INTERIM REPORT ON PHYSIOLOGY AND MECHANISMS OF AIR-BORNE POLLUTANT INJURY TO VEGETATION, 1975 to 1978

by

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ABSTRACT

Biochemical studies conducted under controlled conditions suggested that low concentrations of SO_2 which normally do not produce any visual symptoms on the foliage cause injury to forest vegetation by altering pigment (chlorophyll, phaeophytin and chlorophyllide) metabolism, by inhibiting lipid synthesis and activities of various important enzyme systems and by causing ultrastructural disorganization of cellular membranes.

In general, aqueous and gaseous SO_2 produced results very similar to each other. Plants, when fumigated with SO_2 at the ambient air quality standards, exhibited various biochemical responses that can have a deleterious effect on the normal growth and yield of vegetation. However, 24-48 h after transferring the fumigated plants to an SO_2-free environment, there was a considerable recovery of such functions.

The results obtained so far clearly indicated that chlorophyll/phaeophytin ratio, Mg^{++} content, lipid biosynthesis and acid phosphatase and malate dehydrogenase activities of plant foliage are very sensitive indicators of SO_2 concentrations. Using such techniques, an attempt was made to detect pre-visual SO_2 injury to jack pine and white spruce from the field impingement area (immediate vicinity of GCOS plant). Although a gradient in sulphur content of the collected material was evident, there was no appreciable difference in any of the biochemical and physiological functions that responded to SO_2 under laboratory conditions. However, more experimentation needs to be conducted with some of the sensitive species of vegetation to determine air pollution effects on forest vegetation.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

The growth and yield of vegetation are the direct results of physiological and biochemical activities within the tissues. Environmental factors such as temperature, moisture, light, etc. play an important role in determining the rate of metabolic activity. It has been suggested that air pollutants can affect tree metabolism either temporarily or permanently depending upon concentration and length of exposure (Brandt and Heck 1968; Saunders and Wood 1973; Ziegler 1975; Malhotra and Hocking 1976).

Sulphur dioxide (SO_2) is one of the major air pollutants released from the industrial activity in the Athabasca Oil Sands area and may have the potential to cause damage to the forest vegetation and soils. Other air pollutants released along with SO_2 may also influence such damage. There seems to be a tendency to relate phytotoxicity of pollutants only to visual symptom development (Malhotra and Hocking 1976). However, in many greenhouse studies, SO_2 has been shown to reduce the growth and yield of vegetation in the absence of visual symptoms (Bleasdale 1952; Tingey *et al.*, 1971). This may be the result of disturbances at the ultrastructural or biochemical levels or both.

There are a number of physiological and biochemical events in plant metabolism that are interfered with before the development of visual symptoms of SO_2 phytotoxicity. Once injury to vegetation starts to occur at the visual symptom level, it may be irreversible. It is, therefore, essential that the early detection of air pollutant injury be carried out at biochemical and ultrastructural levels.

The only information available in the literature to determine previsual SO_2 injury to forest species and to establish threshold levels of SO_2 injury originates from other countries. However, due to considerable differences in vegetation type, climate and species present, such information is not directly applicable to the North American boreal forest species.

The objectives of this study are: (1) to study biochemical or physiological threshold levels of air pollutant injury to vegetation; (2) to develop sensitive and reliable methods for detecting air pollutant effects to forest vegetation prior to visual symptom development; and (3) to develop methods for selecting revegetation species on the basis of their true tolerance to air pollutants. In order to estimate the phytotoxic effects of various pollutants it is essential that the above objectives be tested first with each pollutant individually and then in combinations. The results described in this report relate mainly to the effects of SO_2 phytotoxicity; however, studies on combined effects of SO_2 with other pollutants are currently underway.

In the earlier phase of this program when the facilities for fumigating plant material with gaseous SO_2 were not available, research was carried out with aqueous SO_2 to understand the nature of phytotoxicity at the ultrastructural and biochemical levels. The results to date clearly suggest that the aqueous and gaseous SO_2 produce very similar effects.

Several techniques were developed and utilized to detect air pollution injury to forest species around the Alberta Oil Sands Environmental Research Program (AOSERP) study area (Figure 1). Further research is being conducted to establish threshold levels of air pollution injury to forest species. Because of the interim nature of this report, the statements made herein are subject to revision upon completion of the project and release of the final report.

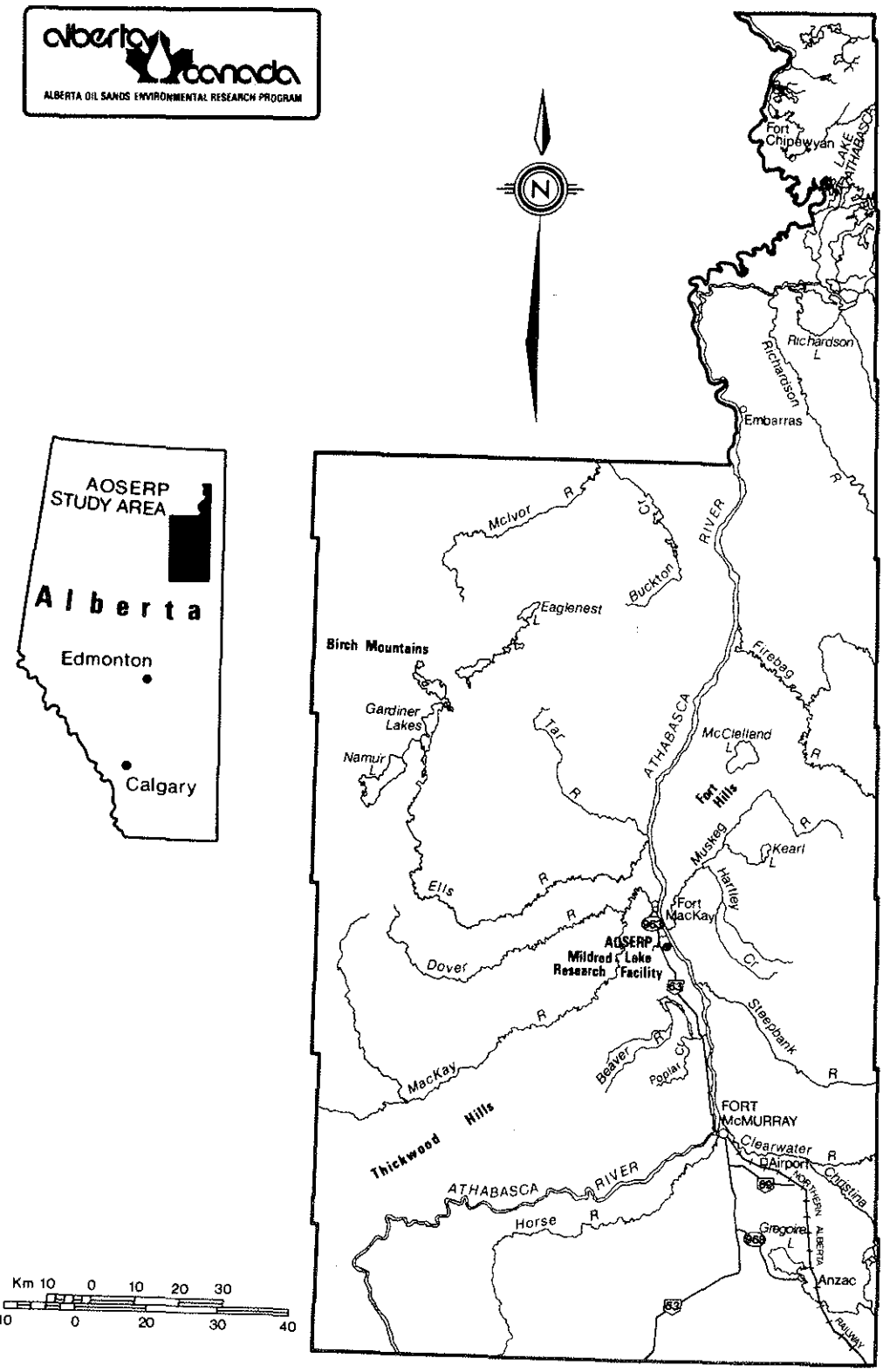

Figure 1. The AOSERP study area.

2. MATERIALS AND METHODS

Most of the research in this project was conducted in the laboratory, greenhouse and fumigation chamber facilities located at the Northern Forest Research Centre, Edmonton. Field specimens to assess the impact of air pollution to jack pine and white spruce were collected from the impingement area at distances of 2.8 (A), 4.2 (B) and 8.3 (C) km south of the Great Canadian Oil Sands (GCOS) plant as shown in Figure 2. These sites were within a sulphur deposition gradient determined from a snow analysis survey (Barrie and Whelpdale 1978).

2.1 GROWTH CONDITIONS

Jack pine seeds (*Pinus banksiana* Lamb.) were treated with 3% H_2O_2 for 10 min after which they were washed with distilled water and dried on paper towels. The seeds were planted in cavity trays in peat moss and irrigated. The trays were placed in the greenhouse and the seedlings grown under supplemented natural lighting (photoperiod 16 h). The seedlings were fertilized once a week with nutrient solution and watered as required.

2.2 SO_2 TREATMENT

It has been suggested that after penetration into plant tissues, gaseous SO_2 becomes hydrated, and it is the hydrated species that affects the various cellular processes (Hocking and Hocking 1977). Therefore, in our earlier studies, when the fumigation facilities were unavailable, we studied the effects of aqueous SO_2 on pigment metabolism and lipid biosynthesis. After acquiring the fumigation facilities in 1977, however, we investigated the effect of gaseous SO_2 on the activities of various important enzymes and lipid biosynthesis.

In equating the effects of gaseous and aqueous SO_2 on the biological activity of the spores of the fungus *Diplocarpon rosae*, Saunders (1966) observed that a X1000 higher concentration of SO_2 had to be employed in the aqueous phase than in the

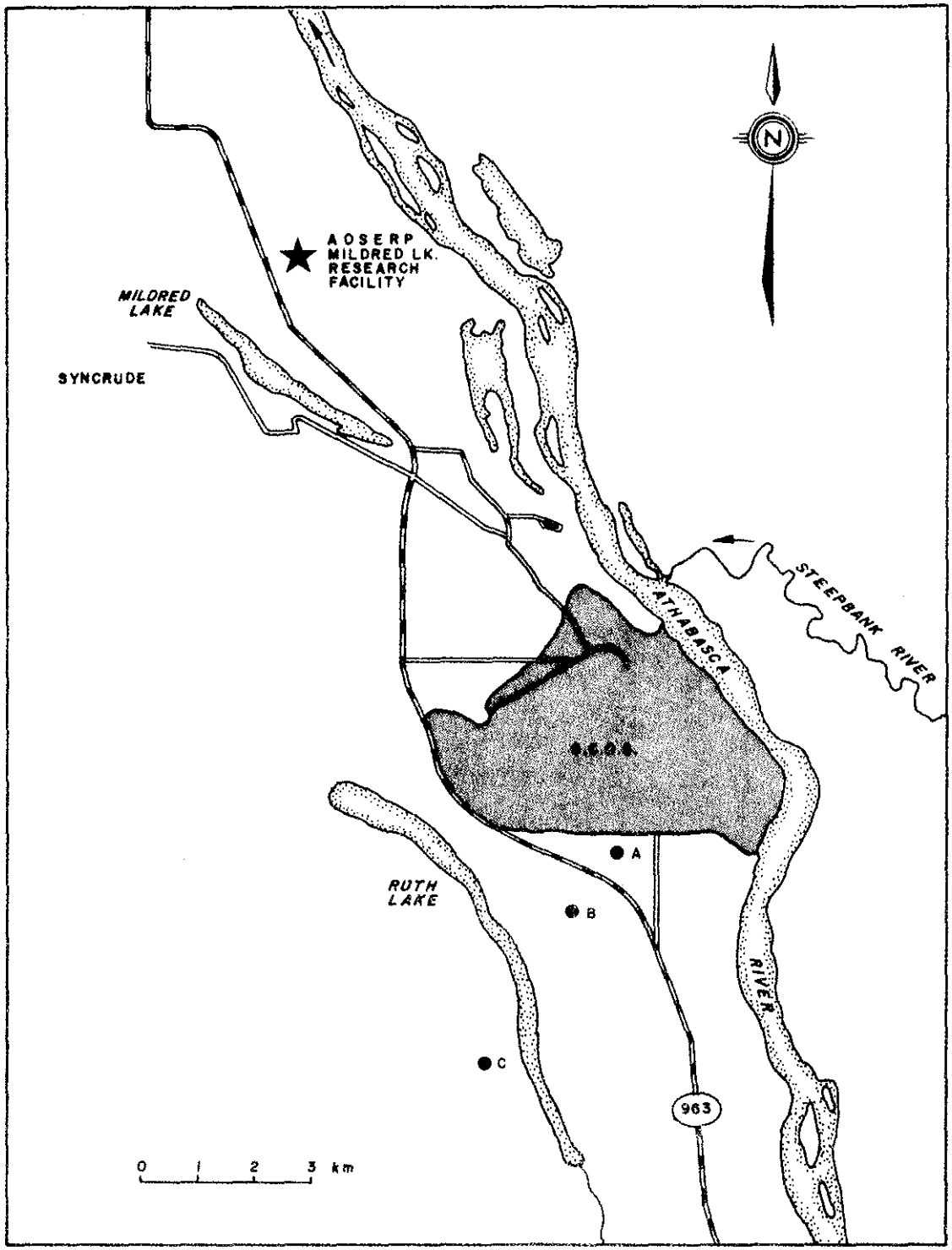

Figure 2. Map of the area around the Great Canadian Oil Sands plant showing sites A, B and C where vegetation samples for biochemical analysis were collected.

gaseous phase. The above relationship is, however, dependent upon temperature and SO_2 concentrations. Recently, Hocking and Hocking (1977) inferred that under moist conditions the dissolved unreacted species of SO_2 ($SO_2.6H_2O$) would be the most likely biologically active species for plant injury. The concentration of dissolved unreacted SO_2 at 25°C for 1, 10 and 100 ppm total SO_2 in water was calculated to be 0.0012, 0.011 and 0.092 ppm, respectively or about 1/1000 of the total SO_2 in water.

2.2.1 Aqueous SO_2 Treatment

Aqueous SO_2 stock solution (1000 ppm) was prepared by bubbling anhydrous SO_2 through a known quantity of 0.1 M Tris buffer (pH 7.2) until a desired gain of weight was reached (Puckett *et al.* 1973). Various concentrations (10, 25, 50, 100, 250, 500 and 1000 ppm) of aqueous SO_2 were prepared by diluting the stock solution. An HCl control adjusted to the same pH as that obtained by 500 ppm SO_2 (3.95-24 h after preparation of aqueous SO_2 solution) was also prepared. Representative samples from excised pine needles from a population of 15-20 seedlings were incubated in these solutions under 200 $\mu E\ m^{-2}s^{-1}$ light source for varying lengths of time at 23°C and then utilized for experimental purposes.

2.2.2 Gaseous SO_2 Treatment

Fumigations were carried out in small acrylic cuvettes using a continuous flow-through system. Flow rates for air and SO_2 were metered and controlled using rotameters and valves before being mixed and introduced into the cuvettes. Further mixing within the cuvettes was achieved by internally mounted fans near the injection ports. The concentrations of SO_2 at the input and output were monitored by means of a Philips PW9700 SO_2 analyzer.

Pine seedlings (15-20 seedlings) were gently pulled out of the styrofoam trays and inserted into vertically slitted

neoprene stoppers so that the foliage extended through the top of the stopper and the peat plugs containing the roots extended through the bottom. The stoppers holding seedlings were inserted through holes cut in the bottom of the cuvette so that the slit closed firmly around the stem and sealed the hole. The cuvette was placed over a water tank filled enough to keep the peat plugs moist for the duration of the experiment. The access ports of the cuvettes were sealed to avoid any leakage. The air flow was 10ℓ min^{-1} and SO_2 flow was adjusted to give the desired concentration at the output of the cuvette.

The cuvettes were placed inside a controlled environmental chamber that maintained uniform light and temperature conditions. Fumigations were carried out at an isothermal temperature of 22°C with light intensity at approx. 280 μE $m^{-2}s^{-1}$ (16 h photoperiod). Relative humidity measured in the output gas stream varied between 60 and 80%.

2.3 PIGMENT RELATED ASSAYS

2.3.1 Magnesium Determination

Loss of magnesium from excised needles (5 g) treated with aqueous SO_2 (22 h) was determined by means of Perkin Elmer atomic absorption spectrophotometer.

2.3.2 Pigment Extraction

Needles (5 g) treated with aqueous SO_2 (22 h) were gently ground by pestle and mortar with the gradual addition of 20 ml of cold 100% acetone. The homogenate was filtered and the residue was washed twice with 80% acetone. The contents of various pigments were quantitatively determined spectrophotometrically according to the method of White *et al.* (1963).

2.3.3 Chlorophyllase Preparation

Aqueous SO_2-treated needles (5 g) for 22 h were throughly homogenized. The homogenate was filtered and residue was washed as above. The residue thus obtained was further washed twice with 20 ml of absolute ethanol-anhydrous ether (3:1, V/V) and twice with 20 ml of anhydrous ether. The residue was dried at room temperature overnight and used for chlorophyllase assay (Holden 1961).

2.4 ELECTRON MICROSCOPY

Aqueous SO_2 treated (22 h) pine needle segments were fixed overnight at 4°C in 3% glutaraldehyde made up in 0.1 M phosphate buffer (pH 7.0). After fixation, the material was washed twice with the buffer and then post-fixed for 2-4 h in 2% osmium tetraoxide dissolved in the phosphate buffer. After rinsing the material with the phosphate buffer it was dehydrated through a graded series of ethanol (50, 70, 85, 95, 100%). The excised needles were then infiltrated (under vacuum) with propylene oxide-araldite mixture (1:1, V/V) for 36 h. After infiltration, the sections were embedded in araldite and polymerised. The sections were cut, stained in 0.27% lead citrate for 2-3 min and examined under an electron microscope.

2.5 GLYCOLIPID DETERMINATION

Total lipids were extracted from the aqueous SO_2 treated (22 h) tissues and evaporated to dryness (Folch *et al.* 1957). These lipids were dissolved in chloroform and an aliquot was analyzed for the quantitative analysis of galactolipids and sulpholipids by thin layer chromatography on silica gel (Roughan and Batt 1968).

2.6 SOLUBLE SUGAR DETERMINATION

After the incubation (22 h) of excised pine needles in aqueous SO_2 the solution was removed and the tissues were washed

2-3 times with distilled water. The incubation solution and the washings from each sample were combined for soluble sugar analysis. HCl was added to an aliquot of the combined solution (final HCl conc. 3%) and centrifuged to remove any residue. The soluble sugars in the supernatant layer were determined according to the method of Dubois *et al.* (1956).

2.7 TISSUE HOMOGENATE PREPARATION

Needles from SO_2 fumigated and control seedlings were excised into 1 cm sections and transferred to a chilled solution (1:10, W/V) containing either 0.05 M Tris-HCl buffer or 0.1 N sodium phosphate buffer (pH 7.4) and 1% PVP (polyvinyl pyrolidone) and 1 mM DTE (dithioerytheritol). The tissue was homogenized at moderate speed for 1.5 min by a Brinkman Polytron homogenizer. The homogenate was passed through two layers of cheese cloth and the filtrate was immediately used as "crude enzyme extract".

2.8 ENZYME ASSAYS

2.8.1 Acid Phosphatase

A typical reaction mixture contained 0.6 mM p-nitrophenyl phosphate (P-NPP), 0.05 M acetate buffer (pH 5.0) and 25 µl of "crude enzyme extract" (from SO_2 fumigated plant material) in a final volume of 2 ml. The reaction was run for 30 min in a water bath at 30°C and stopped by the addition of 2 ml of 10% Na_2CO_3. A control in which P-NPP was added after the addition of Na_2CO_3 was always run with the regular reaction set. This mixture was clarified by centrifugation and the acid phosphatase activity was determined by measuring the difference in optical density between the control and the reaction solution at 410 nm.

2.8.2 Malate Dehydrogenase

The enzyme activity was assayed by following the reverse reaction (L-malate + NAD $\rightleftharpoons$ Oxaloacetate + NADH + H^+) as

described by Ochoa (1955). A typical assay mixture included 0.3 ml of 0.0015M NADH, 0.1 ml of 0.1 M KCN, 0.1 ml of 0.0076M oxaloacetate (cis), 2.5 ml of 0.1 M sodium phosphate buffer (pH 7.5) and 10 µl of enzyme extract. The rate of the reaction was monitored by measuring the decrease in absorbance at 340 nm against a blank containing the reaction mixture without NADH.

2.9 LIPID BIOSYNTHESIS

Excised tissue (0.5 g) from fumigated and control jack pine seedlings was transferred to flasks containing 10 ml water and 10 µCi acetate [$1^{-14}C$]. The incubations were carried out for 3 h in a gyrotary water bath shaker at 30°C and under 200 µE $m^{-2}s^{-1}$ light intensity. After incubation, each tissue sample was removed, washed with distilled water and homogenized in 50 ml (chloroform-methanol (2:1)). The homogenate was then filtered through a glass-wool-packed Buchner funnel, and the residue was washed repeatedly with chloroform-methanol. The chloroform extracts containing lipids were washed with acidic water and evaporated to dryness under reduced pressure and the labelled products analyzed by thin-layer-chromatography.

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3. RESULTS AND DISCUSSION

The experiments for each study were conducted several times and the trends for SO_2 response were found to be highly reproducible. Although considerable effort was made to select uniformly grown plant material, it was difficult to eliminate the biological variability on a day-to-day basis. In order to overcome this difficulty each experiment was designed to include a control (non SO_2-treated plant material) which provided meaningful data on SO_2 response.

3.1 EFFECTS OF AQUEOUS SO_2 ON TOTAL CHLOROPHYLL CONTENT

The pine needles that were incubated in 10, 25, 50 and 100 ppm aqueous SO_2 remained green and looked quite normal whereas the higher concentrations caused increasing degrees of discoloration or bleaching.

The low concentrations (10-100 ppm SO_2) did not produce any appreciable change in total chlorophyll content. However, when SO_2 concentration was increased from 100 ppm to 250 and 550 ppm, there was a sharp drop in pH of the incubation medium as well as in total chlorophyll content (Table 1). At 500 ppm, the pH dropped to 3.95 and the chlorophyll content was reduced to about half that of the control values. From these results it would appear that the decrease in chlorophyll content could be attributed to a drop in pH. The HCl control adjusted to the same pH as produced by 500 ppm SO_2, however, showed approximately 4% decrease in chlorophyll content compared with the regular control. It is therefore evident that the pigment destruction resulted from direct effects of SO_2 in addition to indirect effects such as increase in acidity.

3.1.1 Chlorophyll Breakdown

In order to understand the mechanism of chlorophyll destruction, the effect of aqueous SO_2 on Mg^{++} loss from

Table 1. The effect of various concentrations of SO_2 on total chlorophyll content of pine needles.

Treatment	pH of Incubation Medium	Chlorophyll Content (mg/l)	% of Control
Control	7.20	65.27	100.00
10 ppm SO_2	7.18	64.13	98.25
25 ppm SO_2	7.13	63.50	97.29
50 ppm SO_2	7.10	63.82	97.78
100 ppm SO_2	7.00	63.38	97.10
250 ppm SO_2	6.60	47.98	73.51
500 ppm SO_2	3.95	34.69	53.15
HCl Control	3.95	62.71	96.08

chlorophyll (conversion of chlorophyll into phaeophytin) was determined. Some of the mechanisms of chlorophyll breakdown are shown in Figure 3. Acids such as HF and HCl have been shown to convert chlorophyll into phaeophytin with the release of Mg^{++} (Rao and Le Blanc 1965; Moker 1967). Mg^{++} is replaced by 2 molecules of hydrogen with a resulting change in the light absorption spectral properties of chlorophyll molecules.

Table 2 shows the release of Mg^{++} from excised pine needles as a result of treatment with high concentration of aqueous SO_2. The loss of Mg^{++} from needles treated with lower concentrations of SO_2 (10-50 ppm) was minimal (about 7% at 50 ppm SO_2). However, such loss increased considerably with increasing SO_2 concentration. The data also show that even at very low pH, the conversion of chlorophyll into phaeophytin is due primarily to SO_2 and only to a small extent to acidity.

3.1.2 Effect on Various Pigments

The data in Figure 4 show changes in the content of chlorophyll *a* (A), chlorophyll *b* (B), chlorophyllide *a* (C), chlorophyllide *b* (D), and phaeophytin *a* (E) and phaeophytin *b* (F) as affected by various concentrations of aqueous SO_2. The amounts of chlorophyll *a* and chlorophyll *b* declined with increasing concentrations of aqueous SO_2. However, chlorophyll *a* is more sensitive to SO_2 attack than chlorophyll *b*. At 500 ppm SO_2 chlorophyll *a* was almost completely destroyed, but only one-half of chlorophyll *b* was destroyed.

Low concentrations of SO_2 (10-100 ppm) did not have any appreciable effect on phaeophytin *a* (E) content but the content of this pigment increased considerably at 250 and 500 ppm. Phaeophytin *b* was absent in the control tissue and its content remained unchanged with increasing SO_2, suggesting conversion of only chlorophyll *a* to phaeophytin *a*. Since no chlorophyllide *a* (C) was detected in the control tissue and SO_2 did not show any effect on its content, it is suggested that

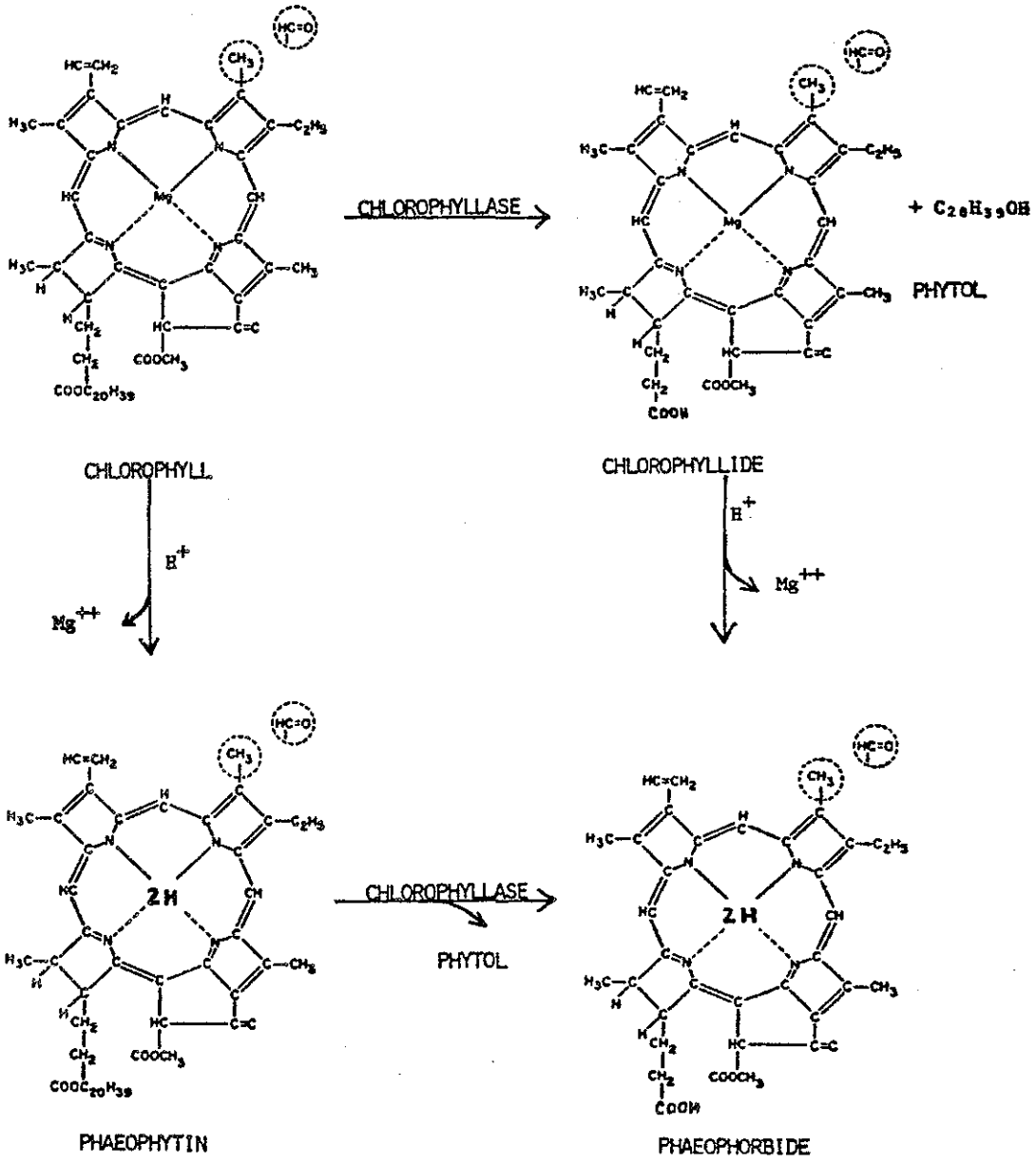

Figure 3. Different mechanisms of chlorophyll breakdown.

Table 2. The effect of various concentrations of aqueous SO_2 on Mg^{++} loss from pine needle chlorophyll.

Treatment	pH of Incubation Medium	Mg^{++} Loss (mg/l)	% of Control
Control	7.20	7.94	100.00
10 ppm SO_2	7.18	8.07	101.64
25 ppm SO_2	7.13	8.26	104.03
50 ppm SO_2	7.10	8.37	105.42
100 ppm SO_2	7.00	9.05	113.98
250 ppm SO_2	6.60	26.81	337.66
500 ppm SO_2	3.95	32.76	412.59
HCl Control	3.95	9.73	112.54

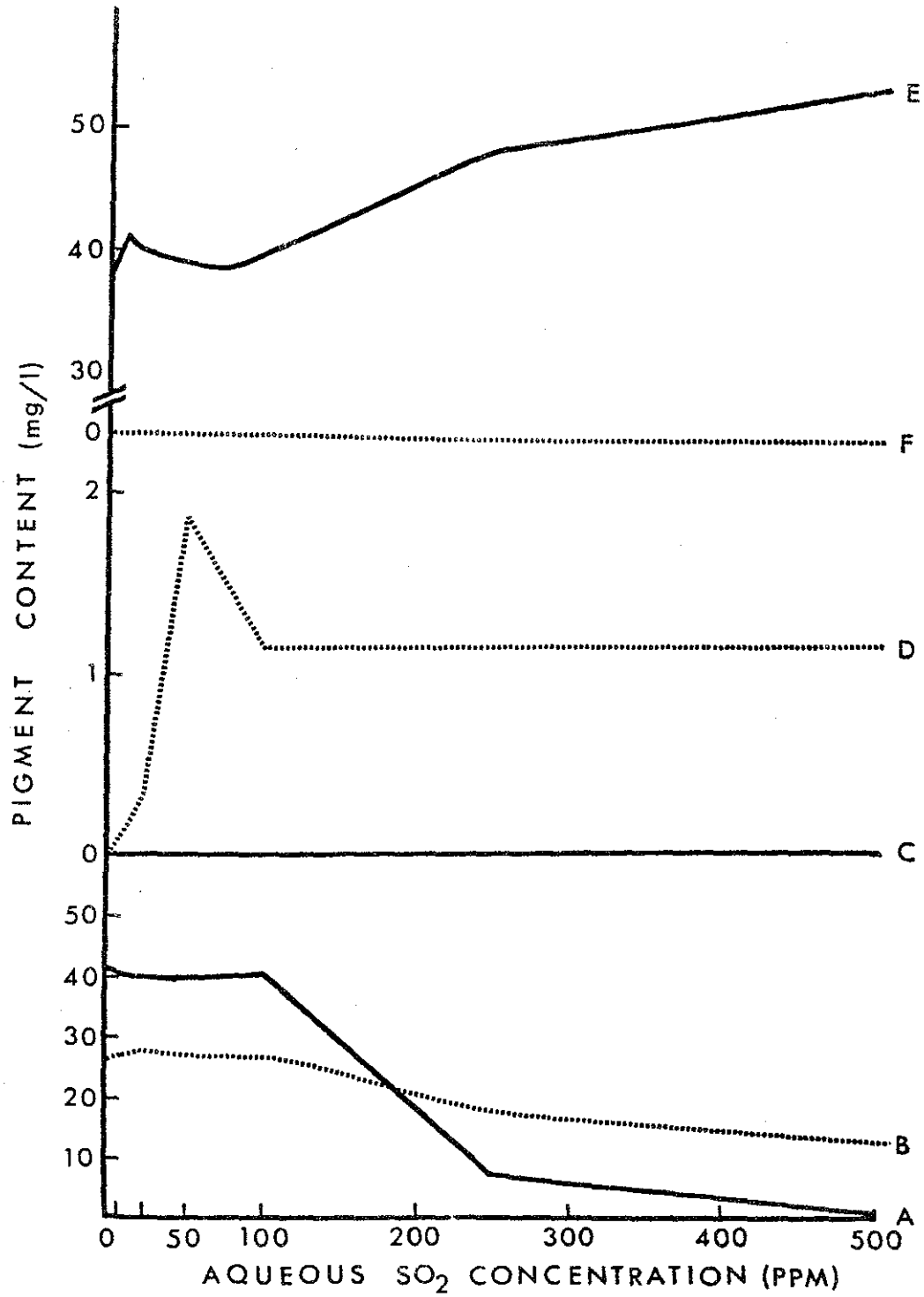

Figure 4. The effect of aqueous SO_2 on chlorophyll a (A), chlorophyll b (B), chlorophyllide a (C), chlorophyllide b (D), phaeophytin a (E) and phaeophytin b (F).

chlorophyll a was destroyed by processes other than simple transformations. The amount of chlorophyllide b (D) increased with increasing concentration of SO_2 to a maximum of 50 ppm followed by a drop at 100 ppm that was sustained up to 500 ppm SO_2. The increase in the amount of chlorophyllide b indicates that at least a part of the chlorophyll b was converted into chlorophyllide b.

3.1.3 Effect on Chlorophyllase Activity

Figure 4 indicates that part of the chlorophyll b was converted into chlorophyllide b and this conversion was maximum at 50 ppm SO_2. Since chlorophyllase is capable of such conversion it was thought desirable to determine if SO_2 has any effect on the activity of pine chlorophyllase. Chlorophyllase activity was measured in terms of chlorophyllide formation.

Concentrations of 10-50 ppm SO_2 stimulated the chlorophyllase activity while higher concentrations (100-500 ppm) inhibited it (Figure 5). Maximum stimulation was obtained at 50 ppm. The activity dropped to a value close to that of control at 100 ppm and then exhibited significant inhibition with increasing concentrations of SO_2. Maximum inhibition (about 30%) was obtained with 500 ppm SO_2. This inhibition was at first attributed to the low pH of the incubation medium caused by high concentration of SO_2, but HCl control showed inhibition of less than 10%. Therefore, the inhibition of chlorophyllase activity at high concentrations of SO_2 is not entirely due to pH effect. It appears that SO_2 has a direct effect on chlorophyllase activity.

These results clearly indicate that the chlorophyllase activity index is a very sensitive indicator of SO_2 concentrations. This method may be useful for detecting SO_2 pollution before the development of visual symptoms.

These biochemical studies suggest that low concentrations of SO_2 which normally do not produce any visual symptoms on the foliage can have an effect on specific enzyme systems such as chlorophyllase. Since low concentrations of SO_2 were shown to

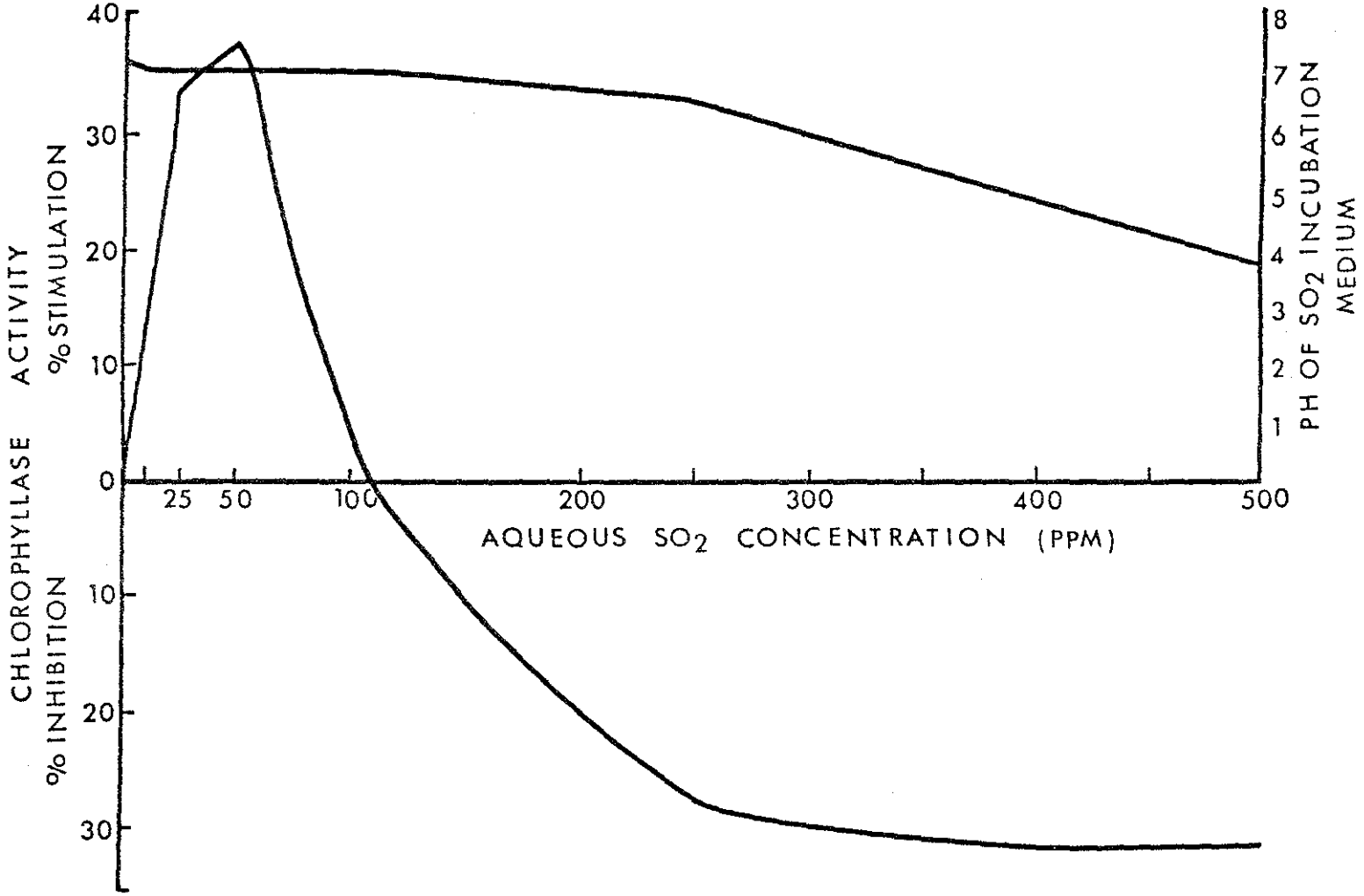

Figure 5. The effect of aqueous SO_2 on the activity of chlorophyllase.

have no appreciable effects on Mg^{++} loss, injury may be a result of increased activity of chlorophyllase. On the other hand, high concentrations of SO_2 that are capable of producing visual symptoms may cause injury by inhibiting the activities of important enzyme systems, by destroying chlorophyll pigments, and thereby resulting in a net drop in photosynthetic efficiency.

3.2 ULTRASTRUCTURAL STUDIES

Electron microscopy has been used to demonstrate the extent of ultrastructural injury caused by air pollutants such as peroxyacetyl nitrate and ozone (Thompson *et al.* 1965, 1966). Little is known about SO_2 effects on ultrastructural organization and metabolic activities in pine tissues. Wellburn *et al.* (1972), however, reported the effects of SO_2 on the ultrastructure of broad bean chloroplasts.

According to cytological examinations, the tip of pine needles is composed of old and fully differentiated cells and the base contains very young and actively growing cells (Linzon, 1967). About 1 cm sections excised from the tip and base of pine needles were incubated in different concentrations of aqueous SO_2 solutions and the ultrastructural changes in chloroplasts were examined by means of electron microscopy.

At aqueous concentrations of 100 and 500 ppm, SO_2 caused swelling of thylakoid discs and desintegrated other intrachloroplast membranes, resulting in the formation of small vesicles (in older, matured tissue). Chloroplast structural injury was more pronounced in old tissues than in younger and metabolically active tissues. In general, electron microscopy showed that older, fully matured tissue was more sensitive to SO_2 injury than the younger actively growing tissue. This was shown by disorientation of chloroplast structure, dislocation of chloroplasts and absence of mitochondria in the older tissue treated with high concentrations of SO_2. The detrimental effects of SO_2 on the ultrastructural organization of pine needle cells appeared to be

markedly different from the effects of low pH alone (HCl control).

Possible relationship between structural disorganization and disruption of biochemical activities of chloroplasts from SO_2-treated young and old tissues were determined by measuring the photosynthetic activity such as Hill reaction (Malhotra 1976) and $^{14}CO_2$ fixation (Malhotra 1977) of isolated chloroplasts. Biochemical observations (photosynthetic activity) were in good agreement with the cytological observations.

3.3 EFFECT OF SO_2 ON TISSUE GLYCOLIPIDS AND PERMEABILITY PROPERTIES

Chloroplast membranes are sites of many enzymatic reactions and are made up of two very important structural components namely lipids and proteins. It is possible that structural disruption due to SO_2 has been preceded by changes in a number of biochemical events.

Chloroplast lipids are mostly glycolipids namely monogalactosyldiglycerides (MGDG), digalactosyldiglycerides (DGDG) and sulphoquinovosyldiglycerides (SQDG) (James and Nichols 1966). These lipids are known to be involved in the structure and function of chloroplast membranes (Shaw *et al.* 1976; Erwin and Bloch 1963); however, nothing is known about the effect of SO_2 on metabolism of these compounds. We therefore decided to study the effect of aqueous SO_2 on (1) the level of these lipids in pine needles and (2) permeability properties of pine needle tissue.

The data in Figure 6 show changes in the levels of glycolipids from fully developed and young needles as affected by various concentrations of aqueous SO_2. Concentrations of MGDG, DGDG and SQDG declined sharply with increasing concentrations of SO_2 up to 25 ppm. Further increase in SO_2 concentration (100 ppm) resulted in only small changes in the concentration of these glycolipids. An interesting observation is that the effect of SO_2 on the level of SQDG was more pronounced in the fully

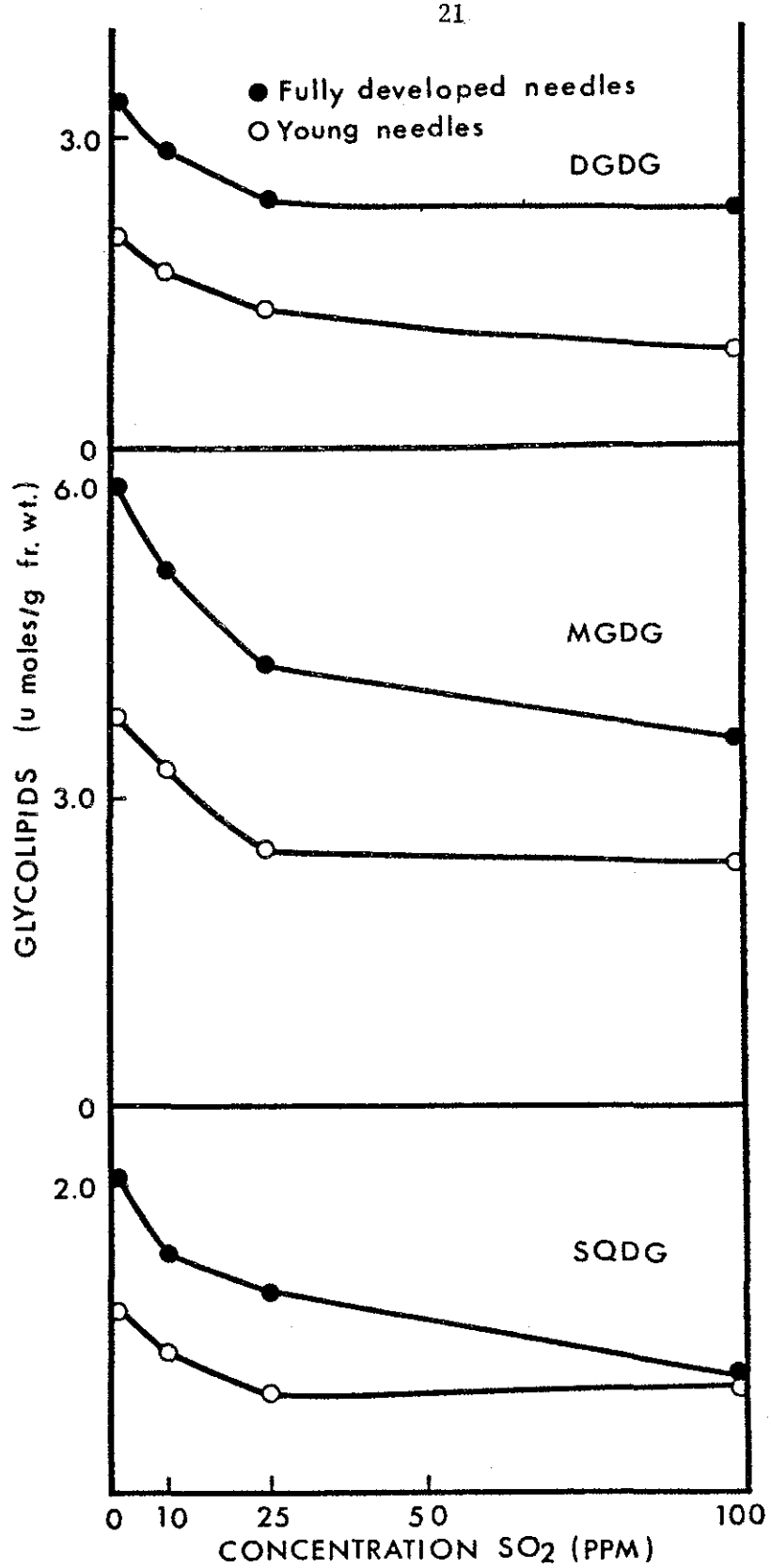

Figure 6. The effect of aqueous SO_2 on the levels of glycolipids in fully developed and young pine needles.

developed tissues than in the young tissues. The reverse was true of the DGDG fraction. These differences clearly indicate that the biosensitivity of various cellular metabolities to SO_2 depends on the age of the needles. It is also interesting to note that a dramatic decrease in the level of all these lipids was obtained at a very low concentration of SO_2 (50 ppm). Such low concentrations did not produce any visual symptoms on pine needles.

The influence of aqueous SO_2 on cellular metabolites such as soluble sugars was determined to ascertain whether the effects of SO_2 discussed were confined to the membrane glycolipids of whether other cellular metabolities such as sugars were also influenced. The effect of SO_2 on the release of sugars would indicate changes in cellular permeability. The incubation of tissues from both fully developed and younger needles resulted in a release of soluble sugars from the tissues, even in the absence of SO_2 (Figure 7). However, the presence of SO_2 in the incubation solution caused a dramatic increase in the amount of sugars released from both the tissues. The release of soluble sugars from tissues of both ages might have occurred due to the decrease in glycolipid concentrations in the SO_2-treated tissues. However, the amount of sugars released from the young tissues was considerably higher than the glycolipid losses, suggesting that SO_2 caused a release of some other bound form(s) of sugar.

Sulphur dioxide was also shown to have an inhibitory effect on other essential plant metabolites such as amino acids and organic acids. The lack of these metabolites can impair important processes such as photosynthesis and respiration.

The difference in biochemical responses to aqueous SO_2 between the fully developed and young pine needle tissues indicates that the phytotoxicity of SO_2 is at least partially dependent upon the stage of plant tissue development. In view of the importance of galactolipids for the structural integrity of the chloroplast thylakoids, any changes in these lipids caused by SO_2 would result in structural and functional injuries to the

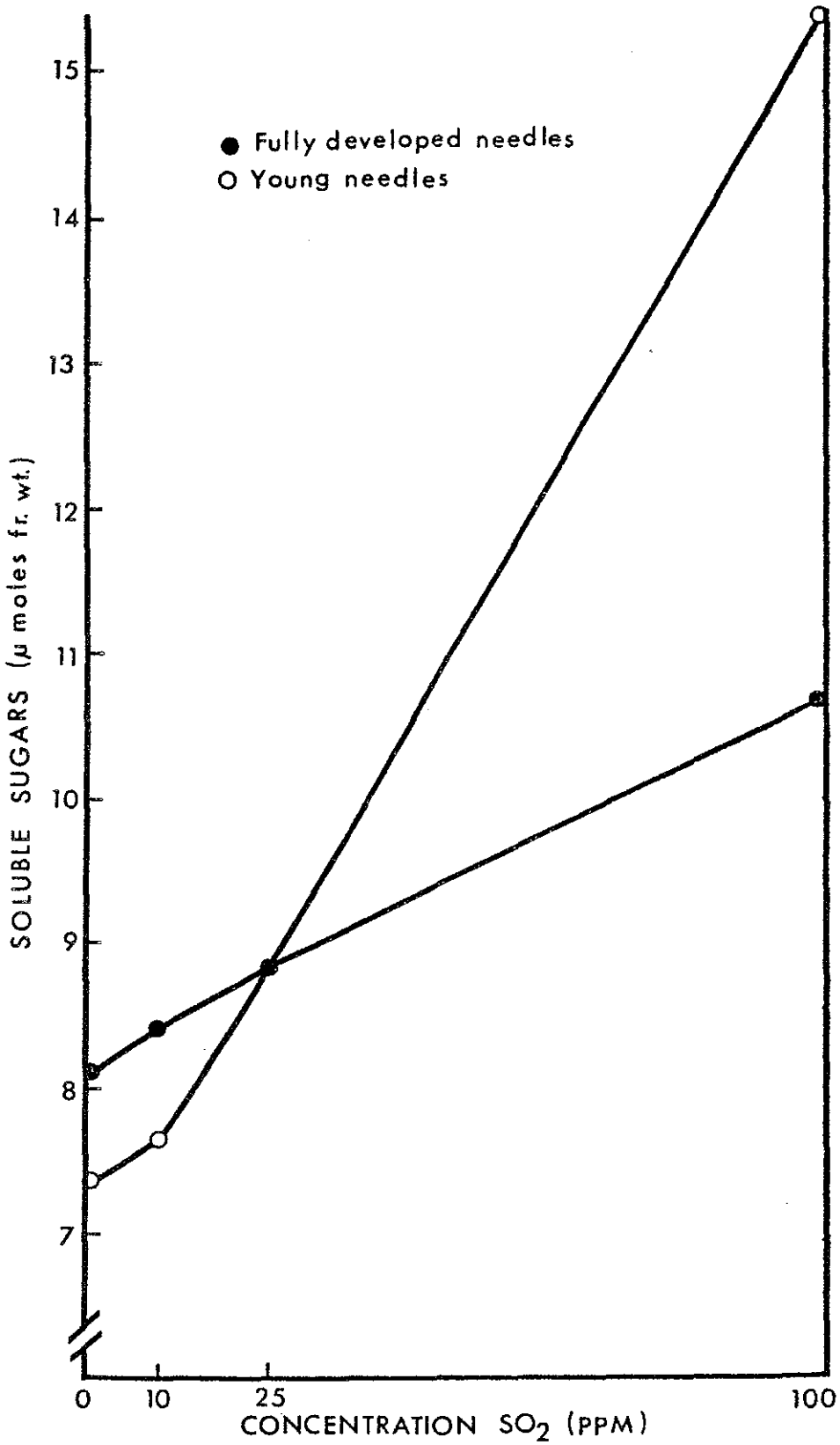

Figure 7. The effect of aqueous SO_2 on the release of soluble sugars from fully developed and young pine needles.

biomembranes. We feel that the process of hidden SO_2 injuries such as swelling of thylakoid discs and disintegration of intra-chloroplast membranes (Malhotra 1976) may be initiated as a result of alteration in glycolipid components. Since very low concentrations of aqueous SO_2 (10-25 ppm) usually do not produce any visual symptoms but cause marked changes in the glycolipid content and composition, the analysis of such lipids may provide an effective tool for determining the extent of hidden SO_2 injury to vegetation.

3.4 EFFECTS OF GASEOUS SO_2 ON PHOSPHATASE ACTIVITY

Under field conditions, vegetation is normally exposed to air pollutants on an intermittant basis. This allows the vegetation to recover at least partially from the air pollutant stress. In order to simulate such field conditions, the plants were fumigated with various SO_2 concentrations for a known length of time and then placed in a SO_2-free environment to determine the percent recovery of impaired biochemical functions.

The results in Table 3 show that exposure of pine seedlings at 0.35 ppm SO_2 for 24 h produced a marked inhibition in acid phosphatase activity in both young (top clustre of new growth) and old (fully developed, approximately 3 month old) needles. Enzyme activity was inhibited more in the older needles than in the younger ones. Inhibition of enzyme activity further increased during the 24 h recovery period in a SO_2-free environment. There was very little visual symptom development after the 24 h fumigation. However, the symptoms were much more obvious during the next 24 h recovery period indicating a lag period requirement for such symptom development. These results, therefore, suggest that fumigation of pine seedlings under above conditions may result in serious impariment of plant metabolism. Rabe and Kreeb (1976) and Schmid and Kreeb (1975) have reported a similar inhibition of acid phosphatase activity in alfalfa plants and lichens after treatment of SO_2.

Table 3. The effects of gaseous SO_2 on acid phosphatase activity in young and old needles of jack pine seedlings and its ability to recover in a SO_2-free atmosphere.

Fumigation Conditions				% of Control Activity	
Conc. (ppm)	Duration (h)	SO_2 Uptake/ tree/h (ppb)	Recovery Period (h)	Young Needles[a]	Old Needles[a]
Expt. 1					
0.35	24	20	0	64	46
0.35	24	20	24	54	36
Expt. 2					
0.33	1	9	0	90	82
0.33	1	9	24	100	100
Expt. 3					
0.10	24	4	0	80	75

[a] Young and old needles represented the pale green top clustre and the dark green 3-4 month old growth, respectively.

The fumigation of pine seedlings at 0.33 ppm SO_2 for only 1 h also inhibited enzyme activity in both young and old tissues by about 10% (Table 3, Expt. 2). However, this inhibition was completely recovered after 24 h in a SO_2-free atmosphere. In this experiment, the treatment of plants with SO_2 resulted in an inhibition of phosphatase activity without development of any visual symptoms of SO_2 phytotoxicity.

Plants fumigated with 0.1 ppm SO_2 for 24 h inhibited the enzyme activity in young needles by 20% and in old needles by 25% (Table 3, Expt. 3). These plants did not produce visual symptoms of SO_2 phytotoxicity. The results clearly suggest that SO_2 when applied at either low concentration for a long period or high concentration for a short period, can inhibit the acid phosphatase activity in jack pine seedlings. This inhibition can become more severe with increasing SO_2 concentration or increasing exposure time.

Table 3 also shows that the effect of SO_2 on acid phosphatase was dependent upon the amount of SO_2 taken up per seedling. The variability in the amount of SO_2 uptake per seedling per hour between Expt. 1 and Expt. 2 (Table 3) was a result of low humidity conditions in the later case. It is suggested that stomata in Expt. 2 were probably only partially open resulting in considerably less SO_2 uptake per seedling.

Our results suggest that the acid phosphatase activity could be used as a very sensitive biochemical indicator of SO_2 phytotoxicity. The ability of the SO_2-fumigated plants to recover their enzymatic activity in a SO_2-free environment suggests a transient nature of SO_2 effect. However, irreversible injury can result at high SO_2 concentrations (Table 3, Expt. 1).

3.5 EFFECT OF GASEOUS SO_2 ON MALATE DEHYDROGENASE

The results in Table 4 demonstrate an inhibitory effect of SO_2 on malate dehydrogenase. The activity of this enzyme declined in relation to the length of fumigation, reaching

Table 4. The effects of SO_2 (0.34 ppm) on malate dehydrogenase activity from pine needles.

Length of Fumigation (h)	Control	Malate Dehydrogenase Activity (units[a]/mg protein)	% Inhibition
19	303	274	10
72	362	226	37
92	315	164	48

[a]One unit is defined as the amount of enzyme required to cause an O.D change of 0.01/min at 340 mμ.

a value almost 50 percent of the control value after 92 h. It is suggested that SO_2 affects the plant metabolism probably by interfering with important enzyme systems such as malate dehydrogenase. The related metabolites such as organic and amino acids also showed a substantial decline after fumigation of jack pine seedlings with 0.34 and 0.51 ppm SO_2.

Malate dehydrogenase assay also has potential as a reliable assessment tool for SO_2 phytotoxicity prior to visual symptom development.

3.6 EFFECT OF GASEOUS SO_2 ON LIPID BIOSYNTHESIS

Exposure of jack pine seedlings for 24 h to 0.18-0.20 ppm SO_2 markedly inhibited the biosysthesis of various lipids (Table 5, Expt. 1, 2). The inhibitory effect among the phospholipids was not specifically greater on any one component. Among the galactolipids, DGDG was generally inhibited more than MGDG. Fumigation of seedlings for 24 h with a high SO_2 concentration (0.35 ppm) drastically inhibited all lipid biosynthesis (Table 5, Expt. 1) and caused needle tip burn. It is therefore suggested that invisible and visible effects of prolonged SO_2 exposure are directly related to impaired cellular metabolism.

3.6.1 Recovery of Fumigated Seedlings

In order to simulate the field conditions, such as varying concentrations of SO_2, as closely as possible, fumigations were carried out in controlled environment cuvettes and followed by different lengths of recovery time in a SO_2-free environment. The experimental conditions chosen and the results obtained are given in Table 5 (Expt. 2, 3). A high SO_2 concentration (0.37 ppm) for a short exposure time (1 h) resulted in much more inhibition of lipid biosynthesis than a low SO_2 concentration (0.20 ppm) for a long period of time (24 h) (Table 5, Expt. 2, 3). The inhibitory effects of either fumigation were partially or completely reversed upon removal of plants from the

Table 5. The effects of gaseous SO_2 on lipid biosynthesis in developing needles from jack pine seedlings and their ability to recover in a SO_2-free environment.

Fumigation Conditions		Recovery Period	% of Control							
Conc. (ppm)	Duration (h)	(h)	PC[a]	PE[a]	PG[a]	DGDG[a]	MGDG[a]	DG[a]	FFA[a]	TG[a]
Expt 1										
0.18	24	0	78.2	74.0	68.6	57.4	81.1	75.6	75.0	80.4
0.35	24	0	30.0	44.0	41.6	29.8	26.4	26.7	15.6	39.0
Expt 2										
0.20	24	0	81.8	60.5	61.5	73.1	81.1	80.6	76.5	88.9
0.20	24	48	82.2	70.3	67.4	100.0	100.0	100.0	100.0	100.0
Expt 3										
0.37	1	0	43.6	37.7	33.3	42.3	44.8	40.7	42.2	39.3
0.37	1	24	63.4	65.4	69.4	66.0	59.2	63.6	115.0	52.4

[a] PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; DGDG, digalactosyldiglycerides; MGDG, monogalactosyldiglycerides; DG, diglycerides; FFA, free fatty acids; and TG, triglycerides.

SO_2 environment. Recovery of biosynthetic ability of plants exposed to a high SO_2 concentration (0.37 ppm) for the short duration (1 h), however, was slower than that of the plants exposed to low SO_2 (0.20 ppm) for a longer period (24 h). It is also interesting to note that at high SO_2 concentration (0.35-0.37 ppm) much of the damage to lipid biosynthesis of tissues occurred during the initial period of exposure (Table 5, Expt. 1 vs Expt. 3). The ability of plants to recover their synthetic activities after removal of SO_2 from the environment is of great significance; however, the recovery is dependent upon the extent of SO_2 exposure, magnitude of metabolic injury, and length of recovery time.

3.7 BIOCHEMICAL ANALYSES ON FIELD SAMPLES

Assessment of air pollution injury to jack pine and white spruce in the impingement area south of GCOS plant (Figure 2) was carried out using some of the more sensitive biochemical and physiological techniques developed in our laboratory. Branches from the two species were collected in a gradient at a distance of 2.8, 4.2 and 8.3 km south of the GCOS plant and analyzed for sulphur content, CO_2 gas exchange capacity (under controlled temperature and light conditions), chlorophyll/phaeophytin ratios, and activity of highly sensitive enzymes such as phosphatase and malate dehydrogenase. Although a gradient in sulphur content was evident, there was no difference in any of these biochemical and physiological functions that responded to SO_2 under laboratory conditions. The highest sulphur levels observed in jack pine needles from the above sites were close to the lower end of the normal sulphur range for such tissues. Low sulphur content and the transient nature of SO_2 injury (demonstrated under laboratory conditions) may be the reasons for not being able to detect SO_2 response in the field collected samples.

The work conducted so far, under controlled conditions, suggested that low concentrations of SO_2 which normally do not

produce any visual symptoms on the foliage can cause injury to forest vegetation by inhibiting lipid synthesis and activities of various important enzyme systems.

4. CONCLUSIONS

Biochemical and ultrastructural responses to aqueous and gaseous SO_2 show that, depending upon the concentration and length of exposure time, SO_2 can cause considerable injury to forest species prior to visual symptom development. However, such injury can either be partially or fully recovered in a SO_2-free environment. The techniques developed to date have great potential as diagnostic tools to: (1) detect SO_2 injury to vegetation before the development of visual symptoms of SO_2 phytotoxicity, (2) establish threshold levels of air pollutant injury to vegetation and (3) screen candidate revegetation species on the basis of their true tolerance to air pollutants.

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