Sulfur Accumulation in Red Maple Leaves Exposed to Sulfur Dioxide

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ABSTRACT

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Investigations were conducted to visualize the localization of sulfur in leaf tissue of red maple trees (*Acer rubrum*) exposed to the common air pollutant, sulfur dioxide (SO_2). Cuttings were exposed to charcoal-filtered air or 0.15 ppm of SO_2 in environmental chambers for 42 days. Scanning electron microscopy and energy dispersive X-ray analysis revealed accumulations of sulfur (S) in the chloroplasts of mesophyll cells exposed to SO_2. Chloroplast perforations were visualized in leaves exposed to SO_2 but not in leaves exposed to charcoal-filtered air. Chloroplast perforation and S accumulation could be preliminary stages of cytolysis reported in previous studies and, in part, explain the mode of action of SO_2 as a phytotoxicant.

Additional key words: chloroplast injury, X-ray digital mapping.

Although many studies of the associations between elemental accumulation from gaseous pollutants and plant damage have been made, no sound relationships between elemental contents of leaves and cellular injury have been described (2). One of the problems has been that direct observations of cellular injury related to cellular accumulation of gaseous pollutants have not existed (1).

Surface changes of plants associated with gaseous air pollution have been reported in several studies (5–7,10–12). Air pollution-induced injury, detected by scanning electron microscopy (SEM), has been described in plants fumigated under laboratory conditions with ozone (O_3) and sulfur dioxide (SO_2) at sublethal concentrations (10,12) and toxic levels (11). Examination of geranium leaves fumigated with O_3 revealed ruptured guard cells and cuticular change. Hybrid poplar leaves, exposed to a combination of O_3 and SO_2, had crystalline inclusions of unknown composition in leaf bundle sheath cells. In the latter, druse-shaped inclusions filled bundle sheath cells (10). Krause and Jensen (11) reported ringed stomata and necrotic lesions on leaves of hybrid poplar after fumigation with a combination of 0.3 ppm O_3 and 0.15 ppm SO_2. Injury to epidermal leaf cells of field grown hybrid poplar (5) were similar to those observed under controlled exposures in the laboratory. Krause (7) further reported that asymptomatic leaves of *Acer rubrum* L. (red maple), grown in ambient air containing high levels of O_3 and SO_2 (polluted air), exhibited collapsed epidermal cells and lacked exfoliate epicuticular wax.

Energy dispersive X-ray analysis (EDX) has been used to detect particulate air pollution on vegetative tree buds (6,8). EDX, a spectrometer when interfaced with an SEM, analyzes characteristic X-rays from a specimen generated by an electron beam. SEM and EDX provide morphological data identifying elements present. Buds of red maple (6) and hybrid poplar (8) grown in areas of high ambient air pollution (O_3 and SO_2) exhibited injured epidermal cells associated with significant levels of particles containing heavy metals, whereas epidermal cells of buds grown in clean air were not injured. Ambient particulate air pollution that was associated with lesions on red maple leaves was characterized with SEM and EDX (9).

Whereas foliar adsorption of S by *A. rubrum* grown in 1 ppm SO_2 for 6 hr was observed (15), no studies have characterized the actual cellular site or sites of S accumulation. Our study was conducted to identify cellular localization of S in leaf tissue of red maple trees, previously exposed to SO_2 as related to cellular injury.

MATERIALS AND METHODS

Plant material. Twenty 1-year-old *A. rubrum* 'Scarlet Sentinel' ramets were potted in a soil-peat-perlite (2:2:1, v/v/v) mixture in 25-cm-diameter plastic pots. All plants were watered as needed and fertilized biweekly with NPK in a ratio of 20/8.6/16.6.

Fumigation treatments. Cuttings were treated in four chambers described by Jensen and Bender (4). The chambers were 1.2-m cubes with an air exchange rate of approximately 0.3 min^{-1}. This was sufficient to maintain the CO_2 concentration near ambient levels (320 ppm) and the pollutants at the selected levels. The light intensity was 730 $\mu E \cdot m^{-2} \cdot S^{-1}$ (photosynthetically active radiation, PAR) and the photoperiod was 16 hr. The temperature was maintained at 24 ± 1 C day and 21 ± 1 C night. Relative humidity was 70 ± 10%. The cuttings were exposed to the following treatments 12 hr/day for 42 consecutive days: 1) control (charcoal-filtered air) and 2) 0.15 ppm SO_2 (charcoal-filtered air after 12 hr). Two replicate chambers were used for each treatment.

The pollutant concentrations were automatically sampled in each chamber at approximately 15-min intervals. The SO_2 concentration was measured with a Model 8450 SO_2 monitor (Monitor Laboratories, San Diego, CA). The instrument was calibrated daily with a Model 8500 SO_2 calibration source (Monitor Laboratories). SO_2 was added to the air stream entering the chambers by micrometering valves that controlled the gas flow from a tank of SO_2.

Electron microscopy. After fumigation, mature leaves, formed during fumigation, with a plastochron leaf age (13) of 10, were sampled 18 hr after each treatment for analysis by SEM and EDX. Ten fresh, hydrated and dehydrated leaf specimens (7) from each treatment and chamber were mounted on carbon planchets with graphite adhesive and attached to aluminum stubs. All samples were placed on a rotating, tilting stage within a vacuum evaporator and coated with carbon. They were subsequently examined with a Hitachi S-500 SEM (Mountain View, CA) equipped with a cold

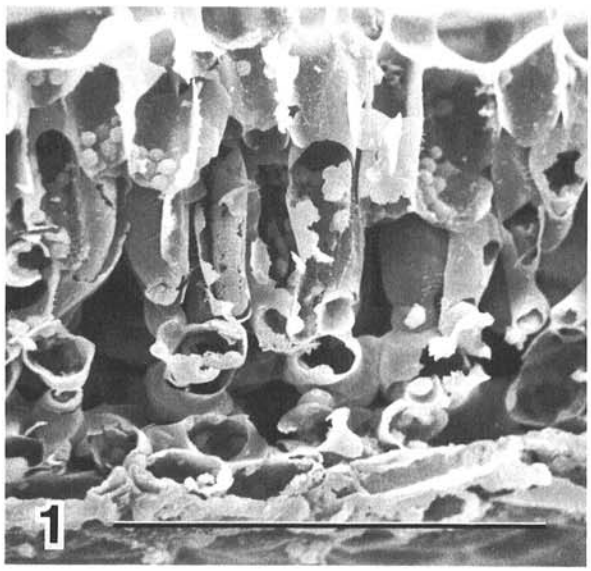
stage (−180 C) to reduce heat artifacts during examination. The SEM was also equipped with a Tracor Northern EDX, Model 2000 (Middleton, WI) with digital beam control hardware with X-ray mapping programs and a continuum subtration program.

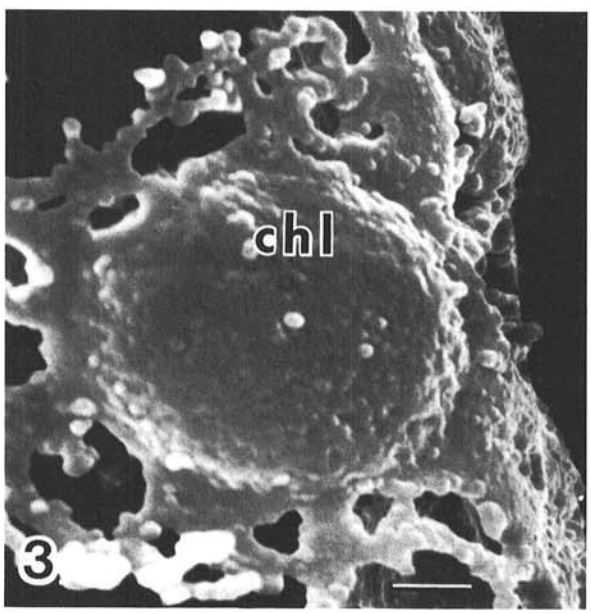
The SEM was operated at 25 kV, tilt angle of 30°, take-off angle of 61°, 100 sec of exposure and mapping at 0.001 sec per point using the X-ray display program from Tracor Northern. A "Hypersense" detector (Tracor Northern) with an Si(Li) crystal of 30 mm, 147 eV resolution, and a 0.3-ml. Be window was used.

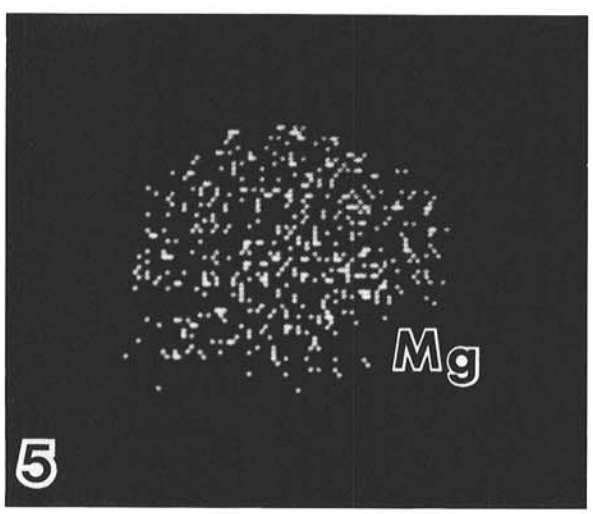
To compensate for inherent problems associated with analyses of samples with irregular surfaces that can produce spurious X-rays and decrease spatial resolution (3), 20 spectra of each treatment were collected and stored. Initially, count rates of the same area scanned by the electron beam were taken at decreasing magnifications. Then, spectra were taken of the same area at various rotation parameters and the number of counts was recorded. If numbers of counts remained constant in the S window, then original spectra were considered to be valid, regardless of specimen surface irregularities or spatial resolution. The EDX techniques used were semiquantitative in this study and cannot be related to real Mg or S values.

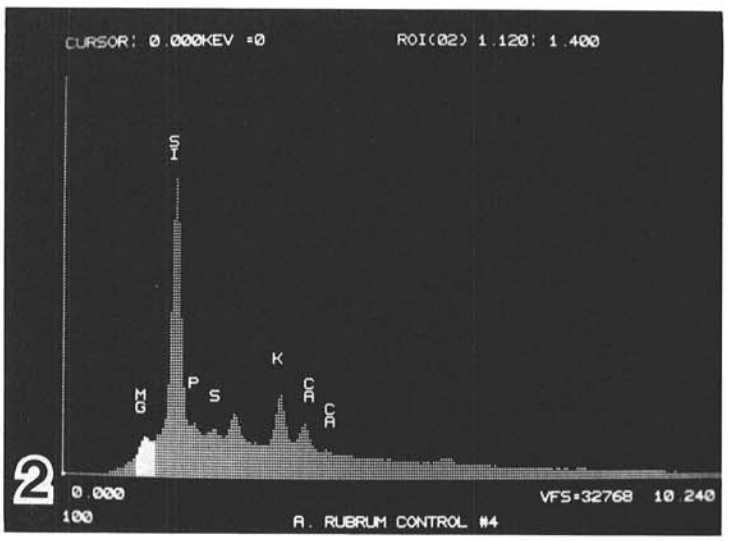
RESULTS AND DISCUSSION

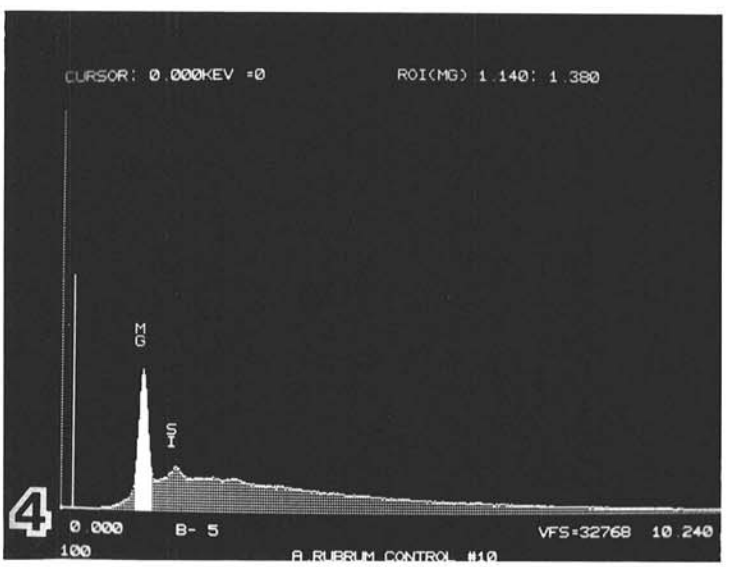
Macroscopic symptoms such as necrosis or chlorosis were not visible on leaves of plants from either treatment. Subsequent SEM examination of upper and lower leaf surfaces from either treatment



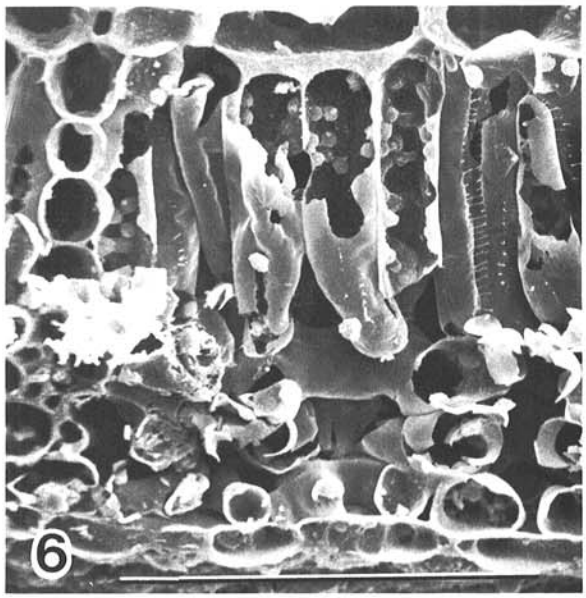


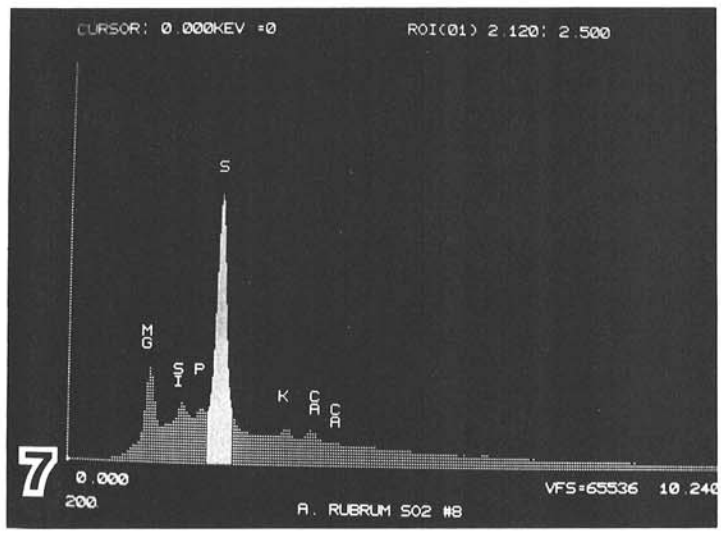


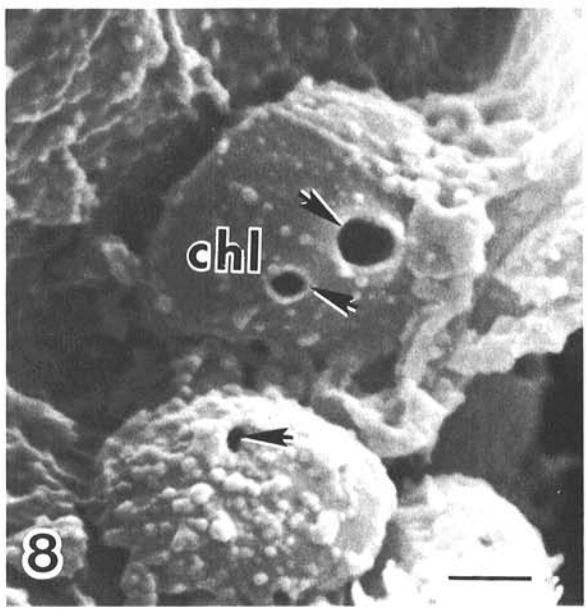


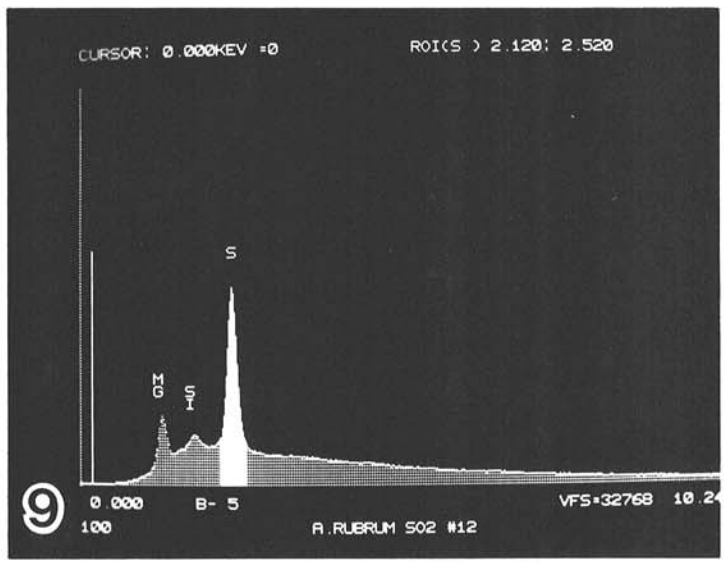


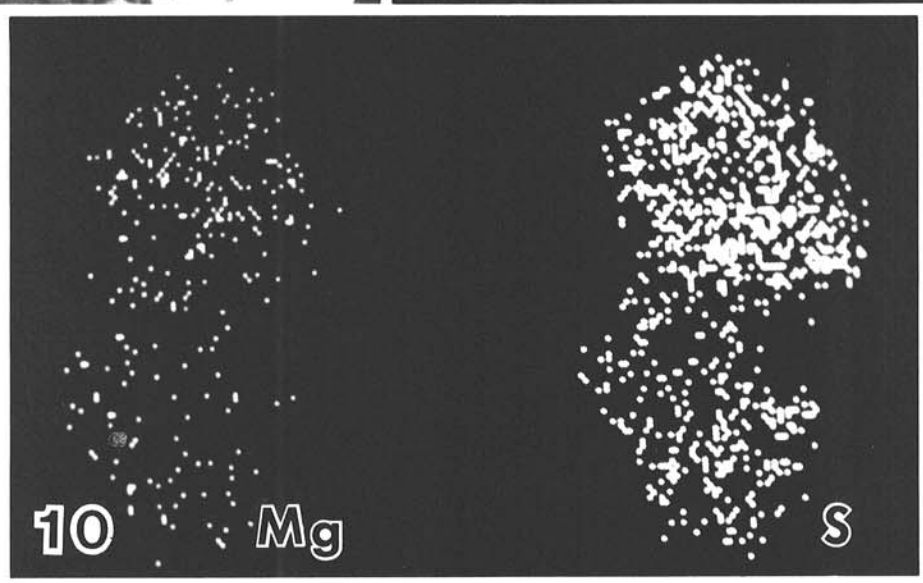
Figs. 1–5. Data from red maple leaves exposed to charcoal-filtered air. **1,** Cross section of leaf. Bar = 50 μm. **2,** Energy dispersive X-ray (EDX) spectrum representative of leaf cross section. Note the presence of Mg, Si, P, S, K, and Ca. **3,** Chloroplasts (chl) were identified as flat, circular organelles, lacking perforations and approximately 1.5–2.5 μm in width. Bar = 0.5 μm. **4,** Representative EDX spectrum of chloroplast above. Note Si and Mg. **5,** X-ray distribution map of Mg in chloroplast from Figure 3.











Figs. 6–10. Data from red maples exposed to 0.15 ppm SO_2 at 12 hr/day for 42 consecutive days. **6,** Cross section of leaf. Bar = 50 μm. **7,** Energy dispersive X-ray (EDX) spectrum representative of leaf cross sections. Note elevated level of S with endogenous elements present that were also in the control (Fig. 2) spectrum. **8,** Chloroplast (chl) with perforations (arrows). Bar = 0.5 μm. **9,** EDX spectrum of chloroplast from leaves exposed to SO_2. Mg and S were detected. **10,** X-ray distribution map of chloroplast from Figure 9. Note separate maps of Mg and S.

did not reveal injury to epidermal cells or stomata or changes to epicuticular wax as previously reported (7). In the current study, the dosage of SO_2 was lower than in the previous study (4), possibly accounting for the lack of macroscopic symptoms or the lack of leaf surface injury as detected by SEM.

Cross sections of leaves from trees exposed to charcoal-filtered air (Fig. 1) revealed turgid epidermal and mesophyll cells with chloroplasts embedded peripherally in the ground plasm containing large central vacuoles as described by Krause (7). EDX spectra were taken of the cross sections and yielded the following background elements: Mg, Si, P, S, K, and Ca (Fig. 2). The unidentified peak in Figure 2 is an escape peak or an artifact. Si was relatively high in the particular control sample described in Figure 2 due to the presence of extraneous cell wall vestiges created by the razor blade. X-ray distribution mapping did not indicate accumulation of any of those endogenous elements in specific organelles. EDX spectra and maps presented are representative of 20 spectra and maps taken of each treatment. At higher magnification, chloroplasts (Fig. 3) were identified as flat, circular organelles approximately 1.5–2.5-μm wide, lacking perforations, lining the walls of mesophyll cells. Additional confirmation that the organelles were chloroplasts was the presence of Mg (16) found within the organelles as displayed in spectra (Fig. 4), and by X-ray distribution mapping (Fig. 5) with Si in the background. Sulfur accumulation in these chloroplasts was not detected by X-ray distribution mapping.

Examination of leaf cross sections from trees exposed to SO_2 (Fig. 6) revealed morphology similar to that previously described (Fig. 1). The presence of S in the mesophyll cells was indicated in spectra (Fig. 7). S was consistently found in all specimens examined, accumulating in the palisade and spongy mesophyll cells. Increased magnification revealed perforations in all chloroplasts (Fig. 8, arrows). Perforations were not quantified. Spectra (Fig. 9) of these chloroplasts, while mostly qualitative, showed the presence of Mg and S as displayed in X-ray distribution maps (Fig. 10) with Si in the background. The level of Mg in chloroplasts exposed to SO_2 (Fig. 9) seems to have decreased when compared to the EDX spectrum from chloroplasts exposed to charcoal-filtered air (Fig. 4). Decreased Mg content, along with intracellular injury expressed as chloroplast perforations, may indicate preliminary stages of lysis and chloroplast disruption. Elevated sulfur content was not detected in other areas of mesophyll cells.

As previously noted (14), it is important to identify "sink" sites of S in plants and to establish dose-response data related to injury of organelles. Depending on its form, S from SO_2 fumigation can accumulate in maple leaves, thereby increasing leaf acidity. Degradation of chlorophyll in mesophyll cell chloroplasts could ultimately result in premature senescence of leaves and, in time, possibly growth reduction. Elucidation of this S association and its chemical composition in mesophyll cells might be suggestive of specific SO_2 diagnostic procedures. On the basis of this study, it seems that the mesophyll chloroplast is the first, or at least one of the first organelles, to be affected when red maples are exposed to elevated levels of SO_2 before macroscopic injury is detected.

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