets. The sulphur content of the leaves was 7.7 ± 0.6 mg S g^{-1}.

Rates of CO_2 assimilation, respiration and transpiration of the youngest fully unfolded leaflet 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 min by a microcomputer. SO_2 concentration was also monitored. Measurements were performed during a prefumigation period of 2 h to obtain stable rates, during a subsequent SO_2 fumigation period of 2 h, and finally during a dark period of 1 h. 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 and 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_n), external CO_2 concentration (C_e), stomatal resistance (r_s) and the experimentally determined boundary layer resistance (r_b): $C_i = C_e - P_n(r_s + r_b)$. 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, Biscoe & Black, 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 cuticula 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):

$$P_n = (P_{max} + R_d)(1 - \exp(-I\varepsilon/(P_{max} + R_d))) - R_d, \quad (1)$$

where P_n = net CO_2 assimilation rate (μg CO_2 m^{-2} s^{-1}),
P_{max} = 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 programme.

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 as (J. Goudriaan, personal communication):

$$P_n = P_{max}(1 - \exp(-g_m(C_i - \Gamma)/P_{max})), \quad (2)$$

where P_n = net CO_2 assimilation rate (μg CO_2 m^{-2} s^{-1}),
P_{max} = 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 $r_m = 1/g_m$ 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 eqn (2) to data on net photosynthesis at different CO_2 concentrations. Equation (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. 1. If the stomatal resistance increases, the internal CO_2 concentration 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 will be $A–A_1–A_3$ 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:

$$r_s = \frac{C_a - C_i}{P_n} - r_b, \tag{3}$$

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; de Wit *et al.*, 1978; Goudriaan, 1982) 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):

$$V = \frac{V_c(C_i - \Gamma)}{C_i + K_c\left(1 + \frac{[O]}{K_o}\right)}, \tag{4}$$

where V is the net photosynthetic rate, V_c is the photosynthetic rate at high CO_2 concentration, Γ is the CO_2 compensation point, K_c is the Michaelis 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:

$$r_m = \frac{\Gamma + K_c}{V_c}\left(1 + \frac{[O]}{K_o}\right). \tag{5}$$

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):

$$\Gamma = t\frac{V_o K_c [O]}{V_c K_o}, \tag{6}$$

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

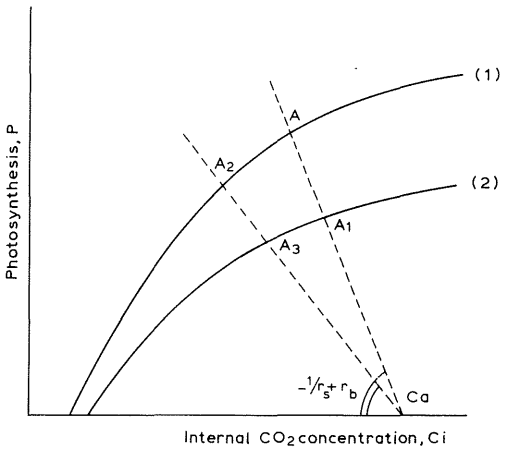

Figure 1. Partitioning of stomatal and non-stomatal contributions to a change in net photosynthesis. When stomatal resistance changes first the trajectory will be $A–A_2–A_3$; when the mesophyll resistance changes first the trajectory will be $A–A_1–A_3$, the dotted lines represent the supply functions ($C_i = C_a - A(r_s + r_b)$ (where r_s and r_b are the stomatal- and boundary layer resistance response), 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).

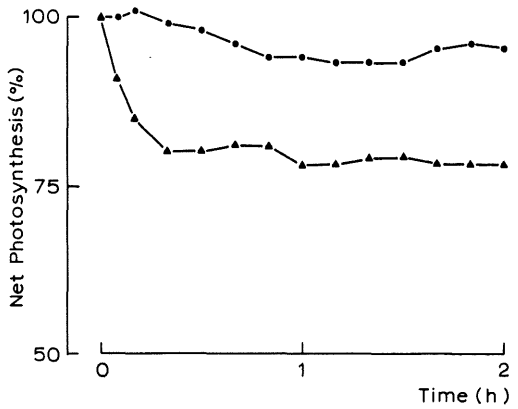

Figure 2. Typical time course of net photosynthesis of *Vicia faba* leaves after the start of fumigation with 800 μg SO_2 m^{-3} for control plants (●) and fumigated plants (▲) at light saturation (mean values of 5 plants; SE ⩽ 4.9% in fumigated plants and 3.2% for control plants).

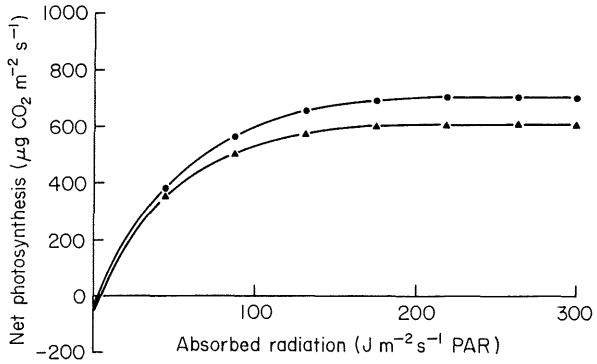

Figure 3. Fitted net CO_2 assimilation light–response curves for *Vicia faba* leaves before (●) and after (▲) a 2-h fumigation period with 400 μg SO_2 m^{-3}.

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:

$$r_m = \frac{\Gamma + K_c}{V_c}\left(1 + \frac{[O]}{K_o} + \frac{[S]}{K_s}\right), \quad (7)$$

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.

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. A strong decrease in net photosynthesis of the fumigated plants occurred within the first 20 min of exposure to SO_2 and stable rates were obtained within 2 h. This pattern is in agreement with the results of Sij & Swanson, 1974; Black & Unsworth, 1979b; Barton *et al.*, 1980; Sisson, Booth & Throneberry, 1981; 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 eqn (1) to the data is presented graphically in Fig. 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 1. The estimated value of P_{max} decreased by 15% as a result of 2 h 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. 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 Hällgren & Gezelius (1982) for pine seedlings.

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 h of fumigation, relative to prefumigation rates, decreased linearly as the rate of SO_2 uptake

Table 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 fumigation with 400 μg SO_2 m^{-3} ($n = 49$)

	Fumigation with SO_2	
	Before	After
P_{max} (μg CO_2 m^{-2} s^{-1})	724 ±21	615 ± 15
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

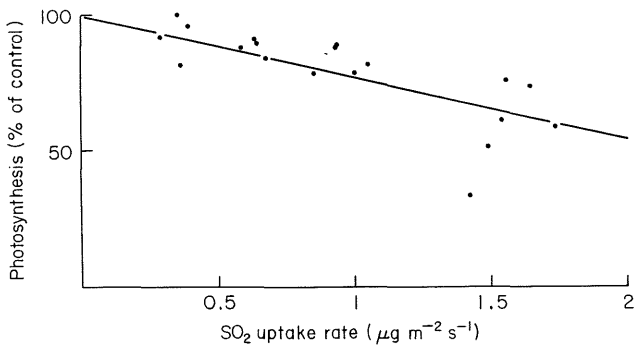

Figure 4. Rates of CO_2 assimilation after a 2-h fumigation period relative to control rates before fumigation in relation to SO_2 uptake rates (F in $\mu g\ SO_2\ m^{-2}\ s^{-1}$). P_n (% of control) = 100–23.19*F($r^2 = 0.55$, $n = 20$).

increased from 0 to 1.5 $\mu g\ m^{-2}\ s^{-1}$ (Fig. 4). This relation is very similar to that reported by Black & Unsworth (1979c) over the range of rates employed here, but they found no further reduction in photosynthesis at higher rates of SO_2 uptake ($>1.5\ \mu g\ m^{-2}\ s^{-1}$). The reduction appeared to be reversible since prefumigation rates of photosynthesis were obtained when plants which had been fumigated with 800 $\mu g\ SO_2\ m^{-3}$ were measured after a 2-h recovery period without fumigation.

The ratio of CO_2 assimilation to transpiration was not significantly affected by SO_2 fumigation (Table 2). The simultaneous reduction of CO_2 assimilation and transpiration may have been caused

Table 2. Ratio of rates of photosynthesis and transpiration (P/T) before and after a 2-h fumigation period with SO_2

n	SO_2 ($\mu g\ m^{-3}$)	P/T	
		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

either directly by an increase in stomatal resistance or indirectly by an increase in mesophyll resistance.

The effect of fumigation with 800 $\mu g\ SO_2\ m^{-3}$ on CO_2 assimilation at light saturation and varying CO_2 concentrations is shown in Fig. 5. The estimated parameter values for the photosynthetic rate at high CO_2 concentrations (P_{max}), the mesophyll conductance (g_m) and the CO_2 compensation point (Γ) of plants fumigated with 800 $\mu g\ SO_2\ m^{-3}$ and of control plants are given in Table 3. The parameter values of the control plants did not change during the 2-h 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. 5). Both the estimated CO_2 compensation point and the mesophyll resistance to CO_2 increased as a result of SO_2 exposure (Table 3). The lack of inhibition of CO_2 assimilation by SO_2 at high CO_2 concentrations was also reported by Carlson (1983) for

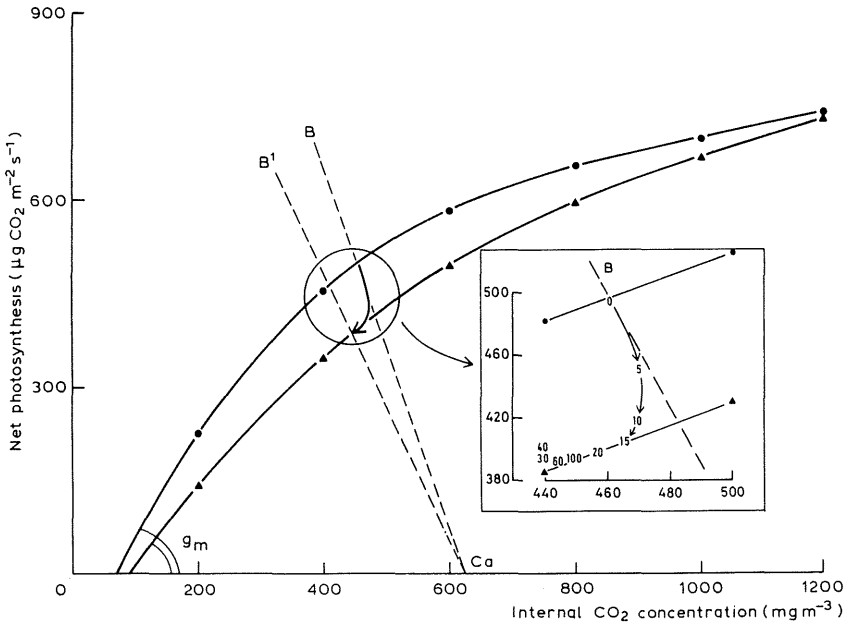

Figure 5. Net CO_2 assimilation rate of *Vicia faba* leaves in relation to calculated internal CO_2 concentration (C_i) before (●), and after 2-h (▲) fumigation with 800 $\mu g\ m^{-3}$ fitted with eqn (2). Dotted lines represent the CO_2 supply functions before (B) and after fumigation (B′) of plants measured at an ambient CO_2 concentration of 340 ppm CO_2 (average values 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 3. Values of CO_2 assimilation at high irradiance and high CO_2 concentration (P_{max}), mesophyll conductance of CO_2 (g_m) and the CO_2 compensation point (Γ) before and after a 2-h fumigation period with 800 μg SO_2 m^{-3} ($n = 22$)

	P_{max} (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)

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.

The role of stomatal resistance in the observed reduction of the rate of CO_2 uptake was analysed by plotting the time course of net leaf photosynthesis versus C_i at an ambient CO_2 concentration of 340 ppm (Fig. 5 inset). A strong reduction in net photosynthesis occurred during the first 10 min 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 4) support the conclusion that stomatal behaviour is not influenced by SO_2. Further analysis of Carlson's (1983) data 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.

Several workers also found stomatal closure in plants of *Vicia faba* 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, Fa & 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 (i.e. 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 vivo* gas exchange measurements it appears that the effects of SO_2 are reversible and suppressed at high CO_2 concentrations (Fig. 5, Black, 1982; Carlson, 1983), which supports the competitive mechanism of SO_2 inhibition suggested by Ziegler (1975). From Table 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

Table 4. The ratio between internal CO_2 concentration C_i and external CO_2 concentration C_a before and after a 2-h fumigation period at high irradiances (300 J m^{-2} s^{-1})

			C_i/C_a	
n*	C_a (ppm)	SO_2(μg m^{-3})	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

* Number of replicates.

separate SO_2 effects on carboxylation and oxygenation of RBP carboxylase/oxygenase.

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References

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

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.

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

Black, V.J. (1984) The effect of air pollution on apparent respiration. In *Gaseous Air Pollutants and Plant Metabolism* (eds M. J. Koziol and F. R. Whatley), pp. 231–248. Butterworths, London.

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. & 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 of *Vicia faba*. *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 vapour pressure deficit. *Journal of Experimental Botany*, **31,** 667–677.

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.

Edwards, G. & Walker, D.A. (1983) *C3, C4: Mechanism, and Cellular and Environmental Regulation of Photosynthesis*, 542 pp. Blackwell Scientific Publications, London.

Farquhar, G.O. & Sharkey, T.O. (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, **33,** 317–345.

Furukawa, A., Natori, T. & Totsuka, T. (1980) The effect of SO_2 on net photosynthesis in sunflower leaf. In *Studies on the Effect of Air Pollutants on Plants and Mechanisms of Phytotoxicity*. Research Reports from the NIES, **11,** 1–8.

Gezelius, K. & Hällgren, J.-E. (1980) Effect of SO_3^{2-} on the activity of ribulose biphosphate carboxylase from seedlings of *Pinus Silvestris*. *Physiologia Plantarum*, **49,** 354–358.

Goudriaan, J. (1977) Crop micrometeorology: a simulation study. In *Simulation Monographs*, 249 pp. Pudoc. Wageningen.

Goudriaan, J. (1982) Potential production processes. In *Simulation of Plant Growth and Crop Production*. F. W. T. Penning de Vries and H. H. van Laar). pp. 98–113. Pudoc, Wageningen.

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.

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

Hällgren, J.-E. & Gezelius, K. (1982) Effects of SO_2 on photosynthesis and Ribulose biphosphate carboxylase in pine trees seedlings. *Physiologia Plantarum*, **54,** 153–161.

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.

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.

Lockyer, D.R., Cowling, D.W. & Jones, L.H.P. (1976) A system for exposing plants to atmospheres containing low concentrations of sulphur dioxide. *Journal of Experimental Botany*, **27,** 397–409.

Louwerse, W. & van Oorschot, J.L.P. (1969) An assembly for routine measurements of photosynthesis, respiration and transpiration of intact plants under controlled conditioning. *Photosynthetica*, **3,** 305–315.

Majernik, O. & Mansfield, T.A. (1971) Effects of SO_2 pollution on stomatal movements in *Vicia faba*. *Phytopathologische Zeitschrift*, **71,** 123–128.

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

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.

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*, (Lancaster) **67,** 539–549.

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.

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.

Sprugel, D.G., 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.

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

Temple, P.J., Fa, C.H. & Taylor, O.C. (1985) Effects of SO_2 on stomatal conductance and growth of *Phaseolus vulgaris*. *Environmental Pollution* (Series A) **37,** 267–279.

Unsworth, M.H., Biscoe, P.V. & Black, V.J. (1976) Analysis of gas exchange between plants and polluted atmospheres. In *Effects of Air Pollutants on Plants* (ed. T. A. Mansfield), pp. 7–16. Cambridge University Press, London.

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 behaviour. *Oecologia* (Berlin), **44,** 296–302.

de Wit, C.T. *et al.* (1978) Simulation of assimilation, transpiration and respiration of crops. *Simulation Monograph,* 148 pp. Pudoc, Wageningen.

Ziegler, I. (1975) The effect of SO_2 pollution on plant metabolism. *Residue Review,* **56,** 79–105.