EFFECTS OF SULPHURDIOXIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *Spinacia oleracea* and *Lycoperscion esculentum*

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CONTENTS

	Page
Certificate	i
Acknowledgement	ii
Physiological and Biochemical Effects of Sulphur dioxide on Higher Plants - A Review	
Materials and Methods	
Results	
Discussion	
Summary	
References	

CERTIFICATE

The research work embodied in this dissertation has been carried out in School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full, for any other degree or diploma of any University.

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Physiological and Biochemical Effects of SO_2 on Higher Plants: A Review

Introduction

Entry and Possible Fate of SO_2 inside Plant Cell

Effect of SO_2 on Chloroplast

- Morphological effects
- Ultrastructural effects
- Interaction of SO_2 with chlorophyll

Effect of SO_2 on Photosynthesis

- Photochemical processes
- Biochemical processes

Ecology of SO_2 Resistance : C_3,C_4 and CAM Plants

Effect of SO_2 on Respiration and Photorespiration

Effect of SO_2 on Photosynthate Allocation and Yield

Task for the Future

INTRODUCTION

Sulphur dioxide pollution has become a worldwide phenomenon due to urban/industrial development. Even in India which still is a developing country, the problem of air pollution is growing due to rapid industrial expansion. During 1966 to 1979, the quantity of sulphur dioxide released from fossil fuel combustion into the atmosphere has tripled. The total sulphur dioxide emission in the country has increased from 1.38 million tonnes in 1966 to 3.20 million tonnes in 1979, an increase of 21 percent more than twice the rate of increase of 8.4 percent experienced in United States during the same period (Varshney and Garg, 1978). There is likelihood of further increase in emission of sulphur dioxide into the Indian atmosphere.

Sulphur dioxide interacts with plants and causes serious damage to vegetation. Various plant processes are adversely affected as well as composition of plant-community is altered (Winner and Bewley, 1978).

Literature on the effects of sulphur dioxide on plants has grown quite voluminous. During the late nineteenth century, the effect of acute sulphur dioxide on plants attracted attention of plant scientists in de-

veloped countries (Hallgren, 1978). Early studies were mostly focussed on documenting visible injury. Such descriptive studies made valuable contributions by stimulating interest in the study of effects of air pollution on plants. In recent years, attention is being focussed to understand the effect of subchronic levels of sulphur dioxide in terms of physiological and biochemical responses (Ziegler, 1973; 1975; Mudd,1975; Malhotra and Hocking, 1976; Horsman and Wellburn, 1976; Davies, 1968; Puckett et al., 1973).

Photosynthesis, which is the main driving force for biomass production, is sensitive to sulphur dioxide pollution. This review isdevoted to the evaluation of the effect of sulphur dioxide on photosynthesis and related organelles in higher plants. Presently, understanding of the effects of sulphur dioxide on photosynthesis in higher plants is far from complete. Consequently, treatment of some of the aspects may appear fragmentary while others may look somewhat speculative.

Entry and Possible Fate of Sulphur dioxide in the Plant Cell

Sulphur dioxide readily enters leaves through stomata. The diffusion of sulphur dioxide into leaf is guided by the same physical processes, namely diffusion

along concentration gradient, which also governs the entry of carbon dioxide into green leaves.

Sulphur dioxide is highly soluble, it forms solution, with surface or tissue moisture in plant leaves. In solution, sulphur dioxide establishes the following equilibria, which have an important bearing on its effects:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3$$

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \quad , \text{pk} = 1.76$$

$$HSO_3^- \rightleftharpoons H^+ + SO_3^- \quad , \quad \text{pk} = 7.20$$

The ionic species formed upon dissolution of sulphur dioxide in water are sulphite (SO_3^{2-}) and bisulphite (HSO_3^-).

The percentage distribution of sulphur species is dependent on pH value. At higher pH the sulphite ion predominates; around pH 4-5, the bisulphite ion; whereas below pH 4, there is an increasing proportion of sulphurous acid H_2SO_3.

Considerable debate has taken place about what species of sulphur dioxide i.e., H_2SO_3, SO_3^{2-}, $S_2O_5^{2-}$, HSO_3^-, is potent for biological injury. Taking physical factors (i.e., external concentration of sulphur

dioxide, temperature, and the pH of the water) which affect flux of sulphur dioxide, Hocking and Hocking (1977) concluded that the major form of dissolved sulphur dioxide at physiological pH values is HSO_3^-.

Hocking and Hocking (1977) has made a comparative evaluation of phytotoxicity of various species of sulphur i.e., H_2SO_3, SO_3^{2-}, $S_2O_5^-$. According to Hocking and Hocking, HSO_3^- is most potent species of sulphur in terms of biological injury.

The various reasons and considerations considered by Hocking and Hocking are as follows:

i. There is negligible H_2SO_3 present in solution at any concentration of total sulphur dioxide (i.e., less than 1/1000 th of the total sulphur dioxide, and in the order of 1/30 th of the unreacted ($SO_2.H_2O$) (Falk and Giguere, 1958; Rabe and Harris, 1963).

ii. There is negligible SO_3^{2-} or $S_2O_5^{2-}$ present in the solution at any time (Falk and Giguere, 1958; Rabe and Harris, 1963).

iii. The principal species tying up sulphur dioxide in aqueous solution is HSO_3^- (to the extent of at least 99 percent of the total sulphur dioxide present at any concentration).

iv. A relatively minor (but significant) component of the total sulphur dioxide is present simply as dissolved unreacted sulphur dioxide i.e., $SO_2.H_2O$ (number of loosely associated water molecules uncertain but may be about 6) (Tammam and Krige, 1925).

v. For a given total concentration of sulphur dioxide in water the amount of unreacted sulphur dioxide arises sharply with temperature (i.e., for $1x10^{-3}$g SO_2/100g H_2O, a factor of almost 4 over 60°C). This is consistent with the rising sulphur dioxide vapour pressure above solution is observed and keeping in with the fact that the fraction of the total sulphur dioxide present in solution is shown to follow Henry's law closely (Johnstone and Leppla, 1934; Arkhipova et al., 1968).

vi. Hocking and Hocking's (1977) data confirm Speeding and Brimblecombe's (1974) criticism of the Hales and Sutter (1973) extrapolation that their non-random error cannot be neglect of pyrophosphates, because of the relative amount produced decreases with concentration of HSO_3^-.

Tanaka (1974) in his discussion of the Hales and Sutter (1973) paper calls attention to the problem of species

distribution. Papers attributing injury mechanisms to HSO_3^-, SO_3^{2-} or other ionic species beg the question of penetration (Rao and Le Blanac, 1965; Puckett et al., 1973). Subsequent to the passage across cell-membrane into cell solute, many reactions become possible including plausible sinks for any species of sulphur dioxide. Rahn and Conn (1944) suggested that undissociated H_2SO_3 is the lethal agency; Vass and Ingram (1949) agreed. But later work, with improved spectroscopic methods showed that H_2SO_3 exists only in negligible amounts (Falk and Giguere 1958; Rabe and Harris, 1963). Hill (1974) recognised Falk and Giguere (1958) conclusion that Sulphur dioxide in solution may be the same molecule as gaseous sulphur dioxide and earlier Hill (1971) hinted that sulphur dioxide itself may be the toxic molecule in soluble, but offered no supporting arguments and did not conceptually separate the stage of penetration with that of active injury.

Hocking and Hocking (1977) inferred that dissolved but unreacted sulphur dioxide is the most active species for initial plant injury in air pollution episodes. Furthermore they showed that the solubility of sulphur dioxide and consequently the equilibrium between atmospheric and aqueous concentrations is dependent on temperature. Thus,

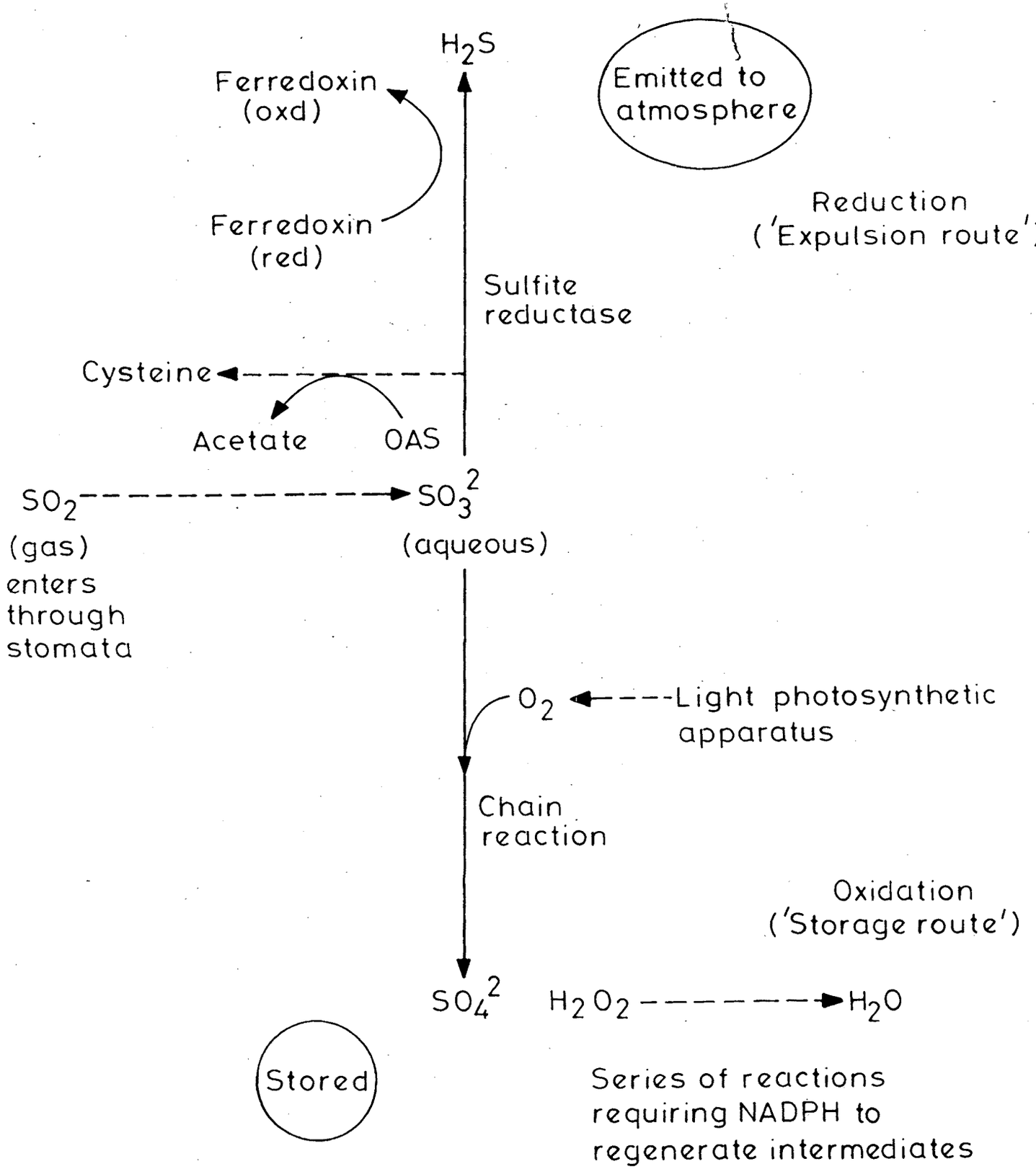


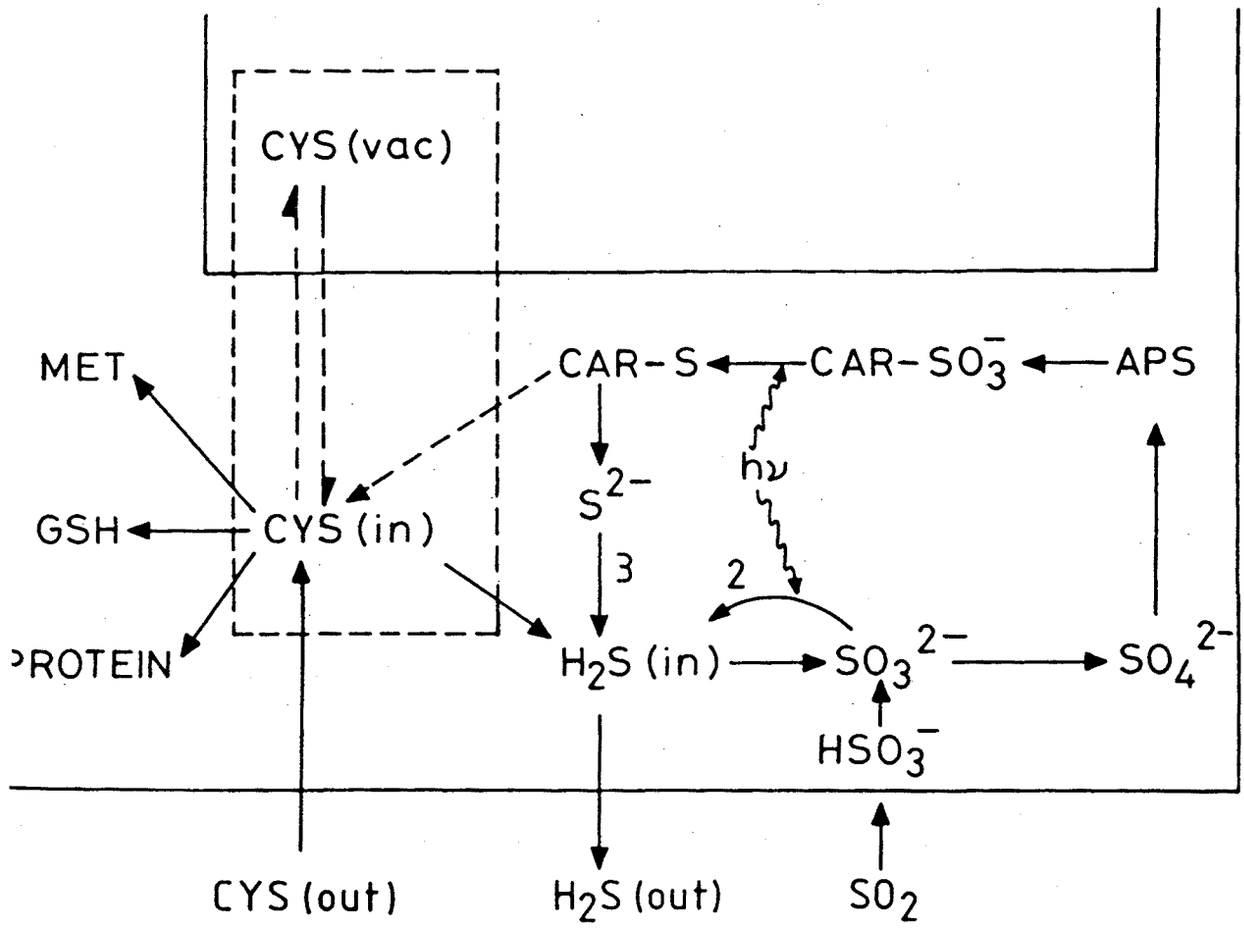
Fig. 1. Possible fates of SO_2 within Plant cell (Source:Koziol & Whatley 1984 pp 196)

if purely physical factors were the only ones affecting flux, gas uptake should be dependent on external concentration, temperature and pH of the water.

Sulfite originating from atmospheric sulphur dioxide may be either oxidized or reduced. Atmospheric sulphur dioxide is oxidized from sulfite to sulfate (storage route) (Weigl and Ziegler, 1962) (Fig.1). Sulfite when converted to sulphate is approximately thirty times less toxic than sulfite and is one of the major products to accumulate (Thomas, 1961). Miller and Xerikos (1979) while working on eight soyabean cultivars found that the four comparatively 'resistant' cultivars converted the sulfite more rapidly than the relatively sensitive cultivars.

Sulfite originating from atmospheric sulphur dioxide is reduced to H_2S gas which can be emitted to the atmosphere (Expulsion route) (de Cormis 1968; de Cormis and Bonte, 1970; Wilson, Bressan and Filner, 1978; Spaleny, 1977; Winner et al., 1981) (Fig.1).

Reactions showing formation of H_2S from sulfite (SO_3^{2-}) a reduction process which takes place within the chloroplast. Three pathways for the formation of H_2S under sulphur dioxide/HSO_3^- stress on plants can be visualized (Fig.2).



ig. 2. Paths of hydrogen sulfide production in leaves (1) Light-independent H_2S synthesis from L-cysteine (2) Light-dependent H_2S synthesis from SO_2 (3) Light-dependent H_2S synthesis from sulfate (Source: Koziol & Whatley, 1984 pp. 301)

i. Light-dependent reduction of sulfate to sulfite may be part of path of H_2S synthesis from sulphur dioxide/ HSO_3^-; subsequent to reduction, sulfite may be split off carrier-bound sulfite, and released as H_2S.(Fig.2).

ii. Alternatively, carrier-bound sulfide may be incorporated into cysteine, from which H_2S may be released by the action of cysteine desuldhydrase(Fig.2).

iii. A third path of H_2S formation may proceed via direct reduction of sulphur dioxide/HSO_3^-(Fig.2).

Oxidation of SO_3^{2-} takes place in plant cells, when they are exposed to sub lethal gaseous sulphur dioxide concentrations, which will cause chronic injury.

When plants are left with excess of sulphur after being incorporated into cysteine and thiol under critical concentration, they release it into the atmosphere. The emission of H_2S may be compared with a pressure valve. Through it excess of sulfur is released out of the intracellular sulfur cycle.

Asada (1967) has partially characterized the enzyme sulfite reductase which catalyzes the reduction. Tamura and Itoh (1974) showed that photosynthetically generated reductant (as reduced ferredoxin) is the physiological

electron donor for the process. Sawhney and Nicholas (1975) and Silivus et al., (1976) demonstrated that process takes place within the chloroplast.

Light-dependent sulfite oxidation takes places within chloroplast (Asada and Kiso,1973). Light-independent sulfite-oxidation is known to take place within mitochondrion (Tager and Rautanen, 1955; Ballantyne, 1977). Asada and Kiso (1973) reported that the presence of either sulphite and/or bisulfite promotes the formation of superoxide radical O_2^- which through a free radical chain reaction initiates oxidation of SO_3^{2-} in illuminated chloroplasts. The rate of sulphite oxidation was higher than the rate of O_2^- production determined by photoreduction of Cytochrome. Asada (1960) indicating that photoreduced O_2^- act as a "trigger" in the oxidation of sulphite in chloroplasts. Halliwell (1981) in his discussion of oxygen-chloroplast interactions, makes the suggestion that the superoxide dismutase (SOD) and ascorbate in the chloroplast are sufficient to 'neutralize' the normal amount of O_2^- produced in vivo. Any increased production of O_2^- would lead to persistence of hydrogen peroxide at concentrations inhibitory to carbon fixation. The following sequence of reactions have

been proposed for sulphite oxidation by O_2^- (Yang, 1970; Tuazon and Johnson, 1977; Asada, 1980).

$$SO_3^{2-} + O_2^- + 3H^+ \longrightarrow HSO_3 + 2OH \quad (1)$$

$$SO_3^{2-} + OH + 2H^+ \longrightarrow HSO_3 + H_2O \quad (2)$$

$$HSO_3 + O_2 \longrightarrow SO_3 + O_2^- + H^+ \quad (3)$$

$$HSO_3 + OH \longrightarrow SO_3 + H_2O \quad (4)$$

$$2HSO_3 \longrightarrow SO_3 + SO_3^{2-} + 2H^+ \quad (5)$$

$$SO_3 + H_2O \longrightarrow SO_4^{2-} + 2H^+ \quad (6)$$

$$2OH \longrightarrow H_2O_2 \quad (7)$$

In the above chain reactions active oxygen species such as H_2O_2 and OH are formed from O_2^- in the cells. This chain can be terminated by the action of superoxide dismutase (SOD) producing hydrogen peroxide. The increased levels of H_2O_2 produced as superoxide dismutase terminates the chain reaction of sulfite photo-oxidation may oxidize the activated enzyme as fructose-1,6-bisphosphatase (FBPase). The oxidation of FBPase may result in inhibition of rate limiting step of Calvin cycle i.e. regeneration of RuBP. The extent to which this would occur would be partially predicted on the rate at which H_2O_2 was removed by the H_2O_2- photoscavenging system

described by Nakano and Asada (1980, 1981) and also by the efficiency with which oxidized form of the enzyme could be re-reduced via the light modulation system.

The active oxygen species such as H_2O_2 and OH formed as a result of free radical chain reaction in the cells. These reduced and excited molecular species of oxygen are highly reactive and oxidize cell components which may cause serious cellular damage in the absence of a suitable scavenger for toxic oxygen species. Different species of active oxygen require specific scavengers such as O_2^- can be scavenged by superoxide dismutase (SOD), H_2O_2 by peroxidase and catalase, O_2 by tocopherols and carotenoids and OH by polyhydroxy compounds such as carbohydrates (Asada, 1980). These scavengers also inhibit further formation of toxic species of oxygen. Tanaka and and Sughara (1980) reported that the sulphur dioxide damage is partly due to the toxicity of active oxygen. Plants protect themselves against such sulphur dioxide induced oxygen toxicity with the help of certain scavenging molecules such as superoxide dismutase and peroxidase. However, their ability to do so varies widely from one species to another. They reported a correlation between high levels of SOD activity and resistance in poplar and spinach leaves to sulphur dioxide. An

increased SOD activity in response to sulphur dioxide fumigation enables plants to counteract sulphur dioxide toxicity. Varshney (1982) found peroxidase activity to be directly correlated with sulphite turnover rate in Zea mays. It may be thus an important factor contributing towards the sulphur dioxide resistance in Zea mays.

Effect of sulphur dioxide on Chloroplast

The chloroplast is the site of photosynthesis. Two distinct types of studies on the effects of sulphur dioxide on chloroplasts have been conducted:

i. isolated chloroplasts have been used as model systems to test reactions to sulphur dioxide stresses, and

ii. chloroplasts isolated from fumigated plants have been investigated

The effects of sulphur dioxide on isolated chloroplasts have been studied using hydration products of HSO_3^- and SO_3^{2-}. Reactions have been discussed at length by Ziegler (1977) and Hallgren (1978). SO_3^{2-} can inhibit electron transport reactions. Libera et al., (1973) showed that SO_3^{2-} inhibited water splitting in chloroplast grana.

In intact chloroplasts SO_3^{2-} appeared to inhibit CO_2 fixation by inhibiting the carboxylation step of Calvin cycle (Libera, Ziegler and Ziegler, 1973). The non competitive SO_3^{2-} inhibition constant is very high for ribulose-1,5 bisphosphate carboxylase (RuBisCo) but the competitive SO_3^{2-} inhibition constant with carbondioxide is lower and closer to the in vivo levels of SO_3^{2-} (1-5 mM). Thus, carbondioxide fixation could be competitively inhibited by SO_3^{2-}. Chloroplast is the site for oxidation and reduction of SO_3^{2-}.

Morphological effects: Studies on this aspect are mainly focussed on Gymnosperms.

Needles of larix. leptolepis (Japanese Larch) fumigated with sulphur dioxide (2.5 ppm for 8 hour during three days) by Mlodzianowski and Bialobok (1977) found gradual changes in the chloroplast profile, from ellipsoidal to oval and then to spherical. These observations were made on mesophyll cells of the middle portion of needles having clear, visible signs of degradation as well. The decrease in the number of grana lamellae was reported by Soikkeli and Tuovinen (1979) in Picea. abies and Soikkeli (1981) in Pinus. sylvestris from mesophyll cells of needles collected from areas polluted mainly by S-compounds Fig. 3(2)

Ultrastructural effects: The chloroplast, a lens-shaped organelle is completely encompassed by a double-membrane. The ground substance of chloroplast, known as stroma is a slightly electron dense granular matrix. Embedded in the stroma is a large number of membrane bounded flattened sacs or thylakoids.

Godzik and Knabe (1973) have reported invaginations from inner membrane of chloroplast envelope, and doubling of the envelope, in needles of Pinus species collected from industrial areas polluted by S-compounds. The same was also observed in Phaseolus vulgaris fumigated with 0.7 ppm sulphur dioxide for 72 hour by Godzik and Sassen (1974). These observations were made from material having no visible symptoms. Chloroplast envelopes of green needles of conifers growing in areas polluted by S-compounds were ruptured at the later stage of cell injury (Soikkeli and Tuovenin, 1979; Soikkeli, 1981).

Godzik and Sassen (1974) described two types of vesicles in the periphery of the chloroplast stroma in sulphur dioxide treated kidney bean leaves. They also found rod-like bundles that may have a connection with crystalline bodies found in stroma of chloroplasts in

Fig.3 The ultrastructure of needles of Norway spruce (Picea abies L. karst.) (1) The chloroplast of a spruce needle from a clean area in winter, (2) The decrease in the number of grana lamellae resulting from sulphur compounds pollution in winter. (3) The increasing lightness in the colour of the plasto-globuli and the accumulation of lipid-like droplets in needles under sulphur compounds pollution in spring. (4) The curling of lamellae in chloroplasts under SO_2 + NO_x pollution in winter. (5) The swelling of lamellae under SO_2 + NO_x pollution in winter - (Magnification in all figures, x 20,000 reduced to two thirds: micrographs by S. Soikkeli) (Source Koziol and Whatley, 1984, pp. 123).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a later stage of damage. Granulation of stroma was observed as the first change induced by sulphur dioxide by Fischer et al., (1973). The granulation has been reported also in Larix leptolepis polluted by sulphur dioxide (Mlodzianowski and Bialobok, 1977), and in Picea abies and Pinus sylvestris growing in S-polluted areas in Finland (Soikkeli and Tuovinen, 1979; Soikkeli, 1981).

Changes described in the chloroplast disks or thylakoids of plants fumigated with sulphur dioxide include swelling of the lamellae and reduction of the grana Fig 3(2) . Wellburn et al., (1972) in Vicia faba (Broad bean), Godzik and Knabe (1973) in some Pinus species, Malhotra (1976) in Pinus contorta and Wong et al., (1977) in Pisum, all described slight swelling of stroma lamellae in the first stage of injury caused by sulphur dioxide. Later the swelling increased and could be detected in the granum thylakoids particularly in those at the 'top' and 'bottom' of the granum stacks. In severe cell injury, all thylakoids were swollen.

Mldozianowski and Bialobok (1977) described two types of injury caused by sulphur dioxide in thylakoids of Larix. leptolepis (Japanese Larch). One involved disappearance of thylakoids and the other their swelling. These authors suggested that the first type prevails in plants which

are more resistant to sulphur dioxide. Godzik and Knabe (1973) in some Pinus species, and Godzik and Sassen (1974) in Phaseolus. vulgaris, reported reduction of grana in otherwise apparently healthy material affected by sulphur dioxide. Later Soikkeli and Tuovinen (1979) in Picea. abies and Soikkeli (1981) in Pinus sylvestris, found that grana were often reduced, consisting of 2-3 lamellae in apparently healthy needles collected from areas polluted by S-compounds. The reduced lamellae were found to swell only at a later stage of injury, after the envelope had shown disintegration.

Plastoglobuli are osmophilic granules which contain, the plastoquinone, an electron-carrier (Bailey and Whyborn, 1963; Lichtenthaler, 1969).

An increase in size and number of plastoglobuli was described in Spinacia. oleracea fumigated with sulphur dioxide by Masuch et al., (1973). They also reported the appearance of many osmophilic granules in close contact with the thyiakoids in sulphur dioxide treated material.

In visibly healthy conifer needles exposed to S-compounds (collected from industrial areas chronically polluted by sulphur dioxide), Soikkeli and Tuovinen (1979) and Soikkeli (1981) reported that the lightening of plasto-

globuli as the first sign of injury Fig. 3(3) . At a later stage, the shape of plastoglobuli changes and their number increased.

Ultrastructural studies have shown that exposure of plants to sulphur dioxide can disrupt the structure of thylakoids and grana Fig. 3(4) , swelling of chloroplast thylakoids Fig. 3(5) , a reduction of grana lamellae, agranulation of chloroplast stroma, stretching of chloroplast envelope. These changes are likely to have important consequences for activities of PSI and PSII and on the light-modulated enzymes of photosynthesis.

Interaction of sulphur dioxide with Chlorophyll

Chlorophyll is the major lipid pigment of thylakoid membranes of chloroplasts in plant-cells. Chlorophyll may undergo several photochemical reactions such as oxidation, reduction, phaeophytinization and reversible bleaching (Vernon and Seely, 1966).

Some of the first experiments of effect of sulphur dioxide on chlorophyll pigment was reported by Rao and Le Blanac (1965) on Lichens (Table 1). They found that laboratory exposure of lichens to lethal doses of sulphur dioxide (5 ppm) resulted in the breakdown of chlorophyll into phaeophytin and Mg^{2+} ions. Similar results were obtained by Coker (1967) with Bryophytes. It is also

Table 1 : Effect of sulphur dioxide pollution on plants with respect to chlorophyll content

Plant species	Habit	Concentration	Duration	Condition	Effect	Reference
Vigna. sinesis	cultivated crop	0.25 ppm SO_2	1.6 hr daily for 40 days	Lab	Decrease in total chlorophyll content	Nandi et al., (1984)
Solanun melogena	cultivated crop	0.5 ppm SO_2	2 hr daily for 42 days	Lab	Decreased in chlorophyll content by 35.5 percent	Agarwal et al., (1983)
Medicago sativa Triticum aestivum Zea mays	cultivated crop	26.6-119.7 ugm^{-3} ground level SO_2 concentration	24 hr for 90 days	Field	Total chlorophyll content reduced	Garg and Varshney (1983)
Medicago sativa Triticum aestivum Zea mays	cultivated crop	218.3 $ugm^{-2}SO_2$ (1.7 gm^{-2}flyash)	1 hr for SO_2	Field and artificially in lab	Increase in total chlorophyll content	Garg and Varshney (1983)
Triticum aestivum	cultivated crop	1 ppm SO_2	2 hr daily for 80 days	Lab	Urea spray increased the amount of chlorophyll	Pandey (1983)
Trigonella foemon graccum	cultivated crop	0.04-1.0 ppm SO_2	3 hr daily for 30 days	Lab	Loss of chlorophyll	Boralkar and Chapekar (1983)
Glycine max Triticum aestivum	cultivated crop	1 ppm	2 hr daily for 60 days	Lab	Glycine less tolerant than Triticum 26.9 percent less toxic	Prasad and Rao (1982)

Table 1. Effects of sulphur dioxide pollution on plants with respect to chlorophyll content

Plane species	Habit	Concentration	Duration	Condition	Remarks	Reference
Butea. monosperma	Wild tree	1,2,3 ppm SO_2	4 hr daily for 15 days	Lab	Maximum decrease in chlorophyll content at 3 ppm	Dubey et al., (1982)
Agropyron smithii	Wild tree	Monthly medium SO_2 conc. 22 ugm^{-3} 175 ugm^{-3}	4 years (1975-78)	Field	Total chl. a and b decreased. Chl a more sensitive than Chl b	Laurenroth and Dodd (1981)
Oryza sativa	cultivated crop	0.25 ppm SO_2	2 hr daily for 90 days	Lab	Decrease in chl content by 27.82 percent	Rao et al., (1981)
Triticum aestivum	cultivated crop	1.0 ppm SO_2	2 hr daily for 80 days	Lab	Decrease in chl. content by 29.6 percent	"
Vicia faba	cultivated crop	0.25 ppm SO_2	2 hr daily for 115 days	Lab	Chl content decrease by 4.06 percent	"
Vigna sinensis	cultivated crop	0.25 ppm SO_2	2 hr daily for 90 days	Lab	Increase in chl content by 12.67 percent was observed by the application of $Ca(OH)_2$	"
Populus americana, Spinacia oleracea	Wild tree, cultivated crop	0.1, 2.0 ppm SO_2			Chl content decrease	Tanaka and Sa hara (1980)
Pinus contorta	conifer tree	100-500 ppm	22 hr at 23°C	Lab 5-6 months old needles incubated in aqueous SO_2 solution	At 100 ppm SO_2 conc. not much effect upon chl a and b. At 250-500 ppm Chl a more sensitive than chl b	Malhotra and Hocking (197

known that light accelerated the phaeophytinization (Krasnowskij, 1969).

Chlorophyll a and chlorophyll b have received most attention in pigment studies; chl a tends to be destroyed at a faster rate than chl b, as can be seen from *in vivo* and *in vitro* studies (Bortitz, 1964; Katz and Shore, 1955).

It has been demonstrated that the effect of sulphur dioxide on pigment breakdown and photosynthesis is a specific effect and is not a function of increased acidity. From experiments with *Pinus contorta*, Malhotra (1977) reported that concentrations below 100 ppm sulphur dioxide in solution had no effect on chl a or phaeophytin a. However, at lower concentrations of sulphur dioxide (10-50 ppm) a significant increase in chlorophyllase activity was detected and chl b was converted to the corresponding chlorophyllide b (The ending -ide indicates the porphyrin without the alcohol side chain). The enzyme converts chlrophylls to chlorophyllide by removal of the phytol group. Chlorophyllase, which was discovered by Willstatter and Stoll (1910), is intimately associated with chlorophyll and its activation is also influenced by light (Holden, 1961). However, it is possible that this enzyme

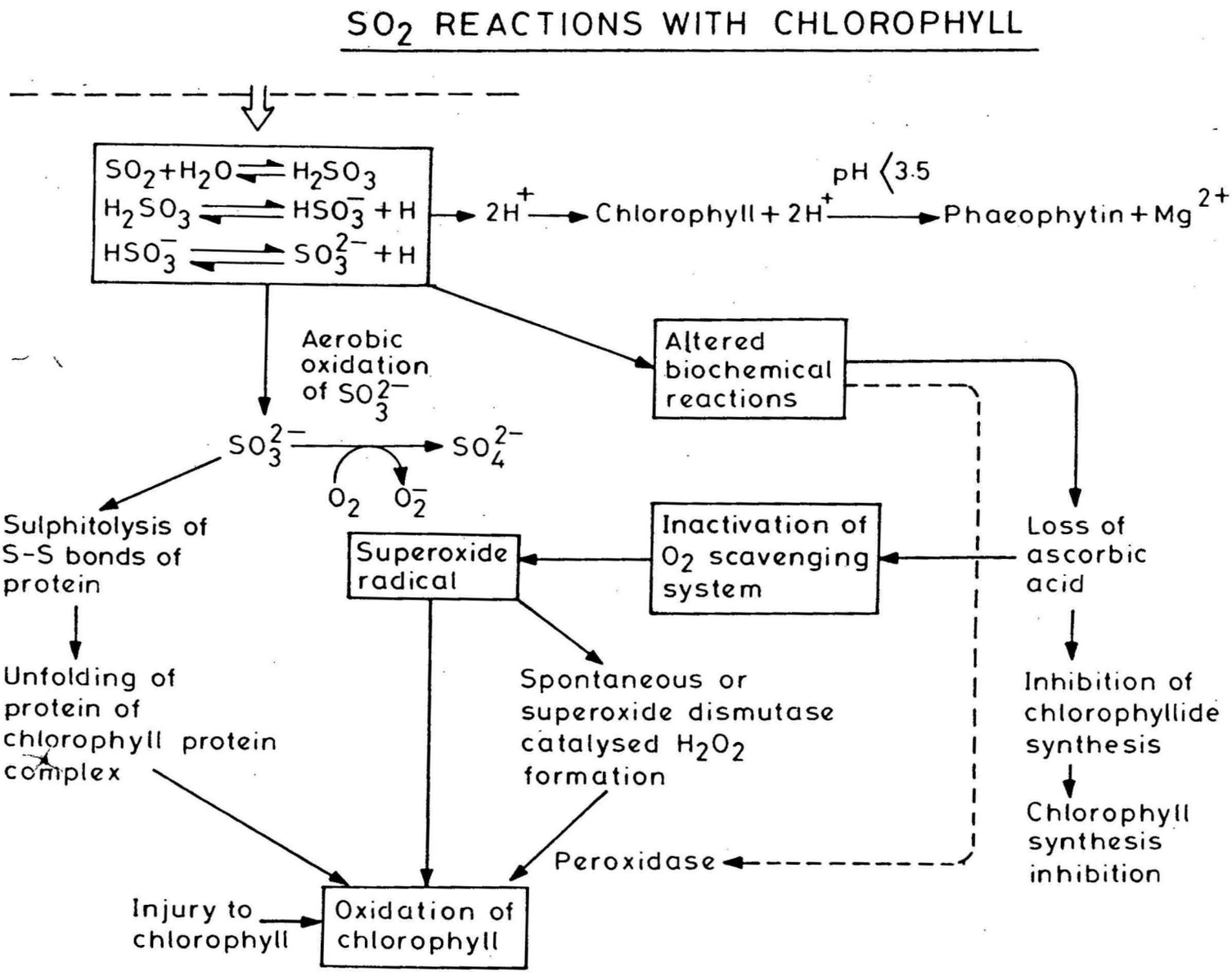

Fig 4. Interaction of SO_2 with Chlorophyll (source: Rao, Air Pollution and Plants A state of the art report, pp-121.)

is associated with a lipoprotein chlorophyll complex and thus is inactive *in vivo*. The maximum activity is obtained when the enzyme is solubilized. The influence of sulphur dioxide on this hydrolytic reaction mediated by chlorophyllase in plants remains to be fully investigated.

The *in vivo* chlorophyll destruction by sulphur dioxide is complex to evaluate, and vast majority of work merely confirms that there is pigment destruction by pH, light and other factors (Fig. 4). Hence, the mechanism of chlorophyll destruction is not explained, although the most likely reaction is an oxidation of pigment molecule. The exact mechanism of oxidation and attack by sulphur dioxide on chlorophylls *in vivo* is not known. It is possible that this may be due to an effect on redox potentials of the pigment-carrier complexes. One possible explanation of the irreversible photoxidation of chlorophyll *in vivo* is that sulphur dioxide formed radicals which inhibit the electron transport chain, thus inhibiting reversible reduction of reaction centre. This might lead to an oxidation of light-harvesting antenna of chlorophyll.

Recently, Sugahara *et al.*, (1980) showed that, *in vitro*, water-soluble protein complexes of chlorophyll

and chlorophyllide were stable and were not destroyed by even 40 mM SO_3^{2-}. The photoconversion of the dark form of chl a and chlorophyllide a protein complex (CP 668) to the illuminated form (CP 743) is influenced, however, inhibited by SO_3^{2-}. The inhibition was apparently due to irreversible denaturation of protein component in the pigment protein complex, probably caused by destruction of disulphide bonds.

$$R\text{-}S\text{-}S\text{-}R + SO_3^{2-} \text{ ------ } R\text{-}S\text{-}SO_3^{2-} + R\text{-}S$$

(Cecil and Mc Phee, 1955)

Rapid in vitro chlorophyll destruction can also be caused by free radicals produced during the oxidation of HSO_3^- catalyzed decomposition of linoleic acid hydroperoxide (Peiser and Yang, 1977, 1978). Recently, Shimazaki et al., (1980) presented evidence that sulphur dioxide fumigation of leaves increases the formation of superoxide radical O_2^- in chloroplasts that in turn destroys chlorophylls (Fig. 4). Superoxide radical has been shown to influence chlorophyll at very low concentrations (10^{-8} to 10^{-7}M) (Asada et al., 1977). In Spinacia. oleracea leaves gaseous sulphur dioxide destroyed chl a more rapidly than chl b, but the loss of chl a was not accompanied by corresponding increase in phaeophytin a (Shimazaki et al., 1980a). Free

radical scavengers inhibited chlorophyll (Polyhydric sugars) breakdown in Spinacia oleracea leaves, it goes to suggest that sulphur dioxide destroys chlorophyll mainly by free-radical oxidation.

Effect of Sulphur dioxide on Photosynthesis:

Photosynthesis is key process which results in harnessing of solar energy into chemical energy through a complex chain of reactions. Various workers have shown on the basis of field and laboratory studies that sulphur dioxide (> 0.2 ppm) have been shown to promote yield in Medicago sativa (Thomas et al., 1943) and net photosynthetic rates (Katz, 1949). Continuous exposure to low concentrations (0.15-0.45 ppm) of sulphur dioxide are known to bring about premature senescence (Guderian, 1977). Libera, Ziegler and Ziegler (1973) demonstrated that exposure of isolated spinach chloroplasts to low concentrations of sulfite below >1 mM) produced a stimulation of carbon fixation. Higher levels of sulfite (upto 3 mM) stimulation photosynthetic electron transport but inhibited carbon fixation. They were able to show that stimulation by low concentrations of sulfite occured at the bisphosphatase step. Ziegler's group extended this approach to the alga Chlorella vulgaris in which it has been shown that the presence

of low concentration of sulfite (1mM) increased growth rates (expressed as cell number, protein, chlorophyll and yield) even under conditions of sulfate sufficiency. This increased yield was accompanied by an increase in the rate of carbon fixation. At higher sulfite concentration rates of carbon fixation were still higher than those of the control. However, yield had dropped below control levels. Miszalski and Ziegler (1979) showed that exposure of whole spinach plants to 0.67 ppm (1.8 mg m^{-3}) sulphur dioxide for 1 hour produced increases in chloroplast membrane thiol groups and an increase in the light activation of NADP-GPD. Paul and Bassham (1971) demonstrated a stimulation of carbon fixation by sulfite in isolated cells of the opium poppy (Papaver somniferum). Pierre (1977) and Pierre and Queiroz (1981, 1982) showed that exposing whole bean plants over a long term to low concentrations of sulphur dioxide (0.1 ppm) increased rates of activity of several enzymes present in the soluble phase of leaf extracts. An increase in serine levels was also observed. These plants also become prematurely senescent as serine levels was also observed.

Taken together, these data suggest that sulfite at low concentrations can stimulate light activation through increasing the concentrations of membrane-bound

thiol groups. This in turn can bring about higher carbon fixation rates, the consequence of which for algal cells can be either increased or decreased productivity. In higher plants, it appears that a consequence of this increased rate of metabolism can be premature senescence. Serine has been implicated in metabolic changes accompanying senescence (Nooden, 1980). Studies on the effect of sulphur dioxide on photosynthesis ranged over a wide spectrum of different photosynthetic reactions, not all of which have been studied with equal intensity and rigour. However, the available information on the effect of sulphur dioxide on photosynthesis have been discussed under two broad categories namely (a) photochemical processes and (b) biochemical processes.

Photochemical Processes

Within chloroplasts, the light reactions which produce ATP and NADPH are mainly associated with the lamellae or thylakoids while the Dark reactions which enzymatically fix CO_2 into acid-stable compounds occur within stroma.

Although chlorophyll is clearly involved in the transformation of radiant energy to chemical energy, details of intermediates and pathways are poorly under-

stood. Three major products are formed as a result of the absorption of radiant energy by chloroplast pigments - oxygen, ATP and NADPH.

The following three aspects regarding the effect of sulphur dioxide on photochemical related events have been mainly investigated:

i. Fluorescence

ii. Hill reaction

iii. Photosynthetic electron transport

i. Fluorescence: Fluorescence refers to the rapid emission of light following chlorophyll excitation. Sulphur dioxide affects chlorophyll fluorescence has been observed by many workers. Arndt (1974) stated that to study, effects of sulphur dioxide, in vivo, chlorophyll fluorescence is one of the useful tool. Several fluorescence parameters are measurable, although fluorescence spectra and relative fluorescence yield are the the most readily studied (Paperogiou, 1975).

The variable fluorescence (Kautsky effect, consists of a fast change and slower fluctuation) will be affected if sulphur dioxide causes blockage on either side of the oxidizing or reducing of the PSII photoreaction. The effects of sulphur dioxide on the variable fluorescence

and the fluorescence spectra have been studied by Hallgren et al., (1978). At pH 8.0 and 1.0 mM SO_3^{2-} there were increases in the fluorescence yield of spinach chloroplasts, but the opposite effect was observed at pH 6.2, where HSO_3^- dominates (Hallgren, 1978). Arndt (1974) has noticed both a slight SO_3^{2-} stimulation of fluorescence at low concentrations and a decrease at higher concentrations ($>$ 1mM 10^{-3}M), indicating two oxidizing and reducing agents) different modes of action of this compound on the electron transport chain in photosynthesis.

ii. Hill reaction: Ultrastructural studies have shown that exposure to sulphur dioxide can disrupt the structure of thylakoids and grana within the chloroplasts, and such disruptions are likely to have important consequences on the activities of PSI and PSII. As PSI and PSII are both localized in the membranes of chloroplasts (Boardman, 1968), a decrease in the Hill reaction (photoproduction of oxygen by chloroplasts).

$$A + H_2O \xrightarrow[\text{chloroplasts}]{h\nu} AH_2 + \tfrac{1}{2} O_2$$

Hill reaction activity was accompanied by swelling and disintegration of chloroplast membranes (Malhotra,1976).

Malhotra (1976) isolated chloroplasts from needles of Pinus contorta (Lodgepole Pine) treated with (50-1000 ppm) concentrations of aqueous sulphur dioxide showed that, at a low concentration (50 ppm), sulphur dioxide stimulated Hill reaction activity, but this activity was completely inhibited at high concentrations (500-1000 ppm).

iii. Photosynthetic electron transport: Recently, Shimazaki and Sughara (1980a, 1980b) studied in detail the effect of gaseous sulphur dioxide on chloroplast photosynthesis in Spinacia oleracea. Fumigation with sulphur dioxide at 1 and 2 ppm for 1 hour produced no effect on 2,6-dichloro-indophenol (DCIP) photoreduction (Hill reaction); however, there was rapid inhibition following longer exposures (for 3-6 hour at 1 and 2 ppm).

Sulphur dioxide did not inactivate the electron flow from the reductant (H_2O) to primary electron acceptor (Q) of PSII. Time-cause analysis of fluorescence intensity of SO_2- treated plants indicated that sulphur dioxide inhibited the accumulation of reduced Q. Furthermore, the addition of 3-(3,4 dichlorophenyl)-1, 1-dimethyl urea (DCMU), an inhibitor acting on reducing site of PSII (Bishop, 1958), caused a rapid increase in

fluorescence in sulphur dioxide inhibited chloroplasts. This suggests that Q was in the oxidized state. This could happen because of sulphur dioxide inactivation of either primary electron donor or the reaction centre, itself in electron transport chain.

Reactions (PSII activity DCIP photoreduction, sulphur dioxide fumigation was performed at 2.0 ppm).

i. H_2O + Ferredoxin --> Inhibition of e^- flow to NADP

ii. DCIP + Na ascorbate + DCMU $\xrightarrow{e^-}$ $DCIPH_2$

iii. Tricine-NaOH + Sucrose + NaCl + NH_4Cl + Chlorophyll + DCIP --> Inhibition of e^- flow

Shimazaki and Sughara (1980a) investigated the site of sulphur dioxide attack (at 2.0 ppm for 5 hour and 1.0 ppm sulphur dioxide for 6 hour) in the electron transport systems by studying both photosystems. Electron flow from H_2O to DCIP was inhibited while that from reduced DCIP to NADP ($DCIPH_2$ - NADP) was not affected under uncoupled conditions. Sulphur dioxide inhibited the overall electron flow from H_2O to NADP to the same degree as the electron flow from H_2O to DCIP. These results, suggest that sulphur dioxide inhibited the electron flow driven by PSII but not that by PSI. A similar effect of sulphur dioxide was observed in photosystems of

Latuca sativa (Garden Lettuce) chloroplasts (Shimazaki and Sughara,1980b). The work with chloroplasts isolated from sulphur dioxide fumigated leaves of Latuca. sativa (Shimazaki and Sughara, 1980b) demonstrated that the site of sulphur dioxide action was located closer to the oxidizing site rather than the reducing site of PSII. This was supported by the observation that the addition of an artificial electron donor for PSII, diphenylcarbazide (DCP), did not change the rate of DCIP reduction in PSII. The work of Shimazaki and Sughara (1980b) also suggests that the results of in vivo effects of sulphur dioxide on both photoelectron transport and photophosphorylation and in vitro effects of treatment of isolated chloroplasts with SO_3^{2-} either produced no overall effect on electron transfer (Asada et al., 1965) or else stimulated a non cyclic type of electron transport (Libera et al., 1973). Non-cyclic electron transport is so-called because of its unidirectional nature - i.e., chlorophyll molecule excited by a captured photon transfers an electron to $NADP^+$ (or ferricyanide).

The effect of gaseous sulphur dioxide seems to be specific and not associated with acidity released decrease in PSII activity, as a decrease in PSII activity due

to low pH could be restored by adding electron donors of PSII but nit in chloroplasts from sulphur dioxide treated plants (Shimazaki and Sughara, 1980b). The differences between *in vivo* effects of sulphur dioxide on both photoelectron transport and phosphorylations, and *in vitro* effects of treatment of isolated chloroplasts with aqueous sulphur dioxide (HCO_3^-, SO_3^{2-} and SO_2) are difficult to reconcile. Shimazaki and Sugahara (1980a) have attributed such differences to production of O_2^- and other radicals during photooxidation of SO_3^{2-}.

Biochemical Processes

Calvin and Bassham (1962) established the sequence of biochemical interconversions within the chloroplast leading to carbon fixation. This phase was initially thought to be light independent, although it used ATP and NADPH produced during the 'light' phase.

In Reductive Pentose Phosphate (RPP) cycle, the first step is the photoassimilation of CO_2 into Ribulose bisphosphate (RuBP) to produce two molecules of 3-phosphoglyceric acid (3-PGA), which via 1,3-bisphosphoglycerate (BPGA) is reduced to the triose phosphate, 3-phosphoglyceraldehyde (3-PGAL). An isomerase converts some to 3-PAGL and some to dihydroxyacetone phosphate

(DHAP). In the presence of fructose-1, 6 bisphosphate (F6P) which is dephosphorylated to yield fructose-6-phosphate (F6P). Part of the F6P recycles to form Ribulose bisphosphate (RuBP) and the balance of which is used either in starch or sucrose synthesis.

In Reductive Pentose Pathway (RPP), five enzymes are activated by light, they are:

1. Ribulose bisphosphate carboxylase (RuBisCo) - This enzyme brings about carboxylation of RuBP.
2. NADP - linked glyceraldehyde-3-P-dehydrogenase (NADP-GPD). This enzyme brings about dehydrogeneration of glyceraldehyde.
3. Fructose bisphosphate phosphatase (FBPase): brings about the removal of phosphate group from C-6 sugar. (dephosphorylation)
4. Seduheptulose bisphoaphate phosphatase (SBPase): brings about the removal of phosphate from C7 sugar. (dephosphorylation)
5. Phosphoribulokinase: Regeneration of ribulose in presence of ATP.

Light modulation refers to light-induced change in chloroplast stromal pH i.e., from pH 7.0 to 8.0 and increase in Mg^{2+} concentration. The function of the light

activation mechanism in photosynthesis, is to ease plant cell in switching over from one function to another, i.e., from carbon fixation (via the reductive pentose phosphate pathway) in light to starch breakdown (via the oxidative pentose phosphate pathway and glycolysis) in the dark. Thus, light modulation enables this mechanism compartmentalization, these two processes within chloroplast envelope itself.

Conformational changes in protein structure (which brings about activation of light-induced enzymes) can be blocked by reaction with sulfite (Koziol and Whatley, 1984). Changes such as these are thought to occur during light modulation of the chloroplast enzymes. Membrane-bound and possibly stromal dithiol groups generated in the light are known to participate in the modulation mechanism.

NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD) and glucose-6-phosphate dehydrogenase (G6P). Glucose-6-phosphate dehydrogenase is the first enzyme in the oxidative pentose phosphate pathway and is inoperative in the light, have been shown to be sulfite-sensitive by Ziegler, Marewa and Schoepe (1976), Anderson and Avron (1976), respectively.

Ziegler (1977) while working on Spinacia oleracea showed that sulfur from either sulphur dioxide or SO_3^{2-} could be incorporated in the chloroplast thylakoids to a much greater extent than sulfate (SO_4^{2-}) sulfur. It has been suggested that SO_3^{2-} could be directly incorporated into the sulfuric groups of sulfolipids (as reported by Benson, 1963) or was taken up at the binding sites in the thylakoids (Schwenn, Depka and Hennies, 1976). Hampp and Ziegler (1977) reported that both SO_3^{2-} and SO_4^{2-} are transported to the inner chloroplast membranes by phosphate translocators and that light modulators this process.

Ziegler and Hampp (1977) presented evidence that the light-induced generation of chloroplast membrane-bound-SH group which is prerequisite for association of SO_3^{2-} with the membranes. This exogenous sulfite gets incorporated into the sulfate assimilation pathway in chloroplasts(through binding with "carrier-SH" (Schiff and Hodson, 1973). This car-SH provides the substrate for Car-S-SO_3 formation.

Anderson and Duggan (1977) extended the study of effects of sulphur dioxide and sulfite on light modulation of chloroplast enzymes. They found that the acti-

vation of NADP-GPD was inhibited by 200 uM SO_3^{2-}, but the activation of SBPase was stimulated enzyme having two functional activities. The same protein can either catalyse carboxylation of the substrate RuBP to form two molecules of 3-phosphogylcerate or it can catalyse react with oxygen to give one molecule of 2-phosphoglycolate and 3-phosphoglycerate. Both these catalytic-reactions are thought to involve a single active site.

Libera, Ziegler and Ziegler (1975) demonstrated that, with isolated chloroplasts and concentrations of sulphite greater than 1 mM, fixation of $^{14}CO_2$ declined rapidly, and at 5mM was reduced to 20 percent. The relative amounts of radioactivity in phosphoglycerate and sugar phosphate were decreased whereas those in aspartate and malate were increased. This indicated a possible shift towards the C_4 dicarboxylic type of fixation and may indicate a higher sensitivity of RuBP carboxylase than of PEP carboxylase towards sulphite. Horsman and Wellburn (1975) exposed Pisum. sativum var. Feltham to known amounts of sulphur dioxide and/or NO_2 for six days. At the end of this period RuBP carboxylase was extracted and assayed. Whilst little change was observed by 5mM sulfite. The activation of NADP-GPD was also inhibited in Pea (Pisum sativum) seedlings which were exposed to 5 ppm sulphur dioxide for

1 hour.

Further insight into this process was developed by Ruth Alscher and Herman (1982). They carried out a study of *in vitro* sulfite effects and so sulphur dioxide *in vivo* on light activation of alkaline, FBPase in two cultivars "Besson" and "Hark" of Soyabean (*glycine max.*) Differential sulfite susceptibility of sulphur dioxide sensitive and sulphur dioxide tolerant soyabean varieties, was found to be associated with the soyabean chloroplast membranes and not with the soluble stromal phase of chloroplasts. The sulfite-sensitive membrane protein described by Mohamed and Anderson (1981) also by Heuber Hansen and Anderson (1982) is likely candidate for this site. Alscher (1984) proposed that one basis for sulfite sensitivity of light activation is due to the binding of sulfite to the chloroplast membrane at a site which is crucial for activation.

Effect of sulphur dioxide on RuBisCO:

RuBP carboxylase is a complex enzyme having two functional activities. Hallgren and Gezelius (1982) showed that fumigations with 'low' sulphur dioxide concentrations (400 ug $SO_2 m^{-3}$, 0.15 ppm) for 8 days, in *Pinus sylvestris*, decreased RuBP carboxylase when expressed on a dry weight basis. This indicates a dec-

rease in the amount of active enzyme present rather than in its specific activity.

Ziegler (1972) found SO_3^{2-} inhibited RuBP carboxylase competitively with respect to bicarbonate; presumably SO_3^{2-} replaces HCO_3^- by reacting at the same enzyme site. SO^{2-} showed a non-competitive inhibition with respect to RuBP and Mg^{2+}. Since sulphur dioxide binds to the enzyme in the same way as CO_2, the degree of inhibition by SO_3^{2-} will be independent of the RuBP and Mg^{2+} concentrations but highly dependent on the concentrations of CO_2 at the reaction site. If this is the case it follows that in plants with the C_4 type of photosynthesis and an increased concentration of CO_2 in the bundle sheath cells, sulphur dioxide should be a less powerful inhibitor.

Photochemical effects are short-term effect studies while yield measurements are long-term studied effects. Therefore, a critical correlation between the results of the effects of sulphur dioxide on photochemical and biochemical processes of photosynthesis has not been attempted. Nonetheless sulphur dioxide effects on photo synthesis at various steps cannot be overlooked.

Effect of sulphur dioxide on the Allocation of Photosynthate Allocation and Yield

Plant growth depends on the coordinated acquisition, allocation and use of carbon, water and nutrient resources to major plant organs (root, stem, leaf, flower and fruit) and to the major classes or metabolic function (vegetative growth, maintenance, defense and reproduction). Air pollutants like sulphur dioxide can directly damage plant tissues and disrupt normal patterns of resource acquisition and allocation.

Not enough attention has been paid to the effect of sulphur dioxide on dry matter distribution in plants.

Bell (1982) assembled data from several studies on the effects of sulphur dioxide on the growth of grasses and found that greater response in roots (decrease in growth) than in shoots often occured. Increases in shoot: root ratio have been found in many dicotyledons fumigated with sulphur dioxide including trees (Freer-Smith 1984). Jones and Mansfield (1982) exposed Phleum pratense to 120 ppb sulphur dioxide for 40 days, beginning 10 days after sowing. Growth analysis were conducted at intervals and the time of appearance of inhibited the growth of roots of seedlings of Zea mays. Both the rates of cell elongation and cell multiplication in roots were reduced.

Two main schools of thought have been pursued to explain effects of sulphur dioxide on assimilate partioning.

i. Brouwer (1963) suggested that proximity of expanding leaves to photosynthesizing leaves, compared with remote location of roots meristems, means that expanding leaves tend to gain priority for assimilate distribution when assimilates are in short supply. Thus, sulphur dioxide which inhibits photosynthesis, causes a redistribution to roots underground organs reducing the normal quota of photosynthates.

ii. Noyes (1980) showed that translocation was inhibited by 39,44 or 69 per cent in Phaselous vulgaris (bean) exposed for 2 hour to 0.1, 1.0 or 3.0 ppm sulphur dioxide (0.26,2.62 or 7.85 ug m^{-3}), respectively while Teh and Swanson (1982) found exposure to 2.9 ppm sulphur dioxide (7.60 ug m^{-3}) for 2 hour inhibited translocation in bean by 45 percent.

In Noyes studies, the quantitative changes of net photosynthesis and translocation in Phaselous vulgaris (sulphur dioxide 100 ppb) appeared to be very differently related to dose of sulphur dioxide. Noyes suggested that because sulphur dioxide decreased translocation (by 39

percent) without corresponding decrease in photosynthesis, it was likely that sulphur dioxide inhibits the mechanism of translocation directly. Autoradiographic studies of his material suggested that sulphur dioxide might inhibit sieve-tube loading.

Like Noyes, Teh and Swanson (1982) found that exposure of the source leaf of Phaselous vulgaris for 2 hour to 2.9 ppm sulphur dioxide inhibited the rate of photosynthesis by 75 percent and the same time the rate of translocation of photosynthate out of leaf fell by 45 percent. Therefore, a larger proportion of the photosynthates were retained. They pointed out that the same proportional effects on translocation in SO_2 polluted leaves was less than predicted i.e., the effect of sulphur dioxide could not be entirely accounted for by its inhibition on photosynthesis. Koziol and Jordan (1978) found increased levels of starch and sugars in the leaves of Phaselous vulgaris of sulphur dioxide polluted plants (3.06 ppm for 24 hour), which is consistent with the inhibition of phloem loading.

Kasana and Mansfield (1986) have pointed out that information on the effect of sulphur dioxide stress on root systems especially in grasses is lacking. Gener-

ally it is assumed that the above ground plant parts are more affected than the roots because they are not directly exposed to pollutant molecules in the atmosphere. Pollutant-induced reductions in root growth are likely to have important consequences in perennials such as grasses, in which regrowth after cutting and grazing is dependent upon the reserve assimilate stored in the roots.

Ecology of Sulphur dioxide Resistance: C_3, C_4 and CAM plants: One of the major objective in the analysis of effects of sulphur dioxide pollution at the physiological and biochemical level is the development of an understanding of what determines relative resistance or susceptibility. Resistance of plants tosulphur dioxide is determined both by sulphur dioxide tolerance and sulphur dioxide avoidance (Levitt 1972; Taylor, 1978). Stress tolerance is further divided into strain avoidance and strain tolerance.

Avoidance involves the exclusion of the pollutant from the plant, primarily by increased stomatal resistance (Mansfield and Freer-Smith, 1984) but increased leaf pubescence (Sharma and Butler, 1973, 1975). Tolerance involves minimising the effects of a pollutant

through detoxification or by metabolic compensations. In the case of sulphur dioxide the absorbed pollutant can be detoxified by oxidation to sulphate (Ballantyne 1977; Varshney, 1982; Garsed and Read, 1977) or reduction to sulfide and emission as H_2S gas (De Cormis, 1968; Filner et al., 1984). Metabolic compensation is perhaps best exemplified by a pollutant-induced transcription of isoenzymes of various constitutive enzymes (Weinstein, 1977). Varshney (1982) found sulphur dioxide to alter isoenzyme profile of glutamate dehydrogenase in P. radiatus, B. nigra and Z. mays exposed to 3, 5 and 10 pphm sulphur dioxide for six weeks.

Winner and Mooney (1980a, 1980b, 1980c, 1982) in their series of papers described a method for partitioning changes in photosynthesis between stomatal and non-stomatal components. For stomatal component they concluded that plants with high conductance will absorb more sulphur dioxide during comparable fumigations than plants with low conductance. They were the first to employ a diagnostic gas exchange technique to explain the effect of sulphur dioxide on ecologically diverse plant species.

Sulphur dioxide resistance is the result of interplay between the ecological, morphological and physiological characteristics of a plant (Winner and Mooney, 1980b). For native species, in polluted area, these characteristics have evolved through natural selection and can be interpreted in evolutionary context. A number of studies have suggested that plant populations growing near sulphur dioxide sources are more sulphur dioxide resistant than populations found in SO_2- free-air. Geranium carolinianum populations differed with respect to the formation of visible injury following an acute sulphur dioxide dose (Taylor and Murdy, 1975) and this intraspecific difference in sulphur dioxide resistance was found to be heritable (Taylor, 1978). Since plants of both G. carolinianum populations absorbed similar quantities of sulphur dioxide during fumigations, the physiological mechanisms accounting for these differences in sulphur dioxide resistance seemed to be related to differences in the capacity of mesophyll tissue to assimilate, detoxify or repair biochemical damage from sulphur dioxide (Taylor and Tingey, 1981).

Populations of Lolium perenne also differ in sulphur dioxide resistance into sensitive plants being associated

plasm. CO_2 provided by decarboxylation of molate aspertate enters chloroplasts of bundle sheath cells where it combines with RuBisCo and C_3 interconversions take place. Thus, this division of labour helps C_4 plants in maintaining internal CO_2 constant with that of ambient CO_2 concentration (Akita and Moss 1972; Goudrien and Von Ler 1978; Louwerse, 1980). C_4 plants show "kranz anatomy" i.e., chloroplasts are concentrated near bundle sheaths (Osmond et al., 1969) which may render them less vulnerable to absorb sulphur dioxide than more uniform chloroplast distribution of C_3 plants. Further, the chloroplast arrangement in C_4 plants may facilitate detoxification of sulphur dioxide absorbed in the immediate vicinity of bundle sheath cells via assimilatory sulfur reduction.

Biochemical contrast between C_3 and C_4 species may also contribute towards differences in their sulphur dioxide responses. The initial CO_2 fixation enzymes differ for these two photosynthetic processes. Ziegler (1972, 1973) with the help of in vitro studies have shown that carboxylating enzymes of both photosynthetic types are competitively inhibited by sulfite with respect to bicar-

bonate. However, PEP carboxylase from corn, a C_4 plant, had greater bicarbonate affinity and therefore better sulphur dioxide exclusion than RuBP carboxylase from spinach, a C_4 plant. Both carboxylating enzymes are found in spinach chloroplasts (Rosenberg et al., 1958) although RuBP carboxylase is primarily responsible for initial fixation of CO_2. In vitro studies carried out by Mukerji and Yang (1974) have shown that PEPCO alloenzyme (some enzymes are built to bind compounds at sites other than the catalytic sites so as to alter the rate of reaction) from spinach chloroplasts was relatively more sensitive to sulphur dioxide than PEPCO alloenzyme from corn (Ziegler, 1973) but was comparatively less sensitive than RuBisCo from spinach (Ziegler, 1972). Significance of such differences in the response of various enzymes at physiological levels is not understood.

Carlson and Bazzaz (1982) measured photosynthetic responses of C_3 and C_4 plants fumigated with sulphur dioxide at elevated CO_2. They proposed that because of stomatal component, C_3 plants, on being fumigated with sulphur dioxide at elevated sulphur dioxide levels, could increase photosynthetic rate with increased CO_2 and compensate for rate reduction caused by sulphur dioxide while C_4 plants are not able to compensate in

can be expected, since C_4 species maintain high CO_2 fixation rates even when stomatal conductance is low. On the other hand, photosynthetic rates of C_3 plants decline in concert with stomatal closure (Wong et al., 1979)(Table 2).

C_4 plants with their physiological and biochemical characteristics, which lead to greater water-use efficiency, are better adapted not only in water-limited habitats but appear to be better adapted for polluted habitats also. C_4 plants have been shown to be generally more tolerant than C_3 plants (Sij and Swanson, 1974; Winner and Mooney, 1980c). Morphological contrasts between C_3 and C_4 may also contribute towards greater intrinsic tolerance of the latter towards sulphur dioxide stress (Winner and Mooney, 1980). A leaf section of C_4 plants show large green bundle sheath cells around vascular bundles flanked by a layer of green mesophyll cells. Such an ordered arrangement is known as "kranz anatomy". It provides C_4 plants with division of labour or compartmentalization. Initial carboxylation reaction i.e., PEPCO enzyme having greater affinity towards carbondioxide than RuBisCo combines with it and the resultant product is Malate aspartate. The above mentioned reaction takes place in mesophyll cells. Malate aspertate enters into bundle sheath cells where it undergoes decarboxylation reaction in cyto-

with habitats with little or no sulphur dioxide (Bell and Mudd, 1976; Horsman et al., 1979). Similar interspecific differences have been found for Rumex obustifolius (Horsman and Wellburn, 1977), Dactylis glomerata, Festuca rubra and Holcus. lanatus (Ayazloo and Bell, 1981). In contrast to the condition of Geranium carolinianum, the differences of sulphur dioxide resistance for these plants seemed at least partly related to sulphur dioxide absorption capacities; plants representing sensitive populations from clean air sites absorbed more sulphur dioxide fumigations than resistance plants from sites with industrial sulphur dioxide (Ayazloo et al., 1982).

Studies on the evolution of sulphur dioxide resistance, are mostly confined on temperate plants but information is lacking on tropical plants. In general 1. it is not known the rate at which sulphur dioxide resistance can change within a species, 2. predicting which species have the greatest potential to adapt to increased level of sulphur dioxide, and 3. the way in which two levels of sulphur dioxide stress will modify community composition.

C_3, C_4 and CAM plants: C_3 and C_4 plants differ in their photosynthetic sulphur dioxide sensitivity(Tu2) This

Table 2 : Effect of Sulphur dioxide on C_3, C_4 and CAM Plants

C_3 (Reductive Pentose Phosphate cycle)	C_4 -dicarboxylic acid cycle	CAM (Crassulacean Acid Metabolism)
Stomatal components		
C_3 plants generally have higher stomatal conductance resulting in higher SO_2 absorption (Winner and Mooney, 1982). In C_3 plants, stomata either stay open resulting in internal CO_2 concentration which remain near to that of air surrounding the leaf or stomata which maintain a constant ratio between external and internal CO_2 concentrations.	C_4 plants, which are better adapted for water conservation have the lower capacity for SO_2 absorption resulting in higher SO_2 resistance (Winnter and Mooney, 1982). In C_4 plants, stomata tend to keep the internal CO_2 concentration constant and therefore independent of external CO_2 concentrations (Carlson and Bazzoz, 1982).	CAM mode results in stomatal opening in dark and not in light. This dark opening provides the opportunity for uptake of SO_2 during the period when physiological mechanisms for SO_2 detoxification are not active (Olszyk and Tingey, 1984).
Morphological		
C_3 plants have chloroplasts which are uniformly distributed throughout mesophyll tissue (Osmond et al., 1969). This arrangements results in decline of photosynthetic rate, when stomata get closed on SO_2 fumigation (Winner and Mooney, 1982).	C_4 plants show "kranz anatomy" i.e. chloroplasts are concentrated around vascular bundle sheaths. Even if, stomata close on SO_2 fumigation, they carry out photosynthesis at usual rate (Winner and Mooney, 1982).	

C_3 (Reductive Pentose Phosphate cycle)	C_4 (dicarboxylic acid cycle)	CAM (Crassulacean Acid Metabolism)
High CO_2		
Fumigation with SO_2 reduced leaf area of C_3 plants more at low CO_2 than at high CO_2. (Carlson and Bazzaz, 1982)	Leaf area of C_4 plants was reduced more at high CO_2 than at low CO_2. These results support that the notion that C_3 species are more sensitive to SO_2 fumigation than are C_4 species at concentrations of CO_2 equal to that found in normal ambient air (Carlson and Bazzaz, 1982).	
Biochemical		
The intiial CO_2-fixation enzymes in C_3 plants is RuBP-carboxylase which has a lower bicarbonate affinity than PEP carboxylase (Ziegler, 1972, 1973). Sulfite-competitively binds to RuBP carboxylase and shows poorer sulfite exclusion than PEP carboxylase (Winner and Mooney, 1980; Ziegler, 1972, 1073).	The initial CO_2-fixation enzyme in C_4 plants is PEP carboxylase which has a higher bicarbonate affinity than RuBP carboxylase of C_3 species (Ziegler, 1972, 1973). Sulfite competitively binds to both enzymes but PEP carboxylase is better-able to discriminate between bicarbonate and sulfite (Ziegler, 1972, 1973; Winner and Mooney, 1980).	The initial CO_2- fixation enzyme in CAM plants is also PEP carboxylase, however, no work has been reported , of SO_2 effect on PEP carboxylase of CAM plants Bisulfite inhibits β-carboxylation and normal pattern of CAM metabolism (Osmand and Avadheri, 1970).

a similar fashion and thus exhibit lower rates of photosynthesis.

Olszyk, Bytneroricz and Fox (1987) studied sulphur dioxide effects on plants exhibiting Crassulacean Acid Metabolism (CAM). Theyfound that Opuntia basilarics (Bearer tail cactus) was the most sensitive species among species surveyed. Injury was found to be associated with a higher stomatal conductance (Table 2).

Olszyk et al., (1987) reported that native vegetation in desert areas of the southwest could be adversely affected by sulfur dioxide emissions from fossil fuel energy generating stations, smelters and other indusdtrial facilities both in the United States and Mexico. While physiological adaptations to arid environment are likely to render plants insensitive to air pollutants during much of the year, some of these adaptations may maximize pollutant sensitivity during those periods when the plants have their greatest metabolic activity due to favourable environmental conditions.

CAM mode results in stomatal opening primarily in the dark and not in the light. The dark opening provides the opportunity for uptake of sulphur dioxide during the period when physiological mechanisms for sulphur dio-

xide detoxification are not active (Olszyk and Tingey, 1984).

It has been suggested that CAM plants are not as sensitive to sulphur dioxide under field conditions. The physiological mechanism of sulphur dioxide toxicity appears to be different for CAM plants compared to C_3 plants. The phytotoxicity of sulphur dioxide has been found to be enhanced in light compared to dark in Opuntia basilaris (Olszyk, Bytneronicz and Fox, 1987). In contrast, the phytotoxicity of sulphur dioxide was decreased in light as compared to dark in Pisum sativum and Lycoperscion esculentum. This maybe due to photo-reduction and photo-oxidation of sulphur dioxide in the light (Olszyk and Tingey, 1984). The mechanism for sulphur dioxide toxicity in the light has not been determined but may be linked to the differential gas uptake and carbon metabolism in dark vs light in CAM plants. Osmond and Avadhani (1970) have shown that sulfite inhibits normal pattern of CAM metabolism under otherwise favourable conditions.

Effect of Sulphur dioxide on Key Physiological Processes

Not many in depth studies have been done on the effect of sulphur dioxide on key physiological processses.

However, results of various studies on the effect of sulphur dioxide on major physiological processes are discussed below.

Information on respiratory response to sulphur dioxide is extremely limited. Thomas and Hill (1937) found no effect of sulphur dioxide on dark respiration in plants exposed to 1 ppm sulphur dioxide for 1 hour. Similar responses to high concentrations have also been observed by Katz (1949), Sij and Swanson (1974) and Furukawa, Natori and Totsuka (1980). Shimazaki and Sugahara (1979) reported that changes in dark respiration, that they observed in plants exposed to 2 ppm sulphur dioxide for 5 hour were too small to have an appreciable effect on two rates of net photosynthesis.

Effects of sulphur dioxide on dark respiration (Table 3) include both inhibition (Taniyama, 1972; Luttge et al., 1972) and a stimulation (Keller, 1957; Bortitz, 1964; Vogl Bortitiz and Polster, 1974; Vogl and Bortitz, 1965; Taniyama et al., 1972; Black and Unsworth, 1979). Enhanced respiratory rates have been observed in a number of pine species and bean (Vicia) exposed to wide range of sulphur dioxide concentrations (0.04 - 2 ppm). These changes in respiratory rates may reflect a number of responses to the pollutants; e.g., process of detoxifica-

Table 3: Effect of sulphur dioxide on Respiration in Plants

Name of the Plant	Habit	Concentration	Duration	Condition	Effect	Reference
Helianthus. annus	herbaceous cultivated crop	1.5 ppm SO_2	30 min	CO_2 was released in air 'Lab'	Decrease in photorespiration	Furukawa et al., (1980)
Pinus banksiana	conifer	0.34 ppm SO_2	24 and 48 hour	Lab	Decrease in activity of enzyme glycollate oxidase	Khan and Malhotra (1982)
Spinacia oleracea, Hordeum vulgare	herbaceous cultivated crop	1 µM 1 mM SO_3^{2-}		Spinach chloroplasts exposed	Inhibition of enzyme glycollate oxidase and accumulation of glycollate; lmM SO_2 does not alter ATP concentration, but 1 mM decreases it	Libera et al., (1974)
Phaseolus vulgaris Zea mays	herbaceous cultivated	30-100 mM Na_2SO_3		Mitochondrial preparation	Inhibition in both plants of ATP formation; Corn mitochondria are as sensitive as bean mitochondria	Ballantyne (1973)

Table 3 : Effect of Sulphur dioxide on Respiration in Plants

Name of the Plant	Habit	Concentration	Duration	Condition	Effect	Reference
Pinus sylvestris	conifer tree	0.75 ul litre^{-1} SO_2	5 days, 6 hr daily	lab. and in field	Dark respiration variation did not have geographical pattern. The process fluctuated in most cases near to the overall average of 1.20 mg CO_2 g^{-1} dry wt h^{-1}	Oleksyn and Bislobok (1986)
Vicia faba	herbaceous cultivated crop	20-200 parts 10^{-9} SO_2	3 days	Glasshouse conditions	Dark respiration rates increased substantially of SO_2 concentration	Black and Unsworth (1979)
Nicotiana tabaccum	"	1.3 ppm SO_2	18 hr	Lab	39 percent increase in glycollate oxidase activity	Soldatini and Ziegler (1979)
Pinus contorta x banksiana	conifer	20 ppm SO_2 and 10-207 ppb	0.5 hr	Artificially in lab and field conditions	Inverse linear relationship between ATP content and SO_2 concentration measured	Harvey and Legge (1978)
Phaseolus vulgaris	herbaceous cultivated crop	0.77, 1.53 3.06, 4.03 6.50, 8.0 parts 10^{-6} SO_2	24 hr continuous	Lab	Respiration increased exponentially with increasing SO_2 concentration	Kozoil and Jordan (1978)

tion, repair mechanism on direct interference with specific respiratory pathways on organelles. Malhotra (1976) reported ultrastructural changes in mitochondria of Pinus contorta (Lodgepole pine) and an inhibition of ATP formation and phosphorylation activity of mitochondria in sulphur dioxide exposed to plants (Ballantyne, 1973; Malhotra and Hocking, 1976; Harvey and Legge, 1979). Nikolarvskii (1966, 1968, cited in Horsman and Wellburn, 1976) reported that exposure of Betula and Acer to 125 ppm of sulphur dioxide for 17 hour resulted in alteration in the activity of the glycolytic and pentose phosphate pathway and the citric acid cycle.

Photo respiration: Photorespiration is the oxidative and irreversible biosynthesis and metabolism of glycolate; few studies have been carried out to study the effect of sulphur dioxide on photorespiration in intact plants. Koziol and Jordon (1978), however, estimated photorespiration from the rate of CO_2 released in the dark period immediately following a light period in which bean (Phaseolus vulgaris) plants had been exposed to 1-8 ppm of sulphur dioxide. They reported exponential increase in photorespiration with increasing sulphur dioxide concentration, which is attribute

to a greater use of energy in repair and replacement processes. Ziegler (1975) found photorespiration to be inhibited by sulphur dioxide. Glycollate oxidase, an important enzyme for the synthesis of glycine and serine was inhibited by low concentrations of SO_3^{2-} in vitro (Zelitch, 1957; Paul and Bassham, 1978; Khan and Malhotra 1982a), and by gaseous sulphur dioxide (Khan and Malhotra 1982). Exposure of Nicotiana tabaccum (tobacco) to high sulphur dioxide concentration (1.3 ppm for 18 hour) induced enhanced the synthesis of glycollate oxidase (Soldatini and Ziegler, 1979).

It has been suggested that a decrease in photorespiration as a result of sulphur dioxide or SO_3^{2-} exposure is due to formation of glyoxylate bisulfite, which is a potent inhibitor of glycollate oxidase (Zelitch, 1957). Glyoxylate bisulfite was found to accumulate in the leaves of Oryza sativa (rice) plants exposed to high concentrations of sulphur dioxide (Tanaka et al., 1972a). Similarly, Pisum sativum exposed to high sulphur dioxide concentration, produced toxic bisulfite compounds of glyceraldehyde, L-ketoglutarate, pyruvate and oxalate (Jiracek et al., 1972).

Effect of sulphur dioxide on Plant Productivity

This topic has been very widely reviewed in literature (Linzon, 1972; Kozlowski and Mudd, 1975; Guderian, 1977; Heck and Brandt, 1977; Jeffree, 1980) (Table 4).

Low sulphur dioxide concentrations (>0.2 ppm) were long shown to cause increases in yield (Thomas et al., 1943) and net photosynthetic rates (Katz, 1949). Prolonged exposure to low concentrations of sulphur dioxide are also known to bring about premature senescence (Guderian, 1977).

Relationship between foliar injury and yield loss are not well understood. Katz and Ledingham (National Research Council of Canada, 1939) found that sulphur dioxide did not affect alfalfa growth until at least 5 percent of the foliage was visibly injured, and Hill and Thomas (1933) reported that yield reductions from acute sulphur dioxide injury were roughly equivalent to plants from which same amount of leaf tissue was removed.

Plant response to short-term high level sulphur dioxide exposures may result in acute foliar injury, whereas low concentration can be beneficial - especially if the soil is deficient in sulphur (Lockyer et al., 1976). Conversely, long term, subacute expo-

Table 4 : Effect of sulphur dioxide on Yield in Plants

Name of the Plant	Habit	Concentration	Duration	Condition	Effects	Reference
Allium-ursinum Anemone nemorosa Arum. maculatum Viola-reichenbachiana	Trees	300 ug SO_2 m^{-3}	4 hr/week	Field	Decrease in productivity and leaf Area Indices (LAI)	Steubig and Fangmeier (1987)
Triticum aestivum Medicago sativa Zea mays	Cultivated crop	218 ug SO_2 m^{-3} (1.7 gm^{-2} flyash)	1 hr for SO_2 and conc. to flyash and combination of SO_2(1 hr) and flyash	Field and artificially in lab	Reduction in area and Biomass of leaf, Total plant biomass	Garg and Varshney (1983)
Agropyron-smithii	Cultivated crop	200 ug SO_2m^{-3}	30 days	Lab	^{14}C translocation and leaf growth increased	Milchumas et
Alianthus altissima	Wild tree	0.1, 0.2 ppm SO_2	1 and 2 weeks	Lab	Decrease in growth and biomass accumulation	Marshall and Furnier (198
Phleum pratense	Wild grass	343 ug SO_2 m^{-3}	5 weeks	Lab	Plants were exposed to two light regimes simulating summer and winter conditions. 50 percent reduction in dry matter for winter light regime	Davies (1980)
Lolium perenne	Wild grass	700 ug SO_2m^{-3}	8 weeks	Lab	Decrease in Yield	Horsman et a (1979)

Name of the Plant	Habit	Concentration	Duration	Condition	Effects	Reference
Nicotiana tabaccum	cultivated crop	0.1 ppm SO_2	8 hr/day, 5 days/week (4 weeks)	Greenhouse exposure chamber	Growth reductions	Reinert et al (1969)
Phleum pratense	wildgrass	0.95 ppm SO_2	8 hr	Field exposure	"	Guderian (196
Trifolium pratense	cultivated crop	"	8 hr	"	"	Guderian (196
Trifolium pratense	cultivated crop	"	12 hr	"	"	Guderian (196
Lolium multiforum	wildgrass	"	12 hr	"	"	Guderian (196
General		0.05-2.0 ppm SO_2	24 hr for growing season	"	Growth suppression, early abscision and reduction in yield	Thomas and Hendricks (19
Lolium perenne	wildgrass	0.1 ppm SO_2	63 days	Ambient air greenhouse	Growth reduction	Bleasdale (19

sures can also result in crop yield losses - often with any accompanying visible foliar injury.

In sulphur-deficient soil, sulphur dioxide acts as a sulphur-nutrient. Bell and Clough(1973) found that continuous exposures to both 12 and 6.7 pphm sulphur dioxide for 9 and 26 weeks respectively, depressed the shoots growth of S23 ryegrass (Lolium perenne) approximately 50 percent. However, Cowling et al., (1973) found that exposure to 4.6 pphm sulphur dioxide for 59 days increased the yield of same variety of ryegrass when it was grown in a sulphur-deficient soil, and later the same research team (Lockyet et al., 1976) reported that exposures of upto 7.3 pphm sulphur dioxide for 77 days had no effect on growth, even when adequate supply of sulphate was present in the soil.

Decreased growth attributable to sulphur dioxide is well presented in numerous publications, but very little information is available to suggest growth reductions unless visible injury occurs. Bell and Clough (1973) found a 46 percent depression in final yield of ryegrass exposed to 0.12 ppm of sulphur dioxide for 9 weeks and a 52 percent depression when plants were exposed to 26 weeks to 0.067 ppm. Exposed plants showed chronic injury but no acute injury. Guderian (1977)

has reported reduced growth in a number of species like Helianthus annus, Zea mays, Pisum sativum, Vicia sativa, when grown singly or in combination with each other, after exposure for 8 to 12 hour to about 1 ppm of sulphur dioxide and in a mixture of three species after 48 hour exposure to 0.4 ppm.

The most extensive data relating to foliar injury occuring in the field in relation to continuous monitoring of ambient sulphur dioxide concentrations are those reported by Dreisinger and Mc Govern (1970) from studies around smelters near Sudbury in Ontario, Canada, and by H.C. Jones et al., (1979) obtained around electric power generating plants in Tennessee Valley. Both investigations demonstrate that considerable variation exists among species in their tolerance to sulphur dioxide. Leaf injury was related to peak concentrations in Sudbury area. To prevent sulphur dioxide injury to most species, the authors concluded that sulphur dioxide concentrations should not exceed 0.70 ppm for 1 hour, 0.40 ppm for 2 hour, 0.26 ppm for 4 hour or 0.10 ppm for 8 hour. Some sensitive species may be injured from 1 and 2 hour exposure to concentrations slightly below than those mentioned above.

Jones et al., (1979) studied foliar effects caused by ambient suphur dioxide levels on native plants as well as crops. After 6,500 field inspections, they concluded that the threshold dose for foliar injury on sensitive species was 0.32 ppm for 1 hour or 0.17 ppm for 3 hour. The probability that foliar effects would occur on any species examined, or that yields of soybean would be reduced, was less than 50 percent for 3 hour exposures to concentrations less than 0.50 ppm. Zea mays, Gossypium sp. Triticum sp. and Nicotiana sp. were much more resistant than Glycine or Pinus taeda and Pinus virginiana.

Research in future should aim at quantifying the importance of sulphur dioxide on crop growth in areas with different pollution characteristics. Field investigations must be backed up by laboratory fumigations, which should closely simulate the ambient situation with respect to climatic conditions, realistic fluctuating levels of different mixtures of pollutants, and normal practices of crop cultivation.

Task for Future Problems Ahead

Detailed studies are needed to correlate results of short-term photochemical studies with that of long-term effects of sulphur dioxide on plant yield.

Laboratory experiments with different combinations of air pollutants are required. As in the real world, outside the laboratory, plants experience all possible interactions with the atmospheric environment. It is also demonstrated that combinations of air pollutants can cause rapid inhibition of CO_2 exchange (Bull and Mansfield, 1974; Ormord, Black and Unsworth, 1981). A challenge for the future will be to describe the relation between deposition velocities of air pollutants, the influence on plant photosynthesis and productivity in the field.

To understand the mechanism of sulphur dioxide action of C_3 type of plants, knowledge is required to understand the effect of sulphur dioxide on different key photosynthetic enzyme systems both *in vitro* and *in vivo*.

In recent years some attention has been paid to a few photosynthetic enzyme systems such as FBPase, SBPase, RuBisCo.

Inspite of some serious studies by Ziegler (1972) in *Spinacia oleracea*, Gezelius and Hallgren in *Pinus sijlvestris* and Hallgren and Gezelius (1982) in *Pine* sps. the behaviour of RuBisCo is far from clear since inhibition by sulphite is a complex and time dependent phenomenon.

Fumigation System

Fumigation chamber

A dynamic fumigation chamber made of glass having $1m^3$ capacity was used in this study (Fig. 5). The chamber was illuminated by light bulbs of 100 W, mounted 30 cm above the chamber. The chamber was air tight having an inlet at the base and an outlet at the top on the opposite side. A small electric fan of 10"x9" size was fixed inside the chamber to ensure uniform mixing. The flow of air-gas mixture into the chamber was monitored with the help of a rotameter and kept constant at 1.55 l min^{-1}.

Sulphur dioxide generation

The sulphur dioxide was generated by bubbling air at a constant rate of 1.55 l min^{-1} in an impinger containing a 100 ml of the desired aqueous solution of sodium metabisulphite (5 mg or 10 mg). The sequence of reactions leading to Sulphur dioxide evolution are as follows:

$$NaHSO_3 \longrightarrow Na^+ + HSO_3^- \qquad (1)$$

$$HSO_3^- + H^+ \longrightarrow H_2SO_3 \qquad (2)$$

$$H_2SO_3 \longrightarrow H_2O + SO_2 \qquad (3)$$

The sulphur dioxide was introduced into the chamber through an inlet. The SO_2 concentration in the air

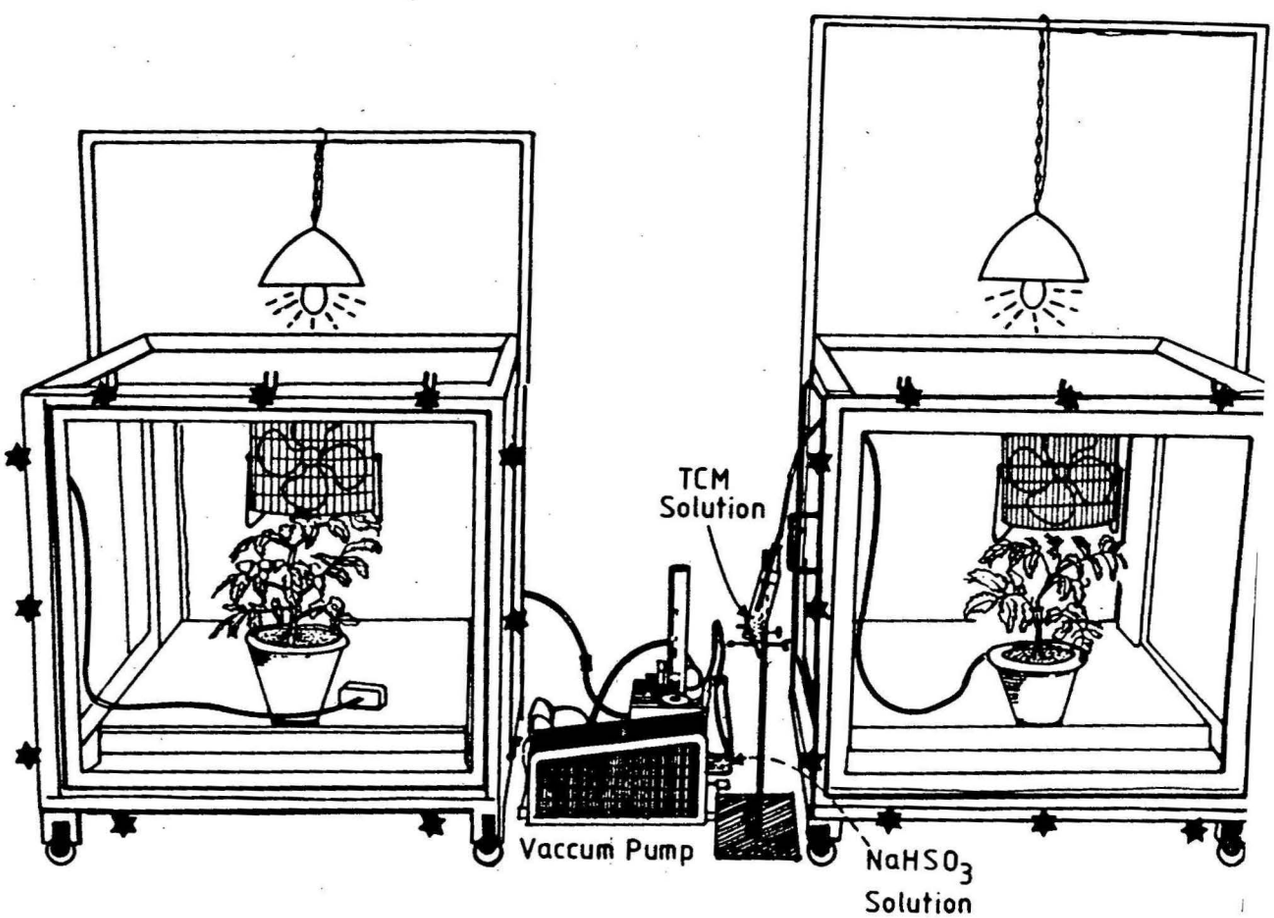

Fig. 5. Schematic representation of SO_2 generation

gas mixture was in the chamber determined by passing out the gas from the exit through a 0.4 percent aqueous solution of tetrachloromercutate (TCM) for ten minutes. A dichloromercurate is formed which reacts with pararosaniline and formaldehyde making a complex of pink colour. The intensity of the colour was measured, spectrophotometrically at 548 nm and optical density (OD) was converted in sulphur dioxide concentration (ppm) using the formula described by West and Gaeke (1956) as

$$\text{ug } SO_2\ m^{-3} = \frac{(A-A_o) \times (10)^3 \times (B_s)}{V_r} \times D$$

where,

A = sample absorbance

A_o = reagent blank

10^3 = conversion of litres to cubic metres

V_r = the sample volume corrected to 25°C and 760 mm Hg litres

B_s = calibration factor, ug/unit of absorbance

D = dilution factor

One ppm SO_2 = 2620 ug m^{-3} SO_2

Complete scrubbing of sulphur dioxide from the air stream was achieved by passing the gas current through two bubblers connected in series containing TCM solution. The concentration of sulphur dioxide in the gas

stream is dependent upon the strength of sodium metabisulphite solution at a given rate of air-flow.

Plant Material

Two plant species, viz., Spinacia oleracea, Lycoperscion esculentum were selected for this study.

Plants were raised from seeds in earthern pots (height 15 cms) filled with sandy-loam soil. Three to four seeds were sown in each pot and twenty five pots were prepared for each species. Plants were exposed to sulphur dioxide when they were 21 days old. Pots were regularly irrigated during the experimental period. Pots were divided into three batches - (1) one batch of control plants, (2) second batch for exposure to 0.1 ppm sulphur dioxide, (3) third batch for exposure to 0.2 ppm sulphur dioxide.

Exposure Schedule

Plants were fumigated daily for 2 hours duration and periodical observations were taken for morphological characters. Plants were fumigated with 0.1 ppm and 0.2 ppm sulphur dioxide. At the end of six weeks, experiment was terminated. Plant observations were made on a number of morphological, physiological and biochemical parametres in addition to biomass measurements.

Morphological Parameters

Number of leaves per plant

Average leaf-area

Shoot biomass (Leaf biomass + stem biomass) and root biomass

Fresh weight of plants were taken to calculate root and shoot biomass.

Physiological and Biochemical Parameters

Chlorophyll content estimation

Net photosynthesis

Ribulose-biphosphate carboxylase (RuBisCo) activity

Morphological Parameters

Number of leaves per plant was counted at the regular intervals, i.e., after 7th, 14th, 21st, 28th, 35th and 42nd day of exposure schedule. Results have been expressed as average number of leaves per plant.

Leaf-area measurements were similarly taken at regular intervals, i.e., 7th, 14th, 21st, 28th, 35th and 42nd day of exposure schedule. Leaf-area was calculated by measuring the length of the leaf and perpendicular line to it was taken as width of the leaf. To minimize error in leaf-area calculations, ten leaves of the same plant were harvested. Outline of leaves were drawn on paper. The same piece of paper was cut into one centimeter square

and weighed. Paper on which leaf-margin is drawn is also weighed. Weight of one centimeter square paper divided by paper weight of leaves gives the multiplying factor. This factor though very small has to be multiplied when making leaf-area measurements.

Physiological and Biochemical Parameters

Chlorophyll estimation:

Fresh leaves weighing 0.5 g were homogenized in 20 ml of 80 percent acetone (acetone: water v/v) in a mortar. The homogenate was filtered through a double layered muslin cloth. The filterate was centrifuged at 3000 g for 15 minutes. The supernatant was made upto 100 ml with 80 percent acetone and the optical density of the extract was measured at 645 and 663 nm wavelength using a Spectronic-20 Bausch and Lamb spectrophotometer (USA). The chlorophyll a and chlorophyll b was determined by using the formula described by Maclachlan and Zalik.

Chlorophyll a (mg g^{-1} fresh leaves) =

$$\frac{12.3\ D_{663} - 0.86\ D_{645}}{dx\ 1000\ x\ w} \times V$$

Chlorophyll b (mg g^{-1} fresh leaves) =

$$\frac{19.3\ D_{645} - 3.6\ D_{663}}{dx\ 1000\ x\ w} \times V$$

where

v = volume of chlorophyll extract in acetone (ml)

d = length of light path (cm)

w = fresh weight g leaves (g)

Net photosynthesis was measured with the help of photosynthesis system, LI-COR Inc., Lincoln Nebrasaka, USA. To measure rate of photosynthesis, a fully sunlit leaf near perpendicular to the sun was chosen. The leaf-chamber of LI-6000 was installed after slightly (one litre size) elevating the concentration in the leaf chamber CO_2. Logging was started with a time step appropriate for a CO_2 draw-down of about 30 ppm. When a page is complete, store it and begin logging again.

Measurement cautions:

i. Keep the leaf chamber in the shade and open with fans running.

ii. Avoid contaminating the leaf chamber with your breath when closing.

iii. Avoid shading of the leaf when closing the chamber, or at any time immediately prior to measurement.

iv. Keep the chamber clean and free of dust or other particulates which may contribute to water adsorption.

v. The range of CO_2 drawn-down should be about 25 ppm. If your CO_2 draw down is too small: (a) use a smaller chamber, or (b) increase observation time, or (c) increase the amount of leaf tissue.

vi. The change in relative humidity should be zero.

vii. Light intensity must be constant during the measurement.

viii. The change in leaf temperature should be less than or equal to 1°C or so; however, this varies strongly with measurement duration and conditions.

Ribulose bisphosphate carboxylase (RuBisCo)

RuBP carboxylase determination by enzymic estimation of D-3-PGA formed, method described by Marco and Tricoli (1983) (Fig. 6).

Ribulose-1-5-bisphosphate (RuBP), Bicine, Mercaptoethanol, Phosphocreatine, creatine phosphokinase, Glyceraldehyde-3-phosphate dehydrogenase (GADPH), Phosphoglycerate kinase (PGK), were obtained from Sigma Chemical Company, USA.

i. 10 ul: Clinipette, Clinicon International GmBH: West Germany.

ii. 50 ul: Clinipette, Clinicon International GmBH: West Germany.

iii. 100 ul: Sigma, Sigma Chemical Company, USA.

iv. P-20: Pipetman: Gilson, Gilson Medical Electronics, (France) S.A.

v. P-1000: Pipetman: Gilson, Gilson Medical Electronics, (France), S.A.

One gram leaves were homogenized in a mortar with glass beads in 10 ml per gram of 100 mM Bicine (pH 8.2) 10 mM $MgCl_2$, 5mM $NaHCO_3$ and 5mM Mercaptoethanol.

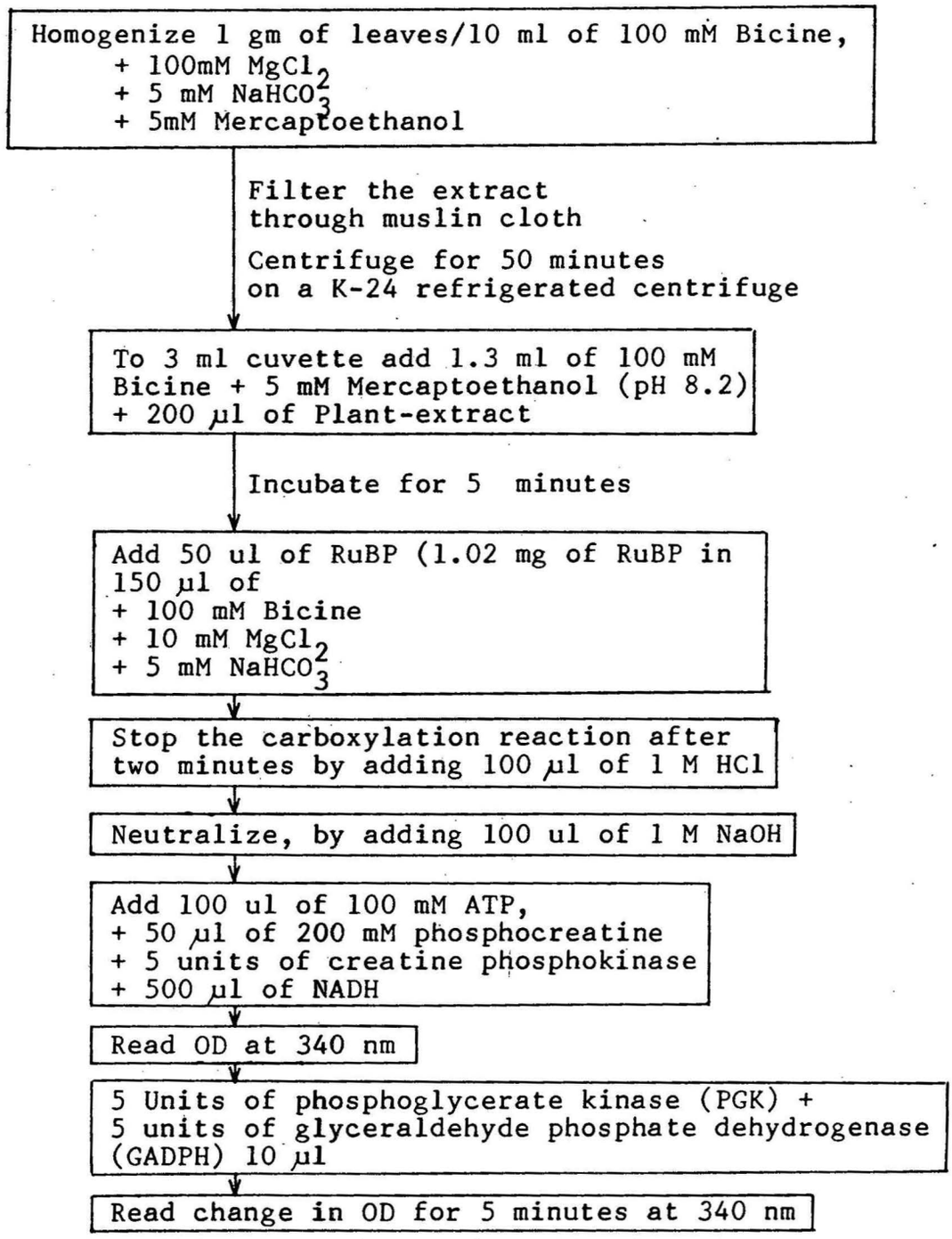

Fig.6 RuBP carboxylase determination by enzymic estimation of D-3-PGA formed

The extract, after filteration through cheesecloth was centrifuged in K-24 at 16,000 x rpm for 50 min at $0^{o}C$. 0.1 ml of crude plant extract was incubated for 5 min. After 5 min of incubation in reaction mixture, reaction was started by adding 0.5 u mol of RuBP in 50 ul of reaction buffer minus $NaHCO_3$. The reaction was stopped after 2 min by adding 100 ul of 1M HCl.

The spectrophotometric assay was effected by performing the carboxylation reaction and then to the reaction mixture adding 100 ul of 1M NaOH with 1.3 ml of 100 mM Bicine pH 8.2, containing 5mM mercaptoethanol. This reaction mixture was transferred to 3 ml quartz cuvette. To this mixture was added 100 ul of 5mM NADH, 100 ul of 100 mM ATP, 50 ul of 200 mM phosphocreatine and 5 units of creatine phosphokinase to give a final volume of 2.4 ml. After recording the absorbance of this solution against a blank containing the same amount of NADH in Bicine on Beckman DU-20 spectrophotometer 5 units of phosphoglycerate kinase (PGK) and 5 units of glyceraldehyde phosphate dehydrogenase (GADPH) as a suspension in ammonium sulphate solution (10 ul) were added. The reduction of D-3-PGA went to completion in about 5 min at $28^{o}C$.

Following precautions were observed:

1. Homogenization of leaves should be carried out in chilled mortar and pastle so that heat caused by friction may not denaturate the enzyme RuBisCo.
2. pH of the reaction mixture should be around 8.2.
3. Mercaptoethanol is to be added to buffer just before making use of the buffer.
4. After centrifugation, the supernatant should be
c crystal-clear, as crude plant-extract is used for determining enzymic activity.
5. RuBP, NADH, ATP, phosphocreatine are to be freshly prepared, i.e., approximately half an hour before the enzyme assay has to be carried out.
6. Ribulose-1-5-bisphosphate dissolves in slightly acidic solution, i.e., pH 4.5 - 5.0.

Statistical Analysis:

The internal variability or dispersion of the data on total biomass, biomass of stem, leaf and root, chlorophyll content was subjected to statistical analysis and standard deviation (σ) was calculated. The sum of squares of the deviations $(x-\bar{x})^2$ from the mean ($\bar{x}$) divided by number of observations (N). The square root of the resultant represents the value of standard deviation (σ).

$$= \sqrt{\frac{\Sigma (x - \bar{x})^2}{N}}$$

where

σ = standard deviation

Σ = sign of algeberic sum

x = observed value

$\bar{x}$ = mean of observed values

N = number of observations

RESULTS

The effect of sulphur dioxide 0.1 ppm (T_1) and 0.2 ppm (T_2) fumigation in S. oleracea and L. esculentum 2 hr daily for six weeks was evaluated. The parameters chosen for studying the response of the above plant species were leaf area, number of leaves per plant, chlorophyll content, biomass content, net photosynthesis and RuBisCO activity.

Leaf Area

In S. oleracea leaf area decreased following SO_2 fumigation (Table 5, Plate 1,2). In control plants the total leaf area was 10.5 cm^2 in fumigated plants it decreased to 9.12 and 9.41 cm^2 in T_1 and T_2 treated plants for two weeks, respectively (Table 5, Fig.7). After three weeks of fumigation the leaf area decreased to 22.8 and 25.4 cm^2 in T_1 and T_2 treated plants, respectively in contrast control set having 29.2 cm^2 leaf area (Table 5, Fig.7). Leaf area increased to 31.7 and 34.5 cm^2 in T_1 and T_2 treated plants, respectively after four weeks of fumigation in contrast to control set having 26.5 cm^2 leaf area. Average leaf area decreased in plants, after five weeks of fumigation, over control value of 34.7 cm^2 to 31.9 and 33.9 cm^2 respectively (Table 5, Fig.7). In plants subjected to T_1 and T_2 treatment for six weeks the leaf area decreased with respect to control value of

Table 5. Effect of 2 hr daily 0.1 and 0.2 ppm of SO_2 fumigation for six weeks on S. oleracea and L. esculentum

Date of sampling	Duration of exposure (days)	Avg. LA of control plants	Avg. LA of plants exposed to 0.1 and 0.2 ppm SO_2	% reduction over control	Avg. LA of plants exposed to 0.2 ppm of SO_2	% reduction over control
S. oleracea						
16.3.88	14	10.5 ± 2.0[a]	9.12 ± 3.97	-13.1	9.41 ± 3.20	-10.3
23.3.88	21	29.2 ± 6.25	22.8 ± 10.6	-21.9	25.4 ± 2.25	-13.0
30.3.88	28	26.5 ± 9.98	31.72 ± 4.49	+16.4	34.5 ± 3.16	+30.1
6.4.88	35	34.72 ± 8.08	31.9 ± 5.66	-8.12	33.9 ± 7.98	-2.36
13.4.88	42	34.78 ±10.05	33.9 ± 5.89	-2.53	33.38 ± 5.89	-4.02
L. esculentum						
16.3.88	14	2.3 ± 1.64[b]	2.6 ± 1.43	+13.0	2.4 ± 1.92	+4.34
23.3.88	21	3.35 ± 2.09	3.03 ± 1.3	-9.5	2.86 ± 0.72	-14.6
30.3.88	28	3.7 ± 0.94	4.76 ± 1.59	+27.0	5.68 ± 1.58	+53.5
6.4.88	35	6.02 ± 1.22	5.07 ± 1.48	-15.7	4.85 ± 3.16	-19.4
13.4.88	42	7.70 ± 1.87	5.80 ± 3.16	-24.6	5.65 ± 1.73	-26.6

LA = Leaf area

[a]Average of twenty reading; [b] Average of six readings

Plate 1. S. oleracea control and treated plants (fumigated with 0.1 ppm SO_2 2 hr daily for six weeks)

Plate 2. S. oleracea control and treated plants (fumigated with 0.2 ppm SO_2 2 hr daily for six weeks)


01
0-1
C
C


0.2
C
C

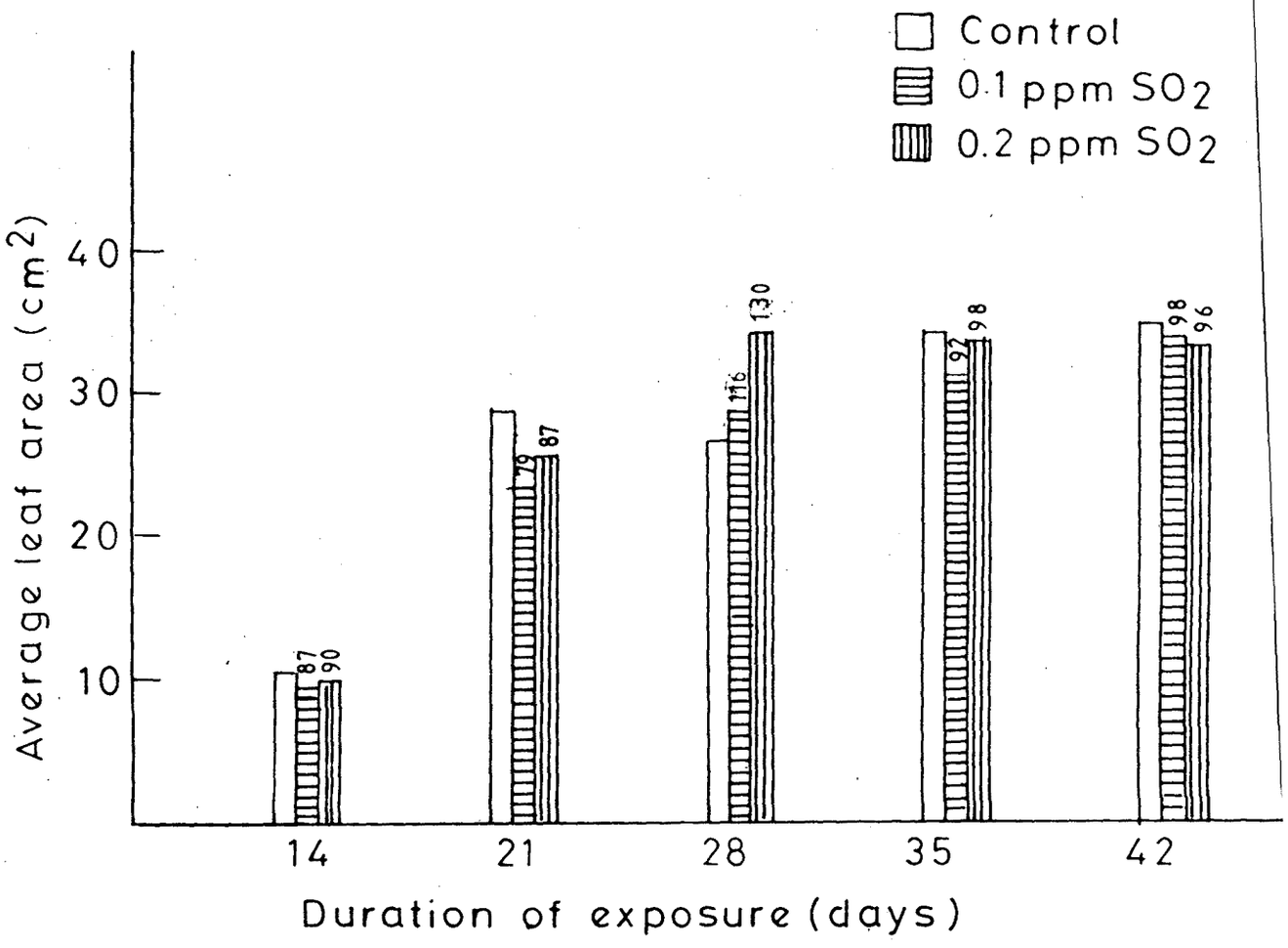

Fig.7 Effect of SO_2 fumigation (0.1 and 0.2 ppm, 2 hr daily for six weeks) on leaf area (cm^2) in Spinacia oleracea

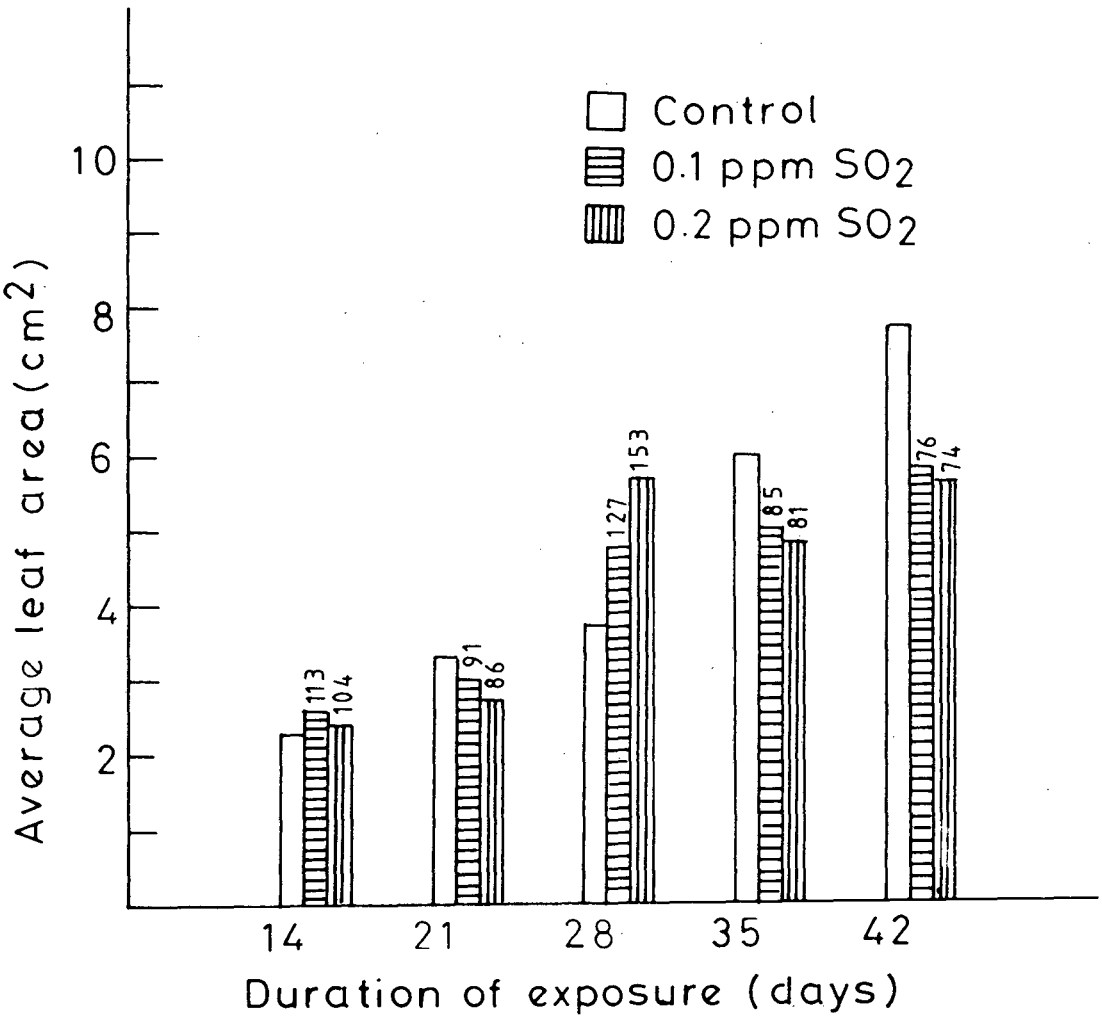

Fig.8 Effect of SO_2 fumigation (0.1 and 0.2 ppm, 2 hr daily for six weeks) on leaf area (cm^2) in Lycoperscion esculentum

34.7 cm^2 to33.9 and 33.3 cm^3, respectively (Table 5, Fig.7).

In L. esculentum average leaf area of plants subjected to T_1 and T_2 sulphur dioxide treatments for two weeks increased over control value of 2.3 cm^2 to 2.6 and 2.4 cm^2, respectively (Table 5, Fig.8). However, after three weeks of fumigation, leaf area decreased to 3.03 and 2.86 cm^2 for T_1 and T_2 treatments, respectively in contrast to control set having 3.35 cm^2 (Table 5, Fig.8). Leaf area increased in T_1 and T_2 treated plants for four weeks of fumigation to 4.76 and 5.68 cm^2. respectively over control value of 3.7 cm^2 (Table 5, Fig.8). Leaf area decreased to 5.07 and 4.85 cm^2 in T_1 and T_2 treated plants for five weeks, respectively in contrast to control set having 6.02 cm^2 leaf area (Table 5, Fig.8). Leaf area decreased to 5.80 and 5.65 cm^2 in plants subjected to T_1 and T_2 treatments, respectively for six weeks, in contrast to control set having 7.70 cm^2(Table 5, Fig.8).

Average Number of Leaves Per Plant

In S. oleracea average number of leaves per plant increased to 6.46 and 7.11 in plants subjected to T_1 and T_2 treatment, respectively in contrast to control set having 6.27 (Table 6, Fig.9). However, after three weeks of fumigation leaf area was reduced to 6.61 and 7.34 for T_1 and T_2 treatments, respectively in contrast to control

Table 6. Effect of 2 hr daily 0.1 and 0.2 ppm of SO_2 fumigation for six weeks on S. oleracea and L. esculentum

Date of sampling	Duration of exposure (days)	Avg. no. of lvs/Plt in control plants	Avg. no. of lvs/Plt in plants exposed to 0.1 ppm of SO_2	% reduction over control	Avg. no. of lvs/Plt in plants exposed to 0.2 ppm of SO_2	% reduction over control
S. oleracea						
16.3.88	14	6.27 ± 1.25[a]	6.46 ± 2.96	+3.0	7.11 ± 2.27	+13.3
23.3.88	21	7.56 ± 1.50	6.61 ± 3.59	-12.5	7.34 ± 3.48	-2.9
30.3.88	28	7.81 ± 2.09	10.6 ± 4.14	+35.7	9.45 ± 7.93	+20.9
6.4.88	35	9.86 ± 4.34	15.2 ± 13.6	+54.1	12.3 ± 8.8	+24.7
13.4.88	42	10.6 ± 4.44	15.8 ± 7.18	+49.1	13.7 ± 3.16	+29.2
L. esculentum						
16.3.88	14	41.0 ± 8.68[b]	30.3 ± 7.21	-26.8	41.1 ± 8.65	+0.24
23.3.88	21	45.5 ± 9.12	43.3 ± 14.6	-4.83	41.3 ± 15.9	-9.23
30.3.88	28	61.5 ± 13.5	47.16 ± 26.3	-23.3	63.0 ± 13.3	+2.38
6.4.88	35	102 ± 98.32	91.25 ± 4.02	-10.5	113·6 ± 120.2	+9.7
13.4.88	42	154 ± 28.5	92.84 ± 45.2	-39.7	129.2 ± 36.7	-16.1

Avg no. of lvs/Plt = Average number of leaves per plant

[a]Average of twenty readings; [b] Average of six readings

set having 7.56 (Table 6, Fig.9). The average number of leaves per plant increased after four weeks of fumigation to 10.6 and 9.45 in T_1 and T_2 treated plants,respectively in contrast to control set having 7.81 (Table 6, Fig.9). The average number of leaves per plant increased to 15.2 and 12.3 in plants subjected to T_1 and T_2 treatments for five weeks, respectively in contrast to control set having 9.86 (Table 6, Fig.9). After six weeks of fumigation the average number of leaves per plant increased to 15.8 and 13.7 in T_1 and T_2 treated plants, respectively with respect to control set having 10.6 (Table 6, Fig.9).

In L. esculentum, the average number of leaves per plant decreeased following sulphur dioxide exposure over the control. The average number of leaves in T_1 and T_2 treated plants for six weeks was 30.3 and 41.1, respectively as compared to control plants having 41.0 (Table 6, Fig.10). The average number of leaves per plant decreased to 43.3 and 41.3 in plants subjected to three weeks of fumigation, respectively with respect to control set having 45.5 (Table 6, Fig.10). After four weeks of fumigation the average number of leaves per plant decreased to 47.1 in plants subjected to T_1 treatment, whereas in plants exposed to T_2 treatment it decreased to 63.0. In control set, the average number of leaves was 61.5 (Table 6, Fig.10, Plate 3, 4).

Plate 3. Growth of L. esculentum plants exposed to 0.1 ppm SO_2 (2 hr daily for six weeks) along with control

Plate 4. Growth of L. esculentum plants exposed to 0.2 ppm SO_2 (2 hr daily for six weeks) along with control


0.1
C


0.2
C

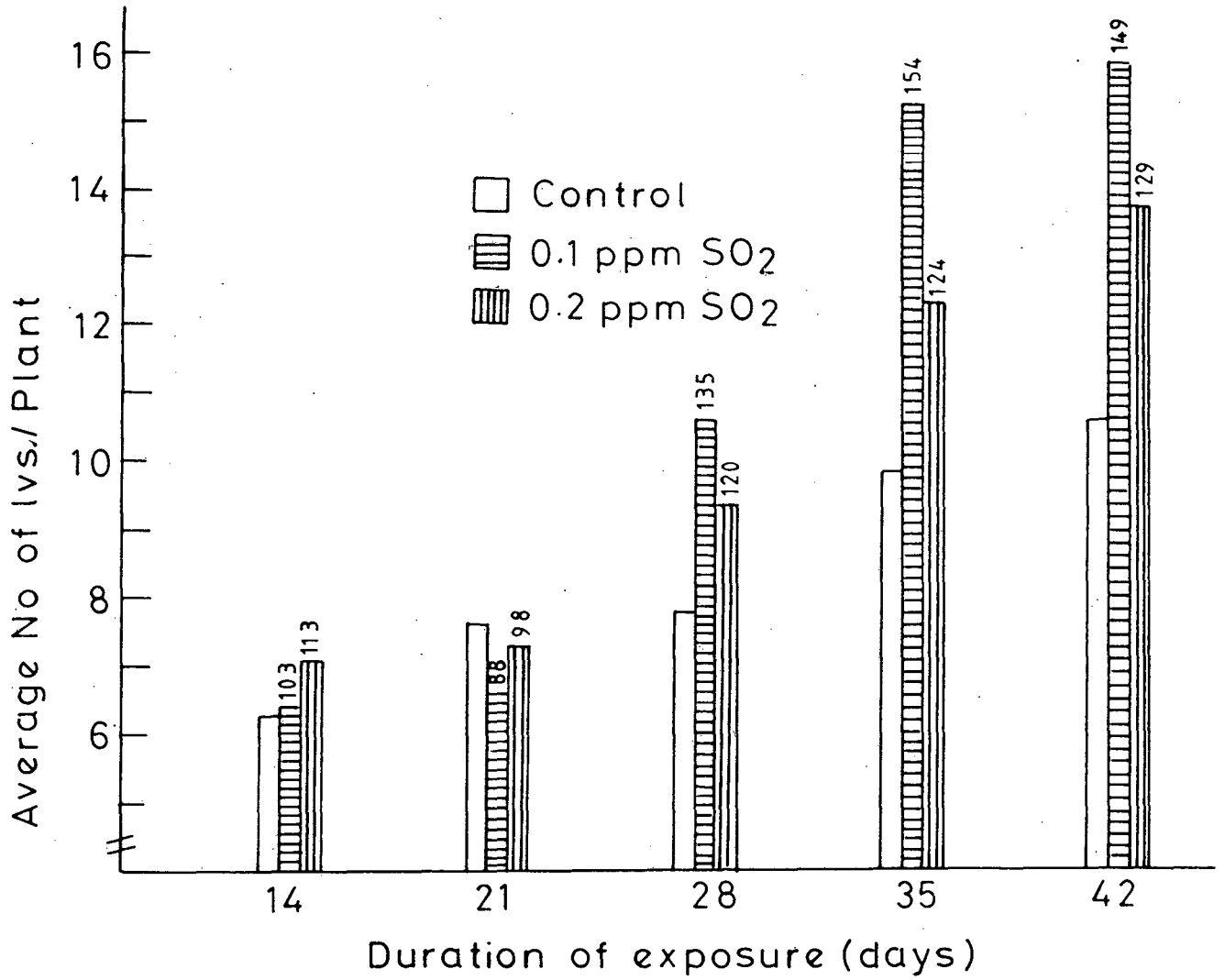

Fig.9 Effect of SO_2 fumigation (2 hr daily for six weeks) on number of leaves in S. oleracea

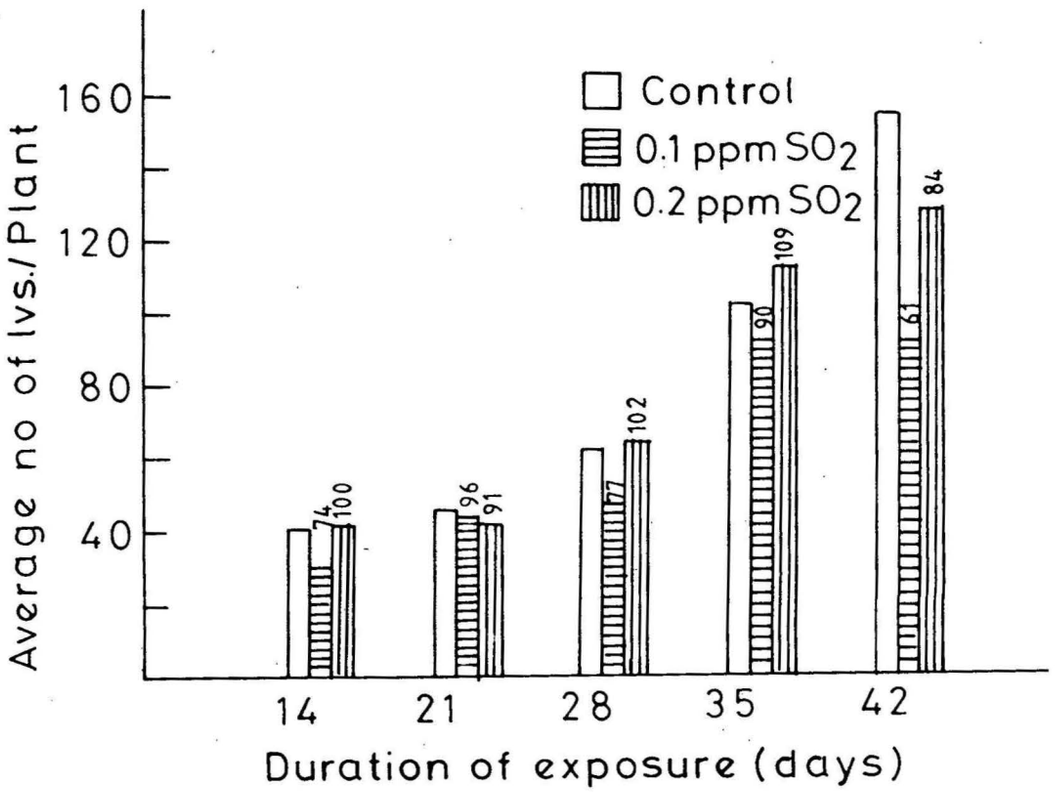

Fig.10 Effect 0.1 and 0.2 ppm SO_2 fumigation 2 hr daily for six weeks on number of leaves in Lycoperscion esculentum

Similarly, after five weeks of fumigation the average number of leaves per plant decreased to 91.25 in plants subjected to T_1 treatment, whereas the average number of leaves per plants increased to 113.6 in plants subjected to T_2 treatment, in contrast to control set having 102 leaves (Table6, Fig.10). The average number of leaves per plant decreased to 92.8 and 129.2 in plants subjected to T_1 and T_2 treatments, for six weeks, respectively in contrast to control set having 154 leaves (Table 6, Fig.10).

Chloroplyll content

In S. oleracea total chlorophyll content decreased to 0.609 and 0.504 mg g^{-1} fresh weight in plants subjected to T_1 and T_2 treatments, for six weeks, respectively in contrast to control value of 0.736 mg g^{-1} fresh weight (Table 7, Fig.11). Chlorophyll a decreased in plants to0.367 and 0.295 mg g^{-1} fresh weight, in plants subjected to T_1 and T_2 treatments for six weeks, respectively with respect to control set having 0.451 mg g^{-1} fresh weight (Table 7, Fig.11). Chlorophyll b decreased in plants subjected to T_1 and T_2 treatments for six weeks to 0.243 and 0.209 mg g^{-1} fresh weight respectively in contrast to control value of 0.285 mg g^{-1} fresh weight (Table 7, Fig.11). Chlorophyll a/b ratio decreased in plants subjected to T_1 and T_2 treatments for six weeks to 1.51 and 1.41,

Table 7. Effects of SO_2 (0.1 and 0.2 ppm 2 hr daily for six weeks) on the absolute and relative (values in parentheses) amounts of Chlorophyll (mg g^{-1} fresh weight) in S. oleracea

Parameter	Chlorophyll content of control plants	Chlorophyll content of plants exposed to 0.1 ppm of SO_2	% reduction over control	Chlorophyll content of plants exposed to 0.2 ppm of SO_2	% reduction over control
Chl a	0.451[b] ± 2.4 (100)	0.367 ± 2.4 (81.3)	-18.6	0.295 ± 0.04 (65.4)	-34.8
Chl b	0.285 ± 2.4 (100)	0.243 ± 0.04 (85.2)	-14.7	0.209 ± 0.01 (78.3)	-26.6
Chl (a+b)	0.736 ± 2.4 (100)	0.609 ± 0.11 (82.7)	-12.7	0.504 ± 0.07 (68.4)	-31.5
Chl a/b	1.58	1.51		1.41	

[b]Average of three readings

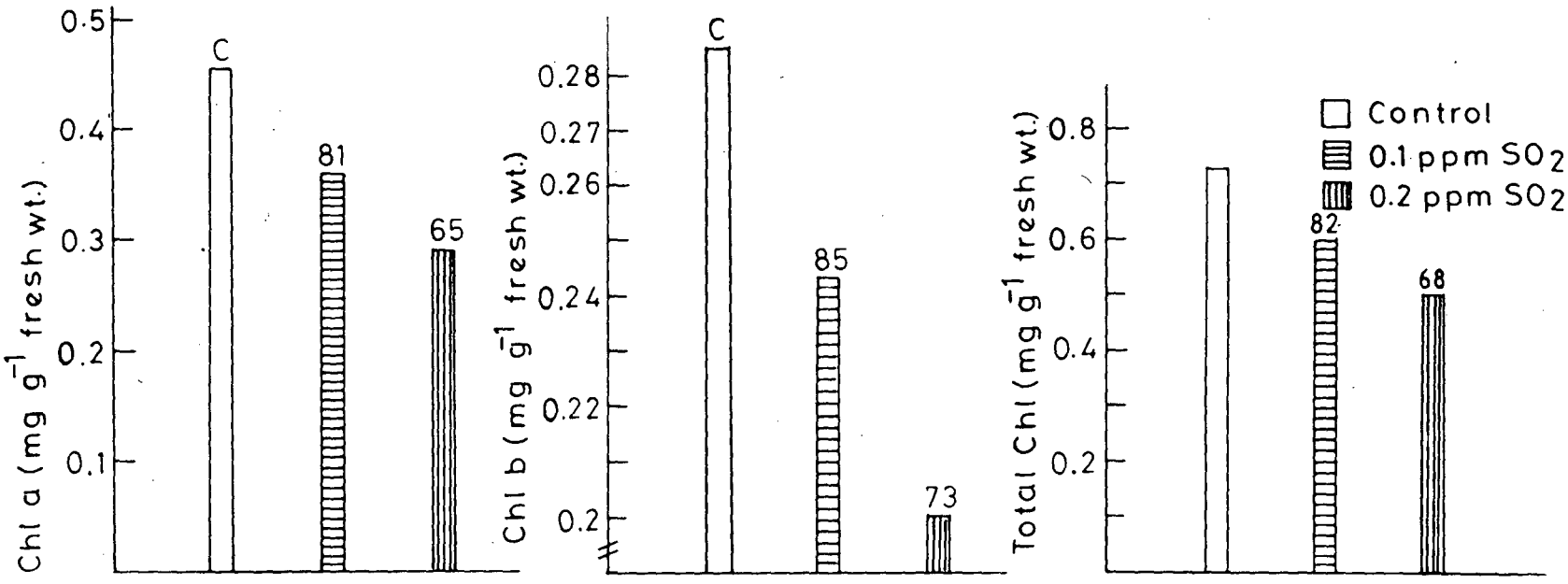

Fig.11 Chlorophyll content of Spinacia oleracea plants fumigated with 0.1 and 0.2 ppm SO_2 (2 hr daily for six weeks)

respectively with respect to control value of 1.58 (Table 7, Fig.11).

In L. esculentum total chlorophyll content decreased after six weeks of fumigation to 0.572 and 0.579 mg g^{-1} fresh weight in plants subjected to T_1 and T_2 treatments, respectively, in contrast to control set having 0.744 mg g^{-1} fresh leaves (Table 8, Fig.12). Chlorophyll a decreased to 0.344 and 0.339 mg g^{-1} fresh leaves in plants subjected to T_1 and T_2 treatments for six weeks respectively in contrast to control set having 0.378 mg g^{-1} fresh weight (Table 8, Fig.12).

Chlorophyll b decreased to0.242 and 0.243 mg g^{-1} fresh weight in plants subjected to T_1 and T_2 treatments for six weeks, respectively in contrast to control value of 0.259 mg g^{-1} fresh weight (Table 8, Fig.11).

Total chlorophyll decreased to 0.572 and 0.579 mg g^{-1} fresh leaves in plants subjected to T_1 and T_2 treatments for six weeks with respect to control value of 0.744 mg g^{-1} fresh weight (Table 8, Fig.11).

Chlorophyll a/b ratio decreased to 1.42 and 1.39 in plants subjected to T_1 and T_2 treatments for six weeks with respect to control value of 1.45 (Table 8, Fig.11).

Biomass Content

In S. oleracea total biomass content increased to

Table 8. Effects of SO_2 (0.1 and 0.2 ppm 2 hr daily for six weeks) on the absolute and relative (values in parentheses) amounts of Chlorophyll (mg g^{-1} fresh weight) in L. esculentum

Parameter	Chlorophyll content of control plants	Chlorophyll content of plants exposed to 0.1 ppm of SO_2	% reduction over control	Chlorophyll content of plants exposed to 0.2 ppm of SO_2	% reduction over control
Chl a	0.378[a] ± 0.23 (100)	0.344 ± 0.07 (91.0)	-8.9	0.339 ± 0.41 (89.6)	-10.3
Chl b	0.259 ± 0.04 (100)	0.242 ± 0.42 (93.4)	-19.0	0.243 ± 0.23 (93.8)	-18.7
Chl (a+b)	0.744 ± 0.65 (100)	0.572 ± 0.36 (76.8)	-23.1	0.579 ± 0.11 (77.8)	-22.1
Chl a/b	1.45	1.42		1.39	

[a] Average of three readings

Table 9. Effect of 0.1 and 0.2 ppm of SO_2 fumigation (2 hr daily for six weeks) on *S. oleracea* (average of twenty readings)

Plant system	Biomass of control Plants	Biomass of Plants fumigated with 0.1 ppm of SO_2	% reduction over control	Biomass of Plants fumigated with 0.2 ppm of SO_2	% reduction over control
Shoot	3.30 ± 3.74	3.77 ± 3.74	+14.2	4.63 ± 3.74	+40.3
Root	0.432 ± 3.74	0.329 ± 3.74	-23.8	0.393 ± 0.20	-9.5
Total	3.73 ± 3.6	3.75 ± 3.74	+0.53	5.33 ± 3.74	+42.8
Root/Shoot ratio	0.130	0.087		0.084	

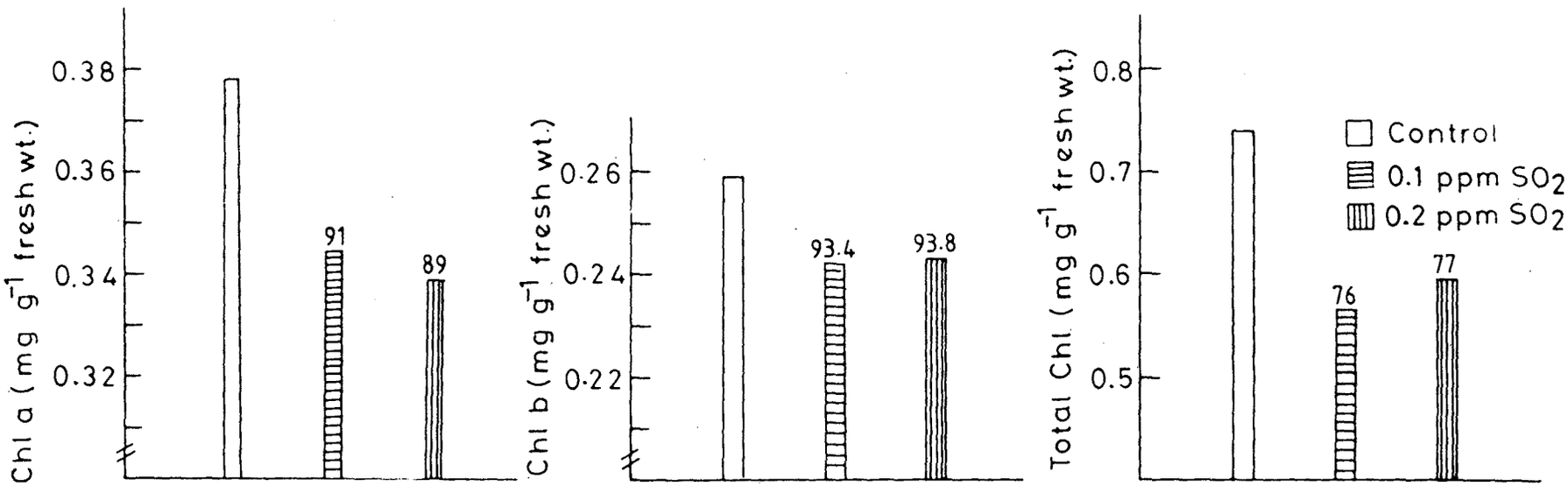

Fig.12 Chlorophyll content of Lycoperscion esculentum plants fumigated with 0.1 and 0.2 ppm SO_2 2 hr daily for six weeks

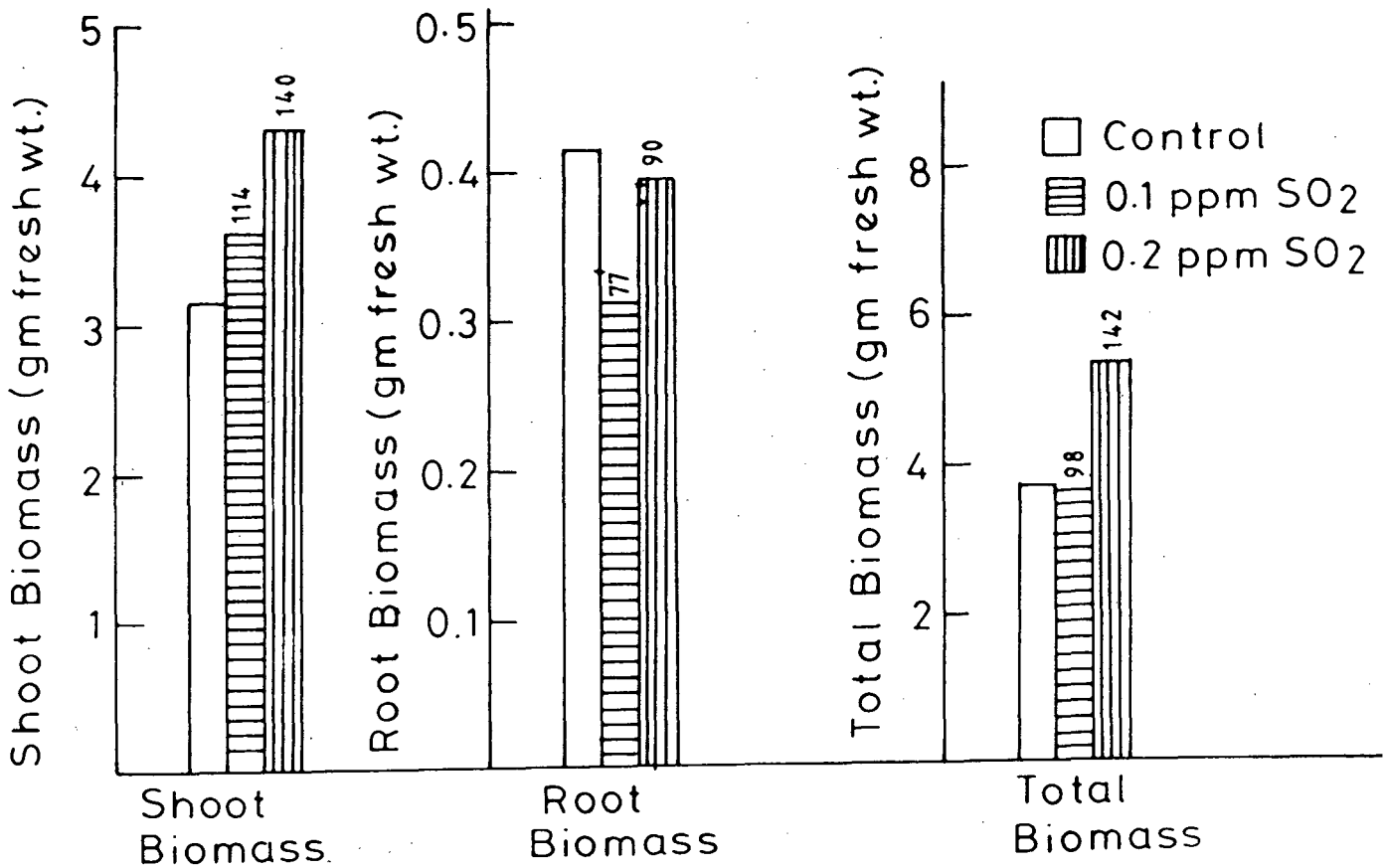

Fig.13 Biomass of Spinacia oleracea plants fumigated with 0.1 and 0.2 ppm SO_2 2 hr daily for six weeks

3.75 and 5.33 g fresh weight in plants subjected to T_1 and T_2 treatments, for six weeks, respectively in contrast to control set having 3.73 g fresh weight (Table 9, Fig.13).

Shoot biomass increased to 3.77 and 4.63 g fresh weight in plants subjected to T_1 and T_2 treatments for six weks over control set having 3.30 g fresh weight (Table 9, Fig.13).

Root biomass decreased to 0.329 and 0.393 g fresh weight in plants subjected to T_1 and T_2 treatments for six weeks as compared to 0.432 g fresh weight in control plants (Table 9, Fig.13).

Root:shoot ratio decreased to 0.087 and 0.084 in plants subjected to T_1 and T_2 treatments for six weeks in contrast to 0.130 R/S ratio in control plants (Plate 5, 6).

In L. esculentum total biomass decreased to 14.0 and 13.9 g fresh weight in T_1 and T_2 treated plants in comparison to control set having 15.4 g fresh weight (Table 10, Fig.14).

Total shoot biomass decreased to 12.7 and 12.8 g fresh weight in plants subjected to T_1 and T_2 treatments for six weeks, respectively in comparison to control set having 13.4 g fresh weight (Table 10, Fig.14).

Stem biomass decreased to 7.27 and 7.65 g fresh weight in T_1 and T_2 treated plants, respectively for six

Table 10. Biomass content (g fresh weight) of L. esculentum fumigated with 0.1 and 0.2 ppm of SO_2 2 hr daily for six weeks (average of six readings)

Plant system	Biomass of control Plants	Biomass of Plants exposed to 0.1 ppm of SO_2	% reduction over control	Biomass of Plants exposed to 0.2 ppm of SO_2	% reduction over control
Stem	8.09 ± 2.23	7.27 ± 2.23	-10.1	7.65 ± 2.23	-5.43
Leaf	5.33 ± 2.23	5.51 ± 2.44	+3.30	5.18 ± 2.23	-2.81
Total shoot	13.42	12.78	-4.76	12.83	-4.39
Root	2.03 ± 2.23	1.22 ± 2.23	-39.9	1.14 ± 0.89	-43.8
Total biomass	15.45	14.00	-2.17	13.97	-12.82
Root:Shoot ratio	0.151	0.095		0.088	
Leaf:Stem ratio	0.65	0.75		0.67	

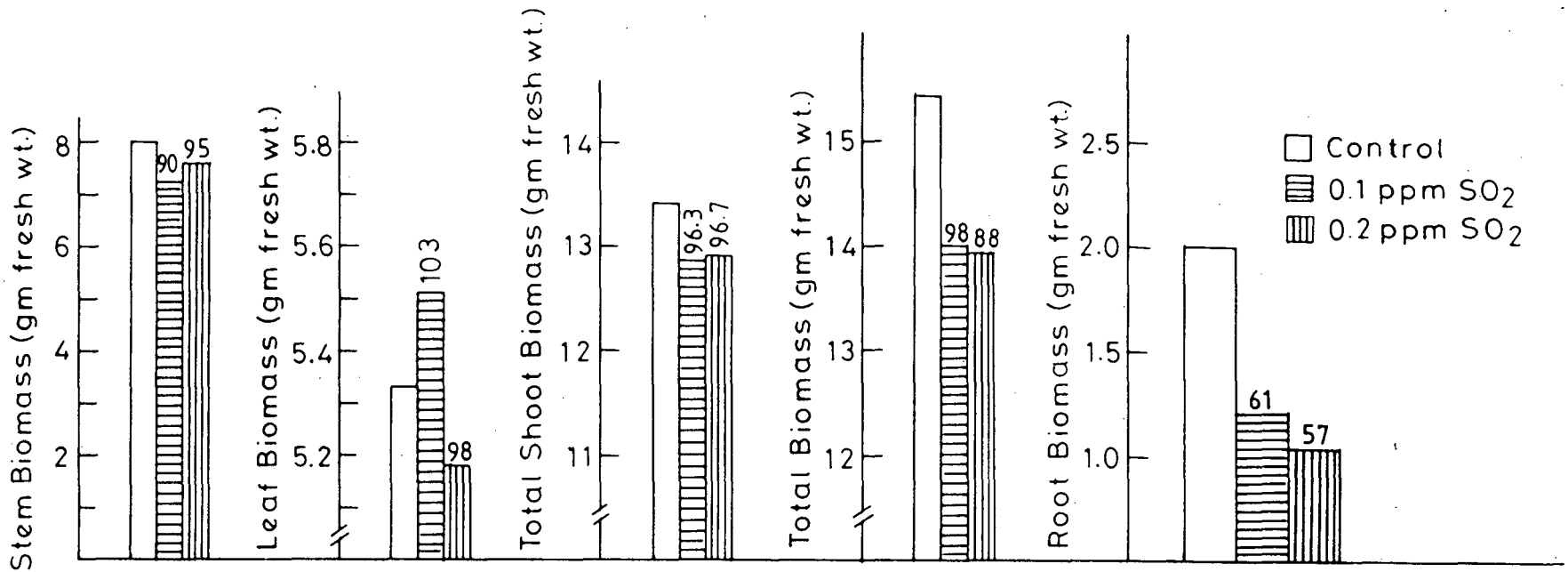

Fig.14 Biomass of Lycoperscion esculentum plants fumigated with 0.1 and 0.2 ppm SO_2 2 hr daily for six weeks

Plate 5. Difference in biomass content of S. oleracea plants exposed to 0.2 ppm SO_2 (2 hr daily for six weeks) along with control

Plate 6. Above uprooted plants of S. oleracea fumigated with 0.2 ppm SO_2 (2 hr daily for six weeks) in pots along with control


C
F


F
F
C

weeks as compared to control set having 8.09 g fresh weight (Table 10, Fig.14).

Leaf biomass increased to 5.51 g fresh weight in plants subjected to T_1 treatment whereas it decreased in T_2 treated plants for six weeks to 5.18 g fresh weight as compared to control set having 5.33 g fresh weight of leaf biomass (Table 10, Fig.14).

Root biomass in T_1 and T_2 treated plants decreased to 1.22 and 1.14 g fresh weight, respectively as compared to control set having 2.03 g fresh weight of root biomass (Table 10, Fig.14).

Root:shoot ratio decreased to 0.095 and 0.088 in plants subjected to T_1 and T_2 treatments for six weeks, respectively in R/S ratio in control plants was 0.151 (Table 10, Fig.14).

Leaf:stem ratio increased to 0.75 and 0.67 in plants subjected to T_1 and T_2 treatments for six weeks, respectively as compared to control set having 0.65 (Table 10, Fig.14).

Net Photosynthesis

In S. oleracea net photosynthesis increased following sulphur dioxide fumigation for six weeks. For first set of observations, it decreased to 0.777 u mol m^{-2} sec^{-1}

Table 11. Effect of SO_2 (0.1 and 0.2 ppm) fumigated (2 hr daily for six weeks) on net photosynthesis (u mol^{-2} sec^{-1}) in *S. oleracea* of CO_2 fixed.
(Average of ten readings)

Set No.	Net photosynthesis of Control plants	Net photosynthesis of plants fumigated with 0.1 ppm of SO_2	% change over control
S_1	0.9539	0.777	-18.5
S_2	0.9539	0.833	-10.9
S_3	0.6028	0.9281	+35.0

Table 12. Observations of Net Photosynthesis in S. oleracea and L. esculentum plants

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PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 7.980 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 304.9 mg H2O/m2/s
INIT INT CO2 = 289.1 ppm
W = -4.458 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	2018.	46.08	33.02	32.60	362.2	8.708	.0000	.0000
2	3	2017.	46.76	33.09	32.64	361.2	8.692	2.069	.8544
3	6	1924.	47.40	33.13	32.69	360.1	8.708	2.089	.8543
4	9	2026.	47.98	33.25	32.75	359.3	8.679	2.106	.6406
5	12	2012.	48.59	33.35	32.80	357.9	8.685	2.138	1.067
6	15	2011.	49.18	33.35	32.86	355.8	8.662	2.198	1.707
7	18	2010.	49.69	33.41	32.91	355.2	8.599	2.102	.4268
8	21	2009.	50.16	33.45	32.96	355.0	8.582	2.057	.2134
M	3	2002.	48.25	33.26	32.78	358.3	8.676	2.108	.8235
R	21	101.3	4.076	0.43	0.36	7.250	.1093	.1416	1.494
IV	0	1997.	46.18	33.03	32.59	362.3	8.726	2.098	1.037
IE	0	22.39	.0540	0.02	0.00	.3058	.0172	.0393	.3887

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PLANT# .0000
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LEAF AREA = 7.600 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 404.1 mg H2O/m2/s
INIT INT CO2 = 318.3 ppm
W = -4.044 ppm/(g/m3)

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1	0	1996.	50.11	34.20	34.48	385.6	8.359	.0000	.0000
2	3	1998.	51.03	34.43	34.52	384.0	8.558	3.379	1.337
3	6	1992.	51.89	34.39	34.56	382.6	8.226	3.391	1.114
4	9	1995.	52.72	34.41	34.61	380.8	8.398	3.471	1.540

6	15	1993.	54.26	34.60	34.68	378.1	8.373	3.272	.6712
7	18	1991.	54.96	34.64	34.73	377.5	8.634	3.578	.4455
8	21	1992.	55.57	34.69	34.78	375.4	8.435	3.542	1.781
M	3	1994.	53.03	34.49	34.63	380.4	8.358	3.442	1.209
R	21	7.187	5.467	0.49	0.30	10.20	.6801	.3057	1.336
IV	0	1997.	50.26	34.27	34.48	385.3	8.329	3.359	1.293
IE	0	.9796	.0788	0.04	0.00	.2626	.1443	.0756	.3670

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CHAMBER VOLUME = 1098. cm3
LEAF AREA = 7.600 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 393.4 mg H2O/m2/s
INIT INT CO2 = 324.5 ppm
W = -3.445 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1925.	43.86	33.61	32.95	368.9	11.03	.0000	.0000
2	3	1948.	44.69	33.70	33.01	368.4	11.01	2.670	.4480
3	6	1932.	45.47	33.91	33.06	367.3	10.99	2.613	.8959
4	9	1976.	46.25	33.87	33.12	367.1	11.01	2.732	.2239
5	12	2032.	46.96	33.86	33.18	365.7	10.97	2.736	1.119
6	15	2031.	47.67	34.08	33.23	363.3	10.93	2.756	2.014
7	18	2036.	48.33	34.15	33.27	363.0	10.93	2.662	.2238
8	21	2017.	48.91	34.19	33.32	362.0	10.95	2.654	.8952
M	3	1989.	46.53	33.92	33.14	365.8	10.98	2.689	.8316
R	21	110.9	5.052	0.58	0.37	6.982	.1010	.1423	1.791
IV	0	1927.	43.98	33.64	32.96	369.4	11.02	2.680	.6080
IE	0	15.64	.0643	0.04	0.01	.3610	.0137	.0435	.5124

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BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 497.1 mg H2O/m2/s
INIT INT CO2 = 312.1 ppm
W = -4.026 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	2015.	48.59	34.08	35.26	375.4	10.82	.0000	.0000
2	3	2021.	49.69	34.23	35.29	374.0	10.83	5.150	1.111
3	6	2017.	50.64	34.34	35.33	372.2	10.89	4.953	1.556
4	9	2023.	51.45	34.53	35.38	370.3	10.85	4.733	1.556
5	12	2023.	52.11	34.47	35.42	368.7	10.78	4.333	1.333
6	15	2028.	52.74	34.67	35.45	367.6	10.75	4.279	.8890
7	18	2030.	53.38	34.79	35.49	367.1	10.71	4.420	.4444
8	21	2035.	54.04	34.78	35.53	366.0	10.60	4.663	.8887
M	3	2024.	51.62	34.49	35.39	370.1	10.80	4.647	1.111

IV	0	2015.	48.94	34.13	35.26	375.0	10.89	5.020	1.556
IE	0	1.571	.1432	0.04	0.00	.3865	.0403	.1934	.2495

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LEAF AREA = 7.600 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 612.7 mg H2O/m2/s
INIT INT CO2 = 317.3 ppm
W = -2.309 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1881.	48.06	35.20	35.34	368.4	8.494	.0000	.0000
2	3	2013.	49.91	35.33	35.39	367.1	8.466	5.808	1.111
3	6	1972.	51.65	35.47	35.45	365.2	8.423	5.936	1.555
4	9	1970.	53.26	35.64	35.49	365.2	8.395	5.669	.0000
5	12	1962.	54.70	35.56	35.53	363.3	8.374	5.514	1.555
6	15	1968.	56.19	35.75	35.56	360.1	8.380	6.057	2.665
7	18	1964.	57.43	35.92	35.61	359.0	8.326	5.237	.8885
I	21	1	1	1	1	1	1	1	1
	0	1	1	1	1	1	1	1	1

PAGE=155
OBS= 8
01 JAN 00:41:47
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 7.600 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 612.7 mg H2O/m2/s
INIT INT CO2 = 317.3 ppm
W = -2.309 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1881.	48.06	35.20	35.34	368.4	8.494	.0000	.0000
2	3	2013.	49.91	35.33	35.39	367.1	8.466	5.808	1.111
3	6	1972.	51.65	35.47	35.45	365.2	8.423	5.936	1.555
4	9	1970.	53.26	35.64	35.49	365.2	8.395	5.669	.0000

6	15	1968.	56.19	35.75	35.56	360.1	8.380	6.057	2.665
7	18	1964.	57.33	35.89	35.61	359.0	8.326	5.237	.8885
8	21	1964.	58.50	35.97	35.65	358.5	8.337	5.476	.4442
M	3	1967.	53.76	35.60	35.50	363.3	8.408	5.671	1.174
R	21	131.6	10.44	0.77	0.31	9.936	.1679	.8203	2.665
IV	0	1949.	48.48	35.23	35.35	368.6	8.481	5.922	1.258
IE	0	24.94	.2110	0.04	0.01	.4864	.0125	.2002	.7225

PAGE=156
OBS= 8
01 JAN 00:45:29
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 7.600 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 516.1 mg H2O/m2/s
INIT INT CO2 = 310.2 ppm
W = -2.909 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1823.	52.57	33.75	34.56	356.3	8.379	.0000	.0000
2	3	1750.	54.01	33.83	34.61	355.5	8.357	6.612	.6685
3	6	1727.	55.31	33.96	34.66	352.3	8.276	6.385	2.673
4	9	1707.	56.55	34.00	34.70	351.8	8.183	6.453	.4455
5	12	1690.	57.65	34.02	34.74	351.2	8.291	6.181	.4455
6	15	1679.	58.67	34.11	34.79	348.3	8.259	6.524	2.450
7	18	1699.	59.68	34.19	34.83	348.0	8.248	6.621	.2226
8	21	1699.	60.58	34.25	34.88	346.1	8.259	6.691	1.558
M	3	1716.	56.92	34.02	34.72	351.2	8.285	6.495	1.209
R	21	144.4	8.006	0.50	0.32	10.20	.1958	.5097	2.451
IV	0	1775.	52.90	33.77	34.56	356.3	8.337	6.398	1.237
IE	0	19.71	.1509	0.02	0.00	.4554	.0340	.1360	.8508

PAGE=157
OBS= 8
01 JAN 00:51:48
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 6.080 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 683.9 mg H2O/m2/s
INIT INT CO2 = 305.3 ppm
W = -3.171 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	2032.	51.57	34.20	35.12	366.0	8.373	.0000	.0000
2	3	2019.	53.09	34.34	35.17	363.3	8.394	11.49	2.780
3	6	1989.	54.48	34.52	35.22	362.2	8.274	11.25	1.112
4	9	1972.	55.75	34.58	35.28	359.8	8.252	11.10	2.501
5	12	1943.	56.97	34.68	35.32	358.7	8.410	11.01	1.111
6	15	1904.	58.06	34.83	35.36	356.9	8.291	10.73	1.945
7	18	1870.	59.07	34.77	35.40	355.2	8.274	10.47	1.667
8	21	1838.	60.02	34.85	35.45	353.8	8.333	11.44	1.388

R	21	193.9	8.445	0.65	0.33	12.08	.1577	1.023	1.669
IV	0	2045.	51.91	34.27	35.13	365.4	8.353	11.33	2.239
IE	0	6.132	.1648	0.05	0.00	.2619	.0402	.2839	.4982

PAGE=158
OBS= 8
01 JAN 01:02:10
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 15.20 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 259.2 mg H2O/m2/s
INIT INT CO2 = 282.3 ppm
W = -3.971 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1829.	53.04	34.53	35.26	364.6	8.346	.0000	.0000
2	3	1801.	54.35	34.77	35.31	362.5	8.352	1.985	.8894
3	6	1780.	55.60	34.98	35.36	360.6	8.395	1.970	.7781
4	9	1789.	56.77	35.02	35.39	356.6	8.390	1.924	1.667
5	12	2010.	57.85	35.07	35.43	354.7	8.415	1.947	.7779
6	15	1961.	58.85	35.07	35.48	354.2	8.362	2.011	.2222
7	18	1951.	59.80	35.28	35.53	352.8	8.340	2.023	.5554
8	21	1944.	60.68	35.22	35.56	351.5	8.313	1.948	.5554
M	3	1883.	57.15	35.01	35.42	357.1	8.371	1.973	.7779
R	21	229.8	7.640	0.75	0.30	13.15	.0747	.0989	1.444
IV	0	1786.	53.30	34.67	35.27	363.9	8.379	1.962	1.139
IE	0	43.79	.1305	0.06	0.01	.7082	.0228	.0296	.3253

PAGE=159
OBS= 8
01 JAN 01:05:13
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 17.10 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 268.3 mg H2O/m2/s
INIT INT CO2 = 283.1 ppm
W = -3.380 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1918.	43.49	34.10	35.14	366.5	8.735	.0000	.0000
2	3	1920.	45.49	34.25	35.15	364.6	8.693	1.652	.6921
3	6	1926.	47.40	34.46	35.17	361.4	8.632	1.679	1.186
4	9	1928.	49.16	34.66	35.21	358.7	8.673	1.681	.9885
5	12	1928.	50.79	34.82	35.25	356.6	8.559	1.672	.7907
6	15	1929.	52.30	34.98	35.27	353.9	8.465	1.603	.9883
7	18	1931.	53.72	35.11	35.32	352.6	8.428	1.649	.4941
8	21	1928.	55.04	35.23	35.34	350.7	8.443	1.575	.6916
M	3	1926.	49.73	34.71	35.23	358.1	8.598	1.644	.8331
R	21	12.75	11.54	1.13	0.20	15.84	.3066	.1057	.6922
IV	0	1921.	43.91	34.12	35.12	366.3	8.744	1.690	1.006
IE	0	1.683	.2061	0.03	0.01	.3880	.0290	.0242	.1740

PAGE=160
OBS= 8
01 JAN 01:07:38
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 14.82 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 309.1 mg H2O/m2/s
INIT INT CO2 = 278.5 ppm
W = -3.338 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1956.	48.40	33.87	35.28	345.6	8.682	.0000	.0000
2	3	1957.	50.20	34.13	35.33	343.4	8.667	2.422	.9121
3	6	1956.	51.91	34.25	35.37	340.2	8.722	2.414	1.368
4	9	1959.	53.48	34.35	35.39	338.1	8.695	2.341	.9119
5	12	1960.	54.94	34.54	35.44	336.2	8.735	2.443	.7978
6	15	1956.	56.40	34.66	35.48	334.8	8.695	2.542	.5698
7	18	1957.	57.58	34.79	35.53	332.4	8.580	2.336	1.025
8	21	1959.	58.75	34.96	35.56	330.3	8.347	2.329	.9114
M	3	1957.	54.01	34.45	35.42	337.6	8.682	2.404	.9281
R	21	4.000	10.34	1.09	0.28	15.30	.1557	.2133	.7981
IV	0	1957.	48.78	33.93	35.28	345.1	8.758	2.433	1.056
IE	0	.8939	.1931	0.02	0.01	.3334	.0747	.0622	.1900

PAGE=161
OBS= 8
01 JAN 01:14:42
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 15.20 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 311.9 mg H2O/m2/s
INIT INT CO2 = 317.5 ppm
W = -3.449 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1979.	50.11	33.89	34.83	368.1	8.753	.0000	.0000
2	3	1979.	52.11	34.08	34.85	366.5	8.748	2.486	.6680
3	6	1982.	53.94	34.27	34.90	364.6	8.760	2.534	.7792
4	9	1983.	55.67	34.50	34.93	362.2	8.605	2.491	1.001
5	12	1984.	57.26	34.56	34.96	359.5	8.604	2.440	1.113
6	15	1984.	58.75	34.75	35.00	356.6	8.544	2.495	1.224
7	18	1986.	60.09	34.88	35.04	354.7	8.558	2.426	.7789
8	21	1982.	61.41	35.03	35.07	352.0	8.623	2.472	1.112
M	3	1982.	56.22	34.50	34.95	360.6	8.653	2.478	.9539
R	21	7.187	11.30	1.14	0.24	16.11	.2158	.1082	.5561
IV	0	1979.	50.54	33.93	34.82	368.8	8.754	2.510	.7595
IE	0	1.178	.2068	0.03	0.00	.3040	.0367	.0253	.1454

PAGE=162

01 JAN 01:20:16
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 15.20 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 291.6 mg H2O/m2/s
INIT INT CO2 = 307.8 ppm
W = -3.913 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	2030.	54.82	34.67	35.54	363.8	8.287	.0000	.0000
2	3	2027.	56.50	34.85	35.57	361.4	8.554	2.575	.9997
3	6	2028.	57.89	35.01	35.60	359.8	8.500	2.392	.6664
4	9	2027.	59.26	35.12	35.64	357.4	8.415	2.503	.9995
5	12	2027.	60.55	35.22	35.66	354.7	8.308	2.450	1.110
6	15	2027.	61.73	35.31	35.68	352.6	8.526	2.387	.8883
7	18	2026.	62.85	35.46	35.72	351.0	8.403	2.557	.6661
8	21	2029.	63.87	35.54	35.75	348.0	8.371	2.461	1.221
M	3	2027.	59.73	35.15	35.65	356.1	8.427	2.475	.9359
R	21	4.000	9.055	0.87	0.21	15.84	.2666	.1878	.5549
IV	0	2028.	55.19	34.73	35.54	363.9	8.428	2.491	.8668
IE	0	.9628	.1631	0.02	0.01	.2079	.0689	.0612	.1726

PAGE=163
OBS= 8
01 JAN 01:28:33
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 15.58 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 305.6 mg H2O/m2/s
INIT INT CO2 = 311.0 ppm
W = -2.006 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1943.	42.25	34.33	35.93	358.7	9.158	.0000	.0000
2	3	1936.	44.20	34.55	35.97	356.9	9.110	1.960	.7576
3	6	1918.	46.08	34.77	35.99	355.8	9.197	1.952	.4328
4	9	1924.	47.79	35.03	36.03	355.0	9.163	1.938	.3246
5	12	1932.	49.45	35.22	36.05	353.4	9.143	1.926	.6491
6	15	1934.	51.01	35.36	36.08	351.5	9.082	1.952	.7573
7	18	1937.	52.48	35.45	36.10	349.9	9.008	1.926	.6490
8	21	1933.	53.84	35.60	36.12	348.3	8.888	1.913	.6490
M	3	1931.	48.43	35.05	36.04	353.7	9.123	1.938	.6028
R	21	24.75	11.59	1.27	0.19	10.47	.1884	.0475	.4330
IV	0	1932.	42.59	34.40	35.94	358.8	9.208	1.961	.5354
IE	0	5.520	.1704	0.05	0.01	.2431	.0481	.0088	.1320

PAGE=164
OBS= 8
01 JAN 01:31:13
SO2 CON .0000
PLANT# .0000

CHAMBER VOLUME = 1098. cm3
LEAF AREA = 20.14 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 324.5 mg H2O/m2/s
INIT INT CO2 = 283.0 ppm
W = -3.679 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1809.	45.84	33.54	35.34	362.0	9.951	.0000	.0000
2	3	1819.	48.98	33.96	35.32	357.7	9.871	2.504	1.342
3	6	1823.	51.82	34.26	35.33	354.2	9.970	2.462	1.090
4	9	1824.	54.45	34.57	35.37	350.4	9.790	2.552	1.174
5	12	1820.	56.82	34.76	35.40	345.6	9.678	2.458	1.509
6	15	1819.	58.94	34.98	35.44	343.2	9.717	2.429	.7548
7	18	1821.	60.92	35.14	35.47	339.4	9.750	2.438	1.174
8	21	1821.	62.68	35.25	35.50	336.2	9.729	2.405	1.006
M	3	1820.	55.17	34.58	35.39	348.5	9.818	2.464	1.150
R	21	15.12	16.84	1.71	0.18	25.78	.2924	.1468	.7550
IV	0	1816.	46.67	33.72	35.30	361.5	9.933	2.522	1.307
IE	0	2.887	.3987	0.08	0.01	.3642	.0473	.0283	.1838

PAGE=165
OBS= 8
01 JAN 01:33:21
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 20.90 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 285.3 mg H2O/m2/s
INIT INT CO2 = 300.4 ppm
W = -2.963 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1927.	44.59	33.47	35.53	370.6	9.715	.0000	.0000
2	3	1929.	47.30	33.78	35.55	367.3	9.695	2.084	.9695
3	6	1930.	49.84	34.03	35.59	363.6	9.775	2.142	1.130
4	9	1930.	52.18	34.29	35.62	361.7	9.875	2.133	.5654
5	12	1939.	54.31	34.44	35.65	359.0	9.908	2.117	.8076
6	15	1924.	56.28	34.65	35.68	356.6	9.908	2.148	.7268
7	18	1929.	58.14	34.81	35.71	353.4	9.775	2.182	.9690
8	21	1928.	59.80	34.98	35.75	351.2	9.801	2.151	.6459
M	3	1930.	52.89	34.32	35.63	360.3	9.807	2.137	.8307
R	21	14.37	15.20	1.51	0.22	19.33	.2128	.0971	.5655
IV	0	1930.	45.22	33.57	35.53	370.0	9.747	2.100	.9724
IE	0	2.939	.3053	0.04	0.00	.3258	.0498	.0168	.1518

PAGE=166
OBS= 8
01 JAN 01:38:04
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 16.34 cm2
BLR = .3000 s/cm

RH IN = .0000 %
INIT TRAN = 310.0 mg H2O/m2/s
INIT INT CO2 = 286.2 ppm
W = -3.452 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1964.	47.96	33.85	35.55	359.0	9.687	.0000	.0000
2	3	1965.	50.03	34.05	35.56	355.2	9.621	2.480	1.446
3	6	1966.	51.94	34.30	35.59	354.7	9.674	2.485	.2066
4	9	1967.	53.72	34.46	35.61	351.0	9.635	2.471	1.446
5	12	1964.	55.36	34.64	35.64	347.7	9.635	2.474	1.239
6	15	1964.	56.87	34.76	35.66	346.7	9.769	2.438	.4132
7	18	1965.	58.26	34.92	35.68	343.4	9.782	2.444	1.239
8	21	1964.	59.58	35.00	35.71	342.4	9.556	2.525	.4131
M	3	1965.	54.28	34.51	35.62	349.9	9.686	2.474	.9150
R	21	3.187	11.61	1.15	0.16	16.65	.1611	.0869	1.240
IV	0	1965.	48.43	33.92	35.54	358.5	9.662	2.471	1.173
IE	0	.6892	.2238	0.04	0.00	.5536	.0542	.0239	.4347

PAGE=166
OBS= 8
01 JAN 01:38:04
SO2 CON .0000
PLANT# .0000
PRESSURE = 1002 mb
CHAMBER VOLUME = 1098. cm3
LEAF AREA = 16.34 cm2
BLR = .3000 s/cm
STM RAT = 2
RH IN = .0000 %
INIT TRAN = 310.0 mg H2O/m2/s
INIT INT CO2 = 286.2 ppm
W = -3.452 ppm/(g/m3)

OB	TIME	QU	RH	LT	CT	C2	FL	CS	PH
1	0	1964.	47.96	33.85	35.55	359.0	9.687	.0000	.0000
2	3	1965.	50.03	34.05	35.56	355.2	9.621	2.480	1.446
3	6	1966.	51.94	34.30	35.59	354.7	9.674	2.485	.2066
4	9	1967.	53.72	34.46	35.61	351.0	9.635	2.471	1.446
5	12	1964.	55.36	34.64	35.64	347.7	9.635	2.474	1.239
6	15	1964.	56.87	34.76	35.66	346.7	9.769	2.438	.4132
7	18	1965.	58.26	34.92	35.68	343.4	9.782	2.444	1.239
8	21	1964.	59.58	35.00	35.71	342.4	9.556	2.525	.4131
M	3	1965.	54.28	34.51	35.62	349.9	9.686	2.474	.9150
R	21	3.187	11.61	1.15	0.16	16.65	.1611	.0869	1.240
IV	0	1965.	48.43	33.92	35.54	358.5	9.662	2.471	1.173
IE	0	.6892	.2238	0.04	0.00	.5536	.0542	.0239	.4347

CO_2 fixed in plants subjected to T_1 treatment, in contrast to control value of 0.9539 mol m^{-2} sec^{-1} CO_2 fixed (Table 11,12, Fig.15). In second set of observations net photosynthesis decreased to 0.8333 and in plants subjected to T_1 and T_2 treatments, in contrast to control value of 0.9539 mol m^{-2} sec^{-1} CO_2 fixed (Table 11,12, Fig.15). For third set of observations, net photosynthesis increased to 0.9281 u mol^{-2} sec^{-1} CO_2 fixed in plants subjected to T_1 treatment for six weeks , in contrast to control set having 0.6028 u mol m^{-2} sec^{-1} CO_2 fixed (Table 11,12, Fig.15).

In L. esculentum net photosynthesis decreased after six weeks of fumigation. In first set of observations, net photosynthesis decreased to 1.209 and 0.8316 u mol m^{-2} sec^{-1} CO_2 fixed in plants subjected to T_1 and T_2 treatments, respectively in contrast to control set having 1.527 u mol m^{-2} sec^{-1} CO_2 fixed (Table 12,13, Fig.16). For second set of observations, net photosynthesis decreased to 1.786 and 1.111 u mol^{-1} sec^{-1} CO_2 fixed in plant subjected to T_1 and T_2 treatment respectively in contrast to control set having 1.8235 u mol^{-2} sec^{-1} CO_2 fixed (Table 12,13, Fig.16). For third set of observations, net photosynthesis decreased to 1.174 u mol $m^{-2}sec^{-1}$ CO_2 fixed in T_2 treated plants as

Table 13 Net photosynthesis (u mol m^{-2} sec^{-1} CO_2 fixed) in L. esculentum fumigated with 0.1 and 0.2 ppm of SO_2 (2 hr daily for six weeks) (Average of ten readings)

Set No.	Rate of photosynthesis of Plants treated as control	Rate of photosynthesis of Plants fumigated with 0.1 ppm of SO_2	% change over control	Rate of photosynthesis of Plants fumigated with 0.2 ppm of SO_2	% change over control
S_1	1.527	1.209	-20.8	0.8316	-45.5
S_2	1.8235	1.786	-2.05	1.111	-39.0
S_2	1.209	1.513		1.174	-2.89

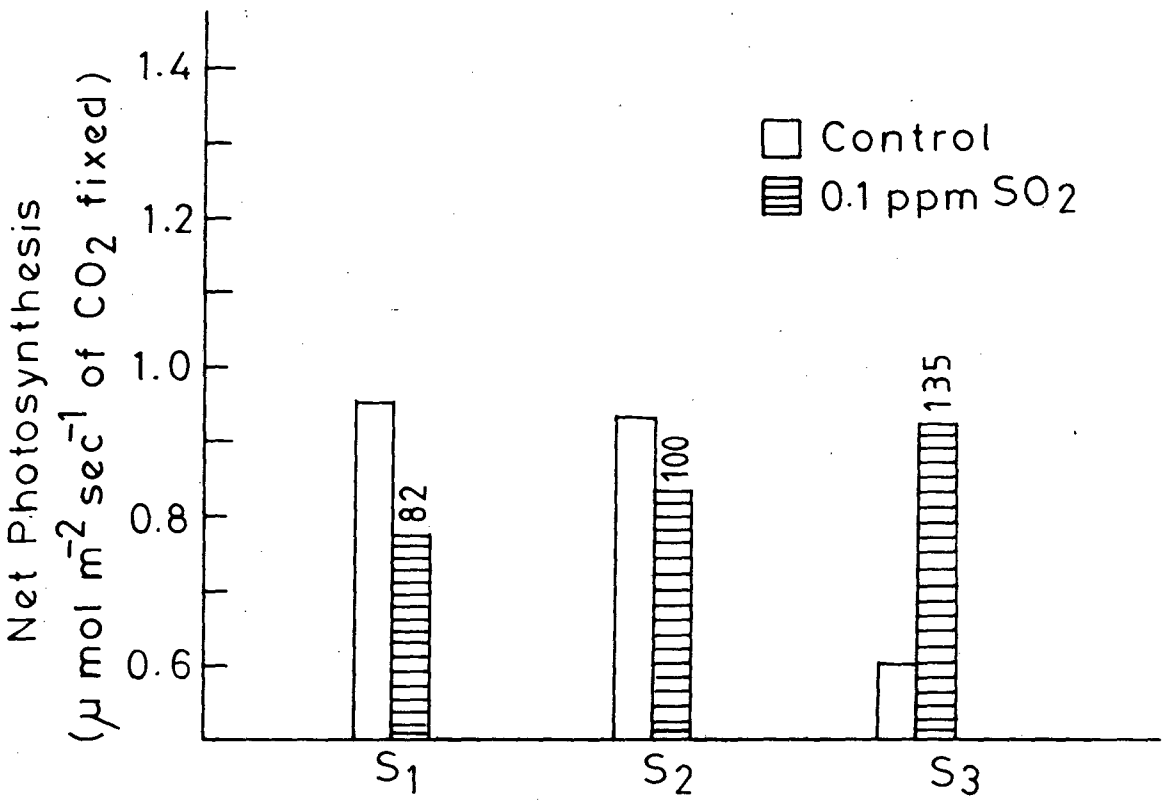

Fig.15 Effect of SO_2 fumigation (0.1 and 0.2 ppm, 2 hr daily for six weeks) on net photosynthesis in Spinacia oleracea

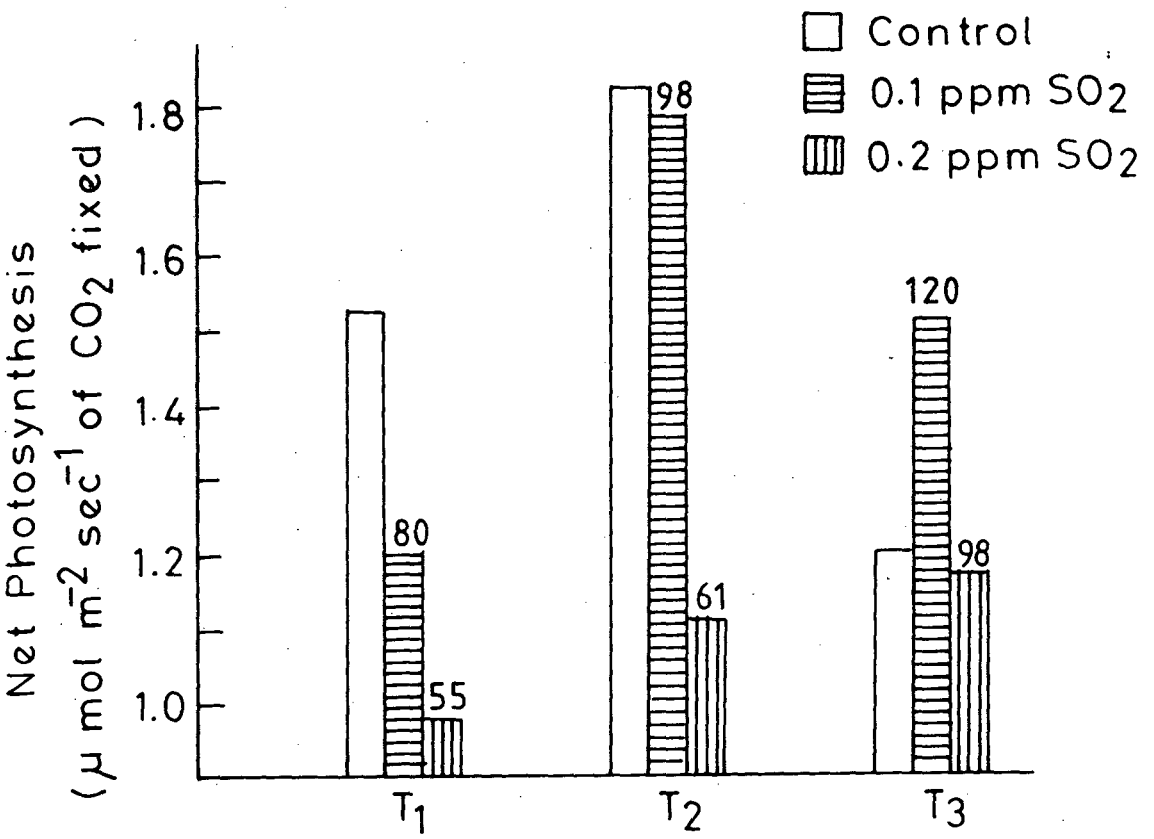

Fig.16 Effect of SO_2 fumigation (0.1 and 0.2 ppm, 2 hr daily for six weeks) on net photosynthesis in Lycoperscion esculentum

compared to control value of 1.209 u mol m^{-2} sec^{-1} CO_2 fixed (Table 12,13, Fig.16).

RuBisCo Activity

The RuBisCo activity of fumigated plants was assayed and compared with control. RuBisCo activity decreased significantly in plants of S. oleracea and L. esculentum subjected to T_1 and T_2 treatments.

The RuBisCo activity in S. oleracea decreased after six weeks of fumigation over control value of 0.95 u mol min^{-1} ml^{-1} NADH oxidized to 0.15 and 0.08 u mol min^{-1} ml^{-1} NADH oxidized in plants subjected to T_1 and T_2 treatments, respectively (Table 14, Fig.17). In second assay, RuBisCo activity decreased over control value of 0.91 u mol min^{-1} ml^{-1} NADH oxidized in plants subjected to T_1 and T_2 treatments for six weeks respectively in contrast to control value of 0.13 and 0.078 u mol min^{-1} ml^{-1} NADH oxidized (Table 14, Fig.17).

In L. esculentum RuBisCo activity decreased to 0.103 and 0.099 u mol min^{-1} ml^{-1} NADH oxidized in plants subjected to T_1 and T_2 treatments, respectively with respect to control value of 0.108 u mol min^{-1} ml^{-1} NADH oxidized (Table 15, Fig.17).

In second assay, RuBisCo activity decreased to 0.102 and 0.087 u mol min^{-1} ml^{-1} NADH oxidized in plants

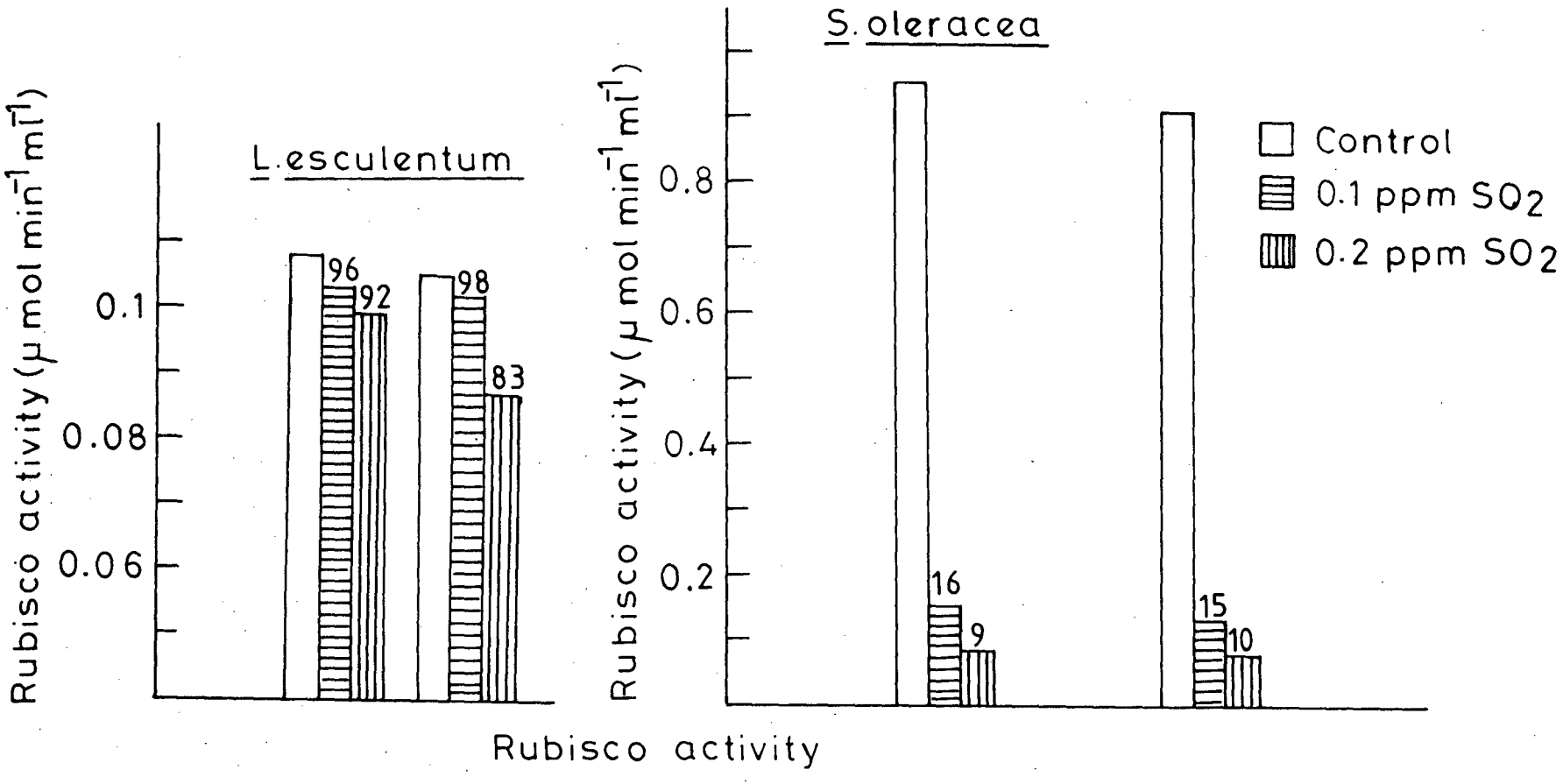

Fig.17 The RuBisCo activity in S. oleracia and L. esculentum plants fumigated with 0.1 and 0.2 ppm SO_2 2 hr daily for six weeks

Table 14. RuBisCo activity (u mol min^{-1} ml^{-1} NADH oxidized) in S. oleracea fumigated with 0.1 and 0.2 ppm SO_2 (2 hr daily for six weeks)

Set No.	RuBisCo activity of control plants	RuBisCo activity of plants exposed to 0.1 ppm SO_2	% reduction over control	RuBisCo activity of plants exposed to 0.2 ppm SO_2	% reduction over control
1	0.95	0.15	-84.2	0.08	-91.5
2	0.91	0.13	-85.7	0.078	-91.4

Table 15. RuBisCo activity in Lycoperscion esculentum fumigated (u mol min^{-1} ml^{-1} NADH oxidized) with 0.1 and 0.2 ppm SO_2 (2 hr daily for six weeks)

Set No.	RuBisCo activity of control plants	RuBisCo activity of plants exposed to 0.1 ppm SO_2	% change over control	RuBisCo activity of plants exposed to 0.2 ppm SO_2	% change over control
1	0.108	0.103	-4.62	0.099	-8.33
2	0.105	0.102	-2.85	0.087	-17.1

subjected to T_1 and T_2 treatments, respectively in contrast to control value of 0.105 u mol min^{-1} ml^{-1} NADH oxidized (Table 15, Fig.17).

Flower Formation

Sulphur dioxide enhanced flower formation in S. oleracea. After six weeks of fumigation with T_1 and T_2 treatments number of flowering plants increased to 4 and 8 as compared to control set having 2 flowering plants (Table 16).

Table 16. Effect of 0.1 and 0.2 ppm SO_2 (2 hr daily for six weeks) on flowering of S. oleracea

	SO_2 fumigation in days				
	14	21	28	35	42
	No. of flowering plants				
Plants fumigated with 0.1 ppm SO_2 for 2 hr daily[2]	-	-	1	2	4
Plants fumigated with 0.2 ppm SO_2 for 2 hr daily[2]	-	1	3	4	8
Control	-	-	-	-	2

Leaf Area

The sulphur dioxide treatment of S. oleracea plants fumigated with 0.1 (T_1) and 0.2 (T_2) ppm 2 hr daily for six weeks has shown that the leaf area of the treated plants is reduced by 2 to 4 percent as compared to control (Table 5).

In L. esculentum plants subjected to T_1 and T_2 treatments, the leaf area decreased. The reduction in leaf area varied between 24 to 26 percent (Table 5).

The reduction in leaf area in plants exposed to sulphur dioxide is also observed by Bell and Clough (1973) in Lolium perenne, Ashenden (1978) in Dactylis glomerata, Ashenden and Mansfield (1977) in Lolium perenne, Laurence (1979) in Zea mays and Triticum aestivum.

Gupta and Ghouse (1987) reported plants of A. esculentum grown in the vicinity of Kasimpur thermal power plant complex have less number of leaves per plant (higher degree of defoliation) and foliar injuries.

Leaf area and yield in cultivated plants have been shown to decrease as a result of sulphur dioxide fumigation in several laboratory and field studies (Davies, 1980; Heck et al., 1981; Ashenden, 1978;

Laurence, 1979; Ashenden and Mansfield 1977). A decrease in leaf area in sulphur dioxide exposed plants also holds true for the native forest floor plants of GieBen (West Germany) namely Allium ursinum, Anemone nemorosa and Arum maculatum by Steubing and Fangmeirer (1987).

Leaves Per Plant

In S. oleracea there was an increase of 29 to 49 percent in the average number of leaves in plants exposed to T_1 and T_2 sulphur dioxide treatments 2 hr daily for six weeks.

In L. esculentum there was a decrease in average number of leaves per plant after fumigation of six weeks. The decrease varied between 16 to 39 percent in plants exposed to T_1 and T_2 treatments 2 hr daily for six weeks as compared to control (Table 5). Observation of the decrease in average number of leaves per plant is supported by studies carried out by Laurence (1978), Gupta and Ghouse (1987) and Steubing and Fangmeirer (1987).

Increase in the average number of leaves per plant of S. oleracea appears to be a compensatory growth mechanism operating to counteract a reduced photosynthetic efficiency (Whitmore and Mansfield, 1983).

Observations made by Farrer et al., (1977) in P. sylvestris by Freer-Smith (1985) by Betula pedula

Garsed et al., (1981) in P. sylvestris by Prasad and Rao (1981) in Triticum sp. by Shanklin and Kozlowski (1984) in Fraxinus pennsylvanica also support the trends obtained in this study with respect to increase in number of leaves per plant. The plants growing in polluted air do appear to be somewhat more leafy. This would be a reasonable compensatory response to sulphur dioxide-induced losses in the photosynthetic capacity of leaves (Winner, Williams and von Caemmrerer, 1985). Plants with more leaves but lower specific photosynthesis will, however, have lower water use efficiency and will be more prone to drought stress; compensatory increase in root for the acquisition of water seems unlikely in view of the overall depression of root growth under pollution stress (Lechowicz, 1987).

In S. oleracea, leaf area in T_1 and T_2 treated plants decreased whereas number of leaves per plant increased. This was primarily due to reduction in leaf-size in T_1 and T_2 treated plants. The total leaf area of treated plants remained much less as compared to control inspite of increase in number of leaves due to their small size.

Chlorophyll Content

Chlorophyll content in treated S. oleracea and L. esculentum plants decreased 12 to 31 percent on exposure to T_1 and T_2 sulphur dioxide treatments for six weeks (Table 7,8).

Total chlorophyll was observed to decrease (12.7 - 31.5%) significantly in plants fumigated 2 hr daily upto six weeks with 0.1 and 0.2 ppm sulphur dioxide of S. oleracea and L. esculentum (Table 7,8). Chlorophyll a was found to be relatively more sensitive than chlorophyll b. Similar pattern of response of chlorophyll to sulphur dioxide was observed by Rao and Le Blanc (1966, 1968), Malhotra (1977), Rabe and Kreeb (1979), Laurenroth and Dodd (1981), Williams et al., (1971), Kondo et al., (1980), Shimazaki et al., (1980).

Nandi, Aggarwal and Rao (1986) reported that chlorophyll b was more sensitive in rice plants to sulphur dioxide damage than chlorophyll a. This they attributed to increase in chlorophyll activity (Malhotra, 1977) and/or inhibition of chlorophyll b synthesis (Aronoff and Kwok, 1977; Castelfranco, 1983).

Mechanism of sulphur dioxide interaction with chlorophyll was suggested as chlorophyll a is converted to phaeophytin following sulphur dioxide fumigation. The conversion of chlorophyll to phaeophytin resulted by replacing the Mg^{+2} with $2H^{+}$ formed due to increased cell acidity due to sulphur dioxide (Rao and Le Blanc, 1966) (Fig.4). However, production of phaeophytin does not seem to be sulphur dioxide specific. Arndt (1971) was

able to get the same type of conversion with other acids, such as hydrofluoric acid and hydrochloric acid.

Hill (1971) suggested that the breakdown of chlorophyll reported by Rao and Le Blanac (1965) was a secondary effect of sulphur dioxide, Puckett et al., (1973) suggested that the toxicity of lichens to sulphur dioxide was due to increased toxicity at low pH, was associated, in part, with the destruction of chlorophyll b by an irreversible oxidation process. Malhotra (1977) reported that chlorophyll a is converted into phaeophytin a and chlorophyll b into chlorophyllide b, in presence of enzyme chlorophyllase into chlorophyllide b following sulphur dioxide fumigation.

Peiser and Yang (1978) showed that free radicals produced from linoleic acid (LooH) decomposition by HSO_3^- were responsible for chlorophyll destruction. Later they observed increased amounts of malandialdehyde (MDA) in leaves damaged by sulphur dioxide. The MDA formation decreases with the reduction of chlorophyll a by the addition of tririon (1.2-dihydroxy benzene-3,5 disulphonate) a scavenger of superoxide (O_2^-) radical (Shimazaki et al., 1980). It has been suggested that the destruction of chlorophyll may be due to the formation of superoxide radical in plants exposed to sulphur dioxide. Chloroplasts produced O_2^- on the reducing side of PSI under

illumination (Asada et al., 1974). Low levels of sulphur dioxide fumigation may not bring about sufficient change in cell pH necessary for converting chlorophyll into phaeoplytin. However, deactivation of chlorophyll by superoxide radical (O_2^-) appears more likely in plants receiving low dose of sulphur dioxide (Fig.4). Thus, the effect of sulphur dioxide on chlorophyll may be considered under two cellular conditions, i.e., at pH values below and above 3.5 (Fig.4). At pH 2.2 to 3.5 the free H^+ ions generated in the cell from splitting of H_2SO_3 into SO_3^{2-} and H^+, displace Mg^{2+} from chlorophyll molecules to degrade from them into phaeophytin molecules (Rao and Le Blanac, 1966). At pH above 3.5 sulphur dioxide may affect the thylakoid membrane of chloroplant by causing oxidation of carotenoids through generation of O_2^- (superoxide radicals) from HSO_3^- (Peiser and Yang, 1978). Once the carotenoid protection is lost, the chlorophyll molecules get oxidized and reduced quantitatively, decreasing the photosynthetic ability of the plant. Also, the free radical (O_2^-) in the presence of superoxide dismutase (SOD) may increase the level of H_2O_2 in the cell which in turn may cause oxidation of chlorophyll molecule in presence of peroxidase and thereby may reduce the level of chlorophyll pigment in the cell.

Biomass

Biomass content of S. oleracea treated plants did not show any definate trend. The biomass of T_1 treated plants did not show any significant change and almost equal to control plants, only marginally 0.53 percent high whereas in T_2 treated plants, biomass was more than the control plants. The effect of sulphur dioxide on biomass data in S. oleracea did not show any relation to experimental treatments. However, root biomass decreased in T_1 and T_2 treated plants (Table 9). The increase in shoot biomass has mainly contributed towards increase in the total biomass content of S. oleracea plants (Table 9). The increase in total biomass content in T_1 and T_2 treated plants appears to be somewhat anamolous type. This deviation can be due to a number of reasons (1) seeds samples may not have same genetic composition as they were purchased from market which did not guarantee same genetic identity in all seeds, (2) period of six weeks fumigation was not sufficient to reveal effect of sulphur dioxide treatment on biomass.

In L. esculentum total biomass content decreased after six weeks of fumigation in T_1 and T_2 treated plants (Table 10). Decrease in root biomass varied between 39-44 per cent as compared to control. however reduction in shoot biomass varied between 4-5 percent, indicating

root tissues are adversely affected by sulphur dioxide pollution as compared to shoot tissue. However, leaf biomass increased in T_1 treated plants whereas it decreased in T_2 treated plants exposed for six weeks (Table 10).

The overall impact of sulphur dioxide pollution stress appears to be greater on roots than on shoot tissues. In L. esculentum, the mean reduction in root biomass is 39.9 and 43.8 percent in plants fumigated with 0.1 and 0.2 ppm of sulphur dioxide compared to 4 percent reduction for shoot biomass during the six weeks period, respectively (Table 10). In S. oleracea too, shoot biomass increased to 14.2 and 40.3 percent compared to reduction in root biomass by 23.8 and 26.1 percent in plants fumigated with 0.1 and 0.2 ppm of sulphur dioxide, respectively (Table 9). The greater suppression of root growth relative to shoot growth is more often apparent in response to either sulphur dioxide or O_3 (Lechowicz, 1987).

Radish (Reinert and Gray, 1981; Reinert et al., 1982) alfalfa (Tingey and Reinert, 1975), blue grass (Poa pratensis) (Whitmore and Mansfield, 1983), perennial rye grass (Lolium perenne) (Bell et al., 1979), Scots Pine (Pinus sylvestris) and Sitka spruce (Picea sitchensis)

(Garsed and Rutter, 1984), all show greater suppression of roots than of shoot biomass. In contrast, root and shoot growth in hardwood tree species appear to be either unaffected or equally affected by sulphur dioxide exposure (Garsed et al., 1979; Roberts, 1975). Norby and Kozlowski (1981), however, did show that the relative effects of sulphur dioxide on root versus shoot growth was temperature dependent in white birch (Betula papyriera), red pine (Pinus resinosa), and T_1 and T_2 two Eucalyptus species. For example, white Birch seedlings grown at 32°C after fumigation had greater suppression of root than shoot growth, but 12°C resulted in a greater suppression of shoot growth. The net effect of these changes in allocation priorities is to reduce the root:shoot ratio under sulphur dioxide pollution regimes. This may have occured because sulphur dioxide inhibits the phloem loading system (Teh and Swanson, 1982). The import of an altered root:shoot ratio lies in the possibility that the acquisition of carbon, energy, water and the nutrient resources will be impaired, thus aggravating deleterious effects of pollutant itself. The polluted plants may be more vulnerable to drought stress since proportionately less root is available to supply water to transpiring leaves (Lechowicz, 1987).

Sulphur dioxide fumigation T_1 and T_2 treatments 2 hr daily for six weeks increased leaf:stem ratio in S. oleracea (Table 13,14) and L. esculentum. Available numeric data (Farrar et al., 1977; Freer-Smith, 1985; Garsed et al., 1981; Prasad and Rao, 1981; Shanklin and Kozlowski, 1984) show a mean 7 percent increase in leaf biomass and a concomitant 5 percent decrease in mean stem biomass in sulphur dioxide fumigated plants. Similar trends are evident for white birch and pin oak (Quercus palustirs) seedlings (Roberts, 1975) and for tobocco but not for cucumber (Cucumis sativus) (Mejstrik, 1980). Since leaves account for essentially all transpiration and have higher nutrient concentrations than stem tissues, any disproportionate change in leaf to stem biomass under sulphur dioxide pollution regimes can potentially amplify the deleterious effects of reduced root:shoot ratios (Lechowicz, 1987).

Net Photosynthesis

Net photosynthesis was affected in S. oleracea and L. esculentum plants fumigated with T_1 sulphur dioxide treatment (Table 11,12,13).

In S. oleracea net photosynthesis decreased in T_1 treated plants. However, for third set of observations, there was an increase of 35 percent in net photosynthesis

as compared to control plants (Table 11). The cause of such variation could not be ascertained as time did not permit for another layout of this experimental schedule.

In L. esculentum, net photosynthesis decreased 2 to 50 percent in T_1 and T_2 treated plants for six weeks (Table 13).

The RuBisCo Activity

The RuBisCo activity was found to be decreased in S. oleracea and to84.2 to 85.7 percent and 91.4 to 91.5 percent in plants fumigated with 0.1 and 0.2 ppm sulphur dioxide respectively (Table 13). The same was true for L. esculentum, RuBisCo activity decreased to 2.85 to 4.62 percent and 6.33 to 17.1 percent in plants fumigated with 0.1 and 0.2 ppm of sulphur dioxide (Table 14). From these results it can be deducted that S. oleracea is more sensitive than L. esculentum.

It has been demonstrated that the levels of certain enzymes decrease while others apparently increase after sulphur dioxide fumigation (Horsman and Wellburn, 1976;1977; Pierre, 1977; Malhotra and Khan, 1980). RuBisCo is reported to decrease after pretreatment with sulphur dioxide (Miszalski and Ziegler, 1980). However, reason for this is not known. Ziegler (1972) proposed that the mechanism

by which sulphur dioxide interfered with photosynthesis was due to the potent and competitive inhibition of RuBisCo with respect to HCO_3^- . However, Gezelius and Hallgren (1980), reported using similar preparationsof RuBisCo from spinach and pine found SO_3^2 associated carboxylase activity to a lesser extent and was non-competitive with respect to HCO_3^-.

Hallgren and Gezelius (1982) suggested that a decrease of RuBisCo in senescing plants has been associated with proteolytic enzyme activity (Peterson and Huffaker, 1975). Whether the sulphur dioxide effect is associated with a stimulation of hydrolytic enzymes or with an increased access of RuBisCo to proteineses, this mimicking senescence, is not known. The lower levels of RuBisCo after sulphur dioxide treatment might also be considered in relation to decreease in protein synthesis (Godzik and Linskens,1974).

Flower Formation

Sulphur dioxide fumigation enhanced flower formation in plants fumigated with T_1 and T_2 treatments, 2 hr daily for six weeks in S. oleracea (Table 15, Plate 1,2). Similar observations were made by Murdy (1979) in Lepidium virginicum. In contrast, sulphur dioxide reduced flower number in

Begonia sp. (Adedipe et al., 1972; Reinert and Nelson, 1980) and in a variety of annual bedding plants (Adedipe et al., 1972).

Above studies clearly show that sulphur dioxide fumigation effects plant metabolism at various steps.

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