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Resistance to Injury by Sulfur Dioxide[1]

CORRELATION WITH ITS REDUCTION TO, AND EMISSION OF, HYDROGEN SULFIDE IN CUCURBITACEAE

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ABSTRACT

In Cucurbitaceae young leaves are resistant to injury from acute exposure to SO_2, whereas mature leaves are sensitive. After exposure of cucumber (*Cucumis sativus* L.) plants to SO_2 at injurious concentrations, illuminated leaves emit volatile sulfur, which is solely H_2S. Young leaves emit H_2S many times more rapidly than do mature leaves. Young leaves convert approximately 10% of absorbed $[^{35}S]SO_2$ to emitted $[^{35}S]H_2S$, but mature leaves convert less than 2%. These results suggest that a high capability for the reduction of SO_2 to H_2S and emission of the H_2S is a part of the biochemical basis of the resistance of young leaves to SO_2.

Plants are injured by far lower doses of SO_2 than are animals (18), in spite of the fact that SO_2 is closely related to one or more intermediates in the path of sulfate assimilation in plants (1, 19), and plants possess sulfite reductase, an enzyme specific for sulfite (13, 20, 23). Above a threshold concentration, usually approximately 0.1 μl/L, a single acute exposure to SO_2 for a few h elicits the acute injury syndrome of interveinal necrotic lesions. Chronic exposure to concentrations somewhat below the threshold can inhibit growth and cause chlorosis. At still lower concentrations, SO_2 can be a nontoxic sulfur source.

When plants are exposed to SO_2, they absorb it rapidly, probably through stomata (22, 29). The SO_2 dissolves in tissue water, whereupon it ionizes to HSO_3^- or SO_3^{2-}. These may be normal intermediates, albeit at lower concentrations, because plants can synthesize SO_3^{2-} from SO_4^{2-} (9, 26). It has been shown in several plant species that most of SO_2 absorbed is oxidized to SO_4^{2-} rapidly and to a lesser extent the sulfur is incorporated into organic sulfur compounds such as cysteine and glutathione (6, 10, 11, 24, 25). On the other hand, light-dependent reduction of SO_2 to sulfide has been suggested as a possible metabolism of SO_2 (6, 17, 21). De Cormis (7) found light-dependent $[^{35}S]H_2S$ emission when plants were given acute exposure to injurious levels of $[^{35}S]SO_2$, and he suggested that H_2S might be the cause of the light-dependent acute injury by SO_2. However, Wilson *et al.* (28) showed that cucurbits that had emitted H_2S in response to light and SO_4^{2-} were not injured, whereas plants that had emitted H_2S at a similar rate in response to light and HSO_3^- were injured. Therefore, H_2S is unlikely to be the cause of acute injury by SO_2, assuming equivalency of the injury mechanisms of gaseous SO_2 and HSO_3^- in H_2O. The work reported in this article is part of a study undertaken in the hope of finding a relationship between metabolism of SO_2 and susceptibility to acute injury by SO_2.

The susceptibility of leaves to injury by SO_2 changes systematically during development (12). In Cucurbitaceae, as in many other species, young leaves are resistant to injury from acute exposure to SO_2 and mature leaves are sensitive (4). The difference in resistance in cucurbits is not attributable to an uptake difference, because young cucurbit leaves actually absorb SO_2 at a higher rate than mature leaves (5). Therefore, we have used cucumber leaves in a search for a difference in metabolism of SO_2 in sensitive and resistant tissues. We have found, after fumigation of cucumber plants with SO_2, a remarkable difference in quantity of H_2S emitted by sensitive mature leaves compared with resistant young leaves.

MATERIALS AND METHODS

Plant Materials. Cucurbit plants (*Cucumis sativus* L. cv. Chipper, formerly called SC25; *C. sativus* L. cv. National Pickling; *Cucurbita pepo* L. cv. Prolific Straightneck squash; and *C. pepo* L. cv. Small Sugar Pumpkin) were grown for 30 to 40 d with modified Hoagland solution (28) in a growth chamber programmed for 16 h of light (max. 8 mw cm^{-2}) at 28°C, followed by 8 h of dark at 16°C. Irradiances were measured with a YSI model 65A radiometer.

Fumigation with SO_2. Plants in plastic pots with the top sealed around the stem with Parafilm were fumigated individually with air containing SO_2 at 24.5 ± 1°C in a closed 40-L Plexiglas chamber. The chamber had an air stirrer built in, and was illuminated with cool-white fluorescent lamps (0.8 mw cm^{-2}). When the plant was placed in the chamber, a beaker containing a mixture which would generate SO_2 upon acidification was also placed in the chamber. After sealing the chamber, lactic acid was added to the beaker contents through a port connected to the beaker by Teflon tubing. The mixture after acidification contained in 30 ml: $KHSO_3$ (60 μmol), Na_2CO_3 (40 μmol), 6.7% (v/v) ethanol, and 12% (v/v) lactic acid.

Measurement of Volatile Sulfur Emission. Immediately after a whole plant was fumigated with SO_2, a leaf to be used for measurement of the sulfur emission rate was detached. The cut end of the petiole of the detached leaf was placed in H_2O in a small sealed vial in a sealed Plexiglas leaf chamber (0.4 L) with two ports, one of which was the air inlet and the other was the air outlet. The vial was sealed around the petiole with Parafilm in order to prevent the possible absorption or release of volatile sulfur compounds via the petiole. The outlet was connected

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through Teflon tubing to a flame photometric sulfur analyzer (Monitor Labs, model 8450). In the case of attached leaves, an attached leaf to be used for measurement was placed in a similar Plexiglas leaf chamber (0.25 L) coupled to the sulfur analyzer. Leaf chambers for detached leaves, as well as those for attached leaves, had built-in heat sinks through which water at a constant temperature could be circulated. Air was drawn at 180 ml min^{-1} through the leaf chamber by the pump of the sulfur analyzer. Emission of volatile sulfur was continuously monitored at 28°C in the light (2 mw cm^{-2}) or in the dark.

Feeding Experiments with $[^{35}S]SO_2$. For the fumigation with $[^{35}S]SO_2$, 0.1 mCi of $[^{35}S]Na_2SO_3$, which was prepared from elemental [^{35}S]sulfur (New England Nuclear) immediately before use by a combustion method (5), was added to the mixture for SO_2 generation. After an intact cucumber plant was fumigated with $[^{35}S]SO_2$ for 30 min in the light (0.8 mw cm^{-2}), leaves were detached and placed in sealed leaf chambers (1-L filter flasks) with 5 ml of H_2O in small vials (petiole solution). Air pushed through the leaf chamber at 60 ml min^{-1} then passed through a trap containing 20 ml of 20 mM NEM[4], then a trap containing 0.1 M zinc acetate and 0.04 M sodium acetate in 20 ml H_2O. Volatile ^{35}S emitted was collected in the traps for 3 h. ^{35}S in aliquots of the NEM trap and zinc trap was determined by liquid scintillation counting. The contents of the NEM trap was concentrated by vacuum-evaporation at 40°C and analyzed by TLC and electrophoresis. The ^{35}S compounds remaining in the leaves after trapping volatile ^{35}S sulfur for 3 h, were extracted, fractionated, and analyzed by TLC or electrophoresis.

Extraction and Fractionation of ^{35}S Compounds. Leaves fed with $[^{35}S]SO_2$ were extracted twice with 10 ml of 80% (v/v) ethanol containing 20 mM NEM (8). The residues were extracted twice with 10 ml of 1% (v/v) TCA. The final residues were transferred to scintillation vials. ^{35}S in aliquots of the ethanol extract, the TCA extract, and in the residues were determined by liquid scintillation counting in scintillation fluid (4 g of PPO, 0.1 g of POPOP, 330 ml of Triton X-100, and 670 ml of toluene). The combined ^{35}S in the three fractions constitutes ^{35}S remaining in leaf tissues. The ethanol extract and the TCA extract, neutralized with 1 N NaOH, were concentrated and subjected to TLC on cellulose plates (Baker-flex, J. T. Baker) developed with *t*-butyl alcohol:88% formic acid:H_2O (14:3:3, v/v) (15), or to electrophoresis on cellulose plates in 0.1 M sodium acetate buffer (pH 4.0) at 600 v for 20 min.

RESULTS AND DISCUSSION

In our early investigations of effects of acute exposure to SO_2 on cucurbits, the plants were exposed in an open system, *i.e.* one in which the SO_2-containing atmosphere was passed once through a chamber containing plants, and then was discarded (4). However, an open system is not well suited to analysis of emitted sulfur compounds because of the high background of unabsorbed SO_2. An open system is also not suited for following the metabolism of $[^{35}S]SO_2$ because it generates large amounts of waste air containing ^{35}S, thereby creating an awkward disposal problem. Also, large volumes of air carrying low concentrations of emitted ^{35}S must be passed through traps in order to have a chance at analysis of the compounds, but in our experience, the traps do not work well under those circumstances.

These considerations led us to prefer a closed fumigation system in which the plants could be loaded with an injurious amount of SO_2. Then the resultant whole plant, or detached leaves, could be transferred to apparatus through which clean air could be drawn and emitted sulfur could either be monitored with a sulfur analyzer, or, if $[^{35}S]SO_2$ had been used, the emitted ^{35}S compounds could be trapped for subsequent analysis.

Table I. *$H_2{}^{35}S$ Emission from Cucumber Leaves Fumigated with $^{35}SO_2$*

Immediately after a cucumber plant with eight leaves was fumigated with $[^{35}S]$-SO_2 for 30 min in the light, two young leaves (leaf area, 88 cm^2), second and third ones from the top, and two mature leaves (239 cm^2), sixth and seventh from the top, were detached and placed in a leaf chamber with the petioles in 5 ml of H_2O in small vials. Volatile ^{35}S sulfur was caught by the NEM trap and the zinc trap for 3 h in the light (0.5 mw cm^{-2}). Leaves after trapping volatile sulfur were subjected to extraction and fractionation of ^{35}S compounds as in "Materials and Methods" and ^{35}S remaining in leaf tissues was determined.

	Young Leaves	Mature Leaves
Fresh weight, g	1.85	5.10
A: ^{35}S remaining in leaves, cpm	10.75×10^6	5.40×10^6
B: $[^{35}S]H_2S$ trapped, cpm	1.587×10^6	0.012×10^6
C: ^{35}S in petiole solution, cpm	0.022×10^6	0.007×10^6
$[^{35}S]H_2S$ as percentage of total absorption (B/A+B+C) × 100	12.9	0.2
^{35}S in petiole solution as percentage of total absorption (C/A+B+C) × 100	0.2	0.1

In preliminary experiments, it became apparent that the metabolism of SO_2 occurred largely in the first couple of h after absorption. We were therefore faced with a dilemma: if we performed fumigations with SO_2 at concentrations commonly used in acute injury studies, approximately 1 to 5 $\mu l\ L^{-1}$, exposure times of 16 h or more would be required to obtain acute injury (4). Such long exposures would have severely limited the information on SO_2 metabolism which we could obtain. If we cut the exposure time to only 1 h or so, at the same concentration, the metabolism could be analyzed, but no injury would occur, so the relationship of the metabolism to injury could not be established. Therefore, we decided to use a brief exposure to a high SO_2 concentration in order to get enough exposure to cause acute injury. It is well established that acute injury is a function of exposure concentration (above a threshold that is characteristic of the species) and the duration of exposure (see Ref. 12). Because we were interested in the biochemical basis of the difference in resistance of young and mature cucurbit leaves to injury from acute exposure to SO_2, we determined the conditions for brief exposure that would injure mature cucumber leaves but not young leaves, in the same manner as the 16-h exposure used in our earlier studies (4). In the closed system, with a plant having approximately 600 cm^2 of leaf area, this could be achieved with an initial concentration of 22 $\mu l\ L^{-1}$. The plant absorbed approximately 80% of the SO_2, or 30 μmol, within 30 min. Five to 10 h after a 30-min fumigation, necroses symptomatic of acute injury were observed on mature leaves, but none were evident on young leaves. This injury pattern is comparable to that caused by SO_2 at 3 to 3.5 $\mu l\ L^{-1}$ for 16 h, which was the treatment that gave the largest difference between young and mature leaves in our previous study using an open fumigation system (4). In effect, the plants were exposed to an injurious pulse of SO_2 in the experiments reported here.

There is evidence that conditions which cause stomatal closure can protect plants from acute injury by SO_2 (3), and there are reports that under some conditions acute exposure to SO_2 can cause stomatal closure (2, 14). However, it is not established that either genetically or developmentally determined differences in sensitivity to SO_2 are attributable to differences in stomatal behavior. On the contrary, in the case of the difference between young and mature cucurbit leaves, we have established previously that absorption differences are not responsible for the sensitivity difference. In fact, the more resistant young leaves actually absorb substantially more SO_2 than the sensitive mature leaves (Table I;

[4] Abbreviation: NEM, *N*-ethylmaleimide.

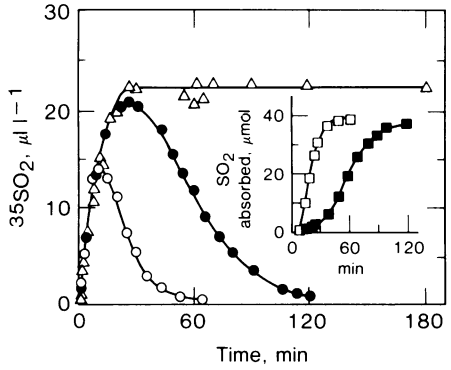

FIG. 1. Changes in SO_2 concentration in fumigation chamber and SO_2 absorption by cucumber plant. After generating SO_2 as described in "Materials and Methods," in absence of a cucumber plant in the light (△), or in presence of a plant with 600 cm^2 of leaf area in light (○) or in dark (●), 20 ml of air in fumigation chamber was taken by plastic syringe at time indicated and introduced into sulfur analyzer. SO_2 concentration was calculated from a calibration curve made with known concentrations of SO_2. Amount of SO_2 absorbed by a whole plant in light (□) or in dark (■) was calculated from changes in SO_2 concentration in leaf chamber.

also, see Fig. 3 in Ref. 5). Furthermore, stomates act by modulating fluxes. Under the conditions of pulse exposure used here, in which almost all of the available SO_2 was absorbed by the plant, stomatal status cannot have much effect on the amount of SO_2 absorbed, hence on differences in injury between young and mature leaves.

In the closed system, plants were illuminated with 0.8 mw cm^{-2} of light because it was sufficient for development of the injury symptoms, which is a light-dependent process (4). Higher light intensities, more closely approximating full sunlight, or even growth chamber intensities, would have created a serious heating problem in the closed system. Although it would have been nice to be able to expose plants to SO_2 at high light intensities, a thermostatted closed-system chamber would only have replaced an acceptable problem with an unacceptable one: condensation of transpired water on the cooled surfaces of the chamber. Because of the high solubility of SO_2 in water, such condensation would have made it virtually impossible to control the amount of SO_2 available for absorption by the plant. Therefore, rather than construct a large thermostatted chamber for whole plants, we elected to keep the light intensity low enough to prevent excessive heating, but high enough to allow development of the acute injury symptoms.

The closed-system chamber attained the concentration of 22 μl L^{-1} SO_2 within 15 min after the sulfite reservoir was acidified. The concentration remained constant for at least 5 h if there was no plant in the chamber, or if there was a pot with soil and a root system, the soil being covered with Parafilm (Fig. 1). At least 90% of ^{35}S introduced as $[^{35}S]SO_3^{2-}$ could be recovered from the gas phase of the chamber plus the contents remaining in the reservoir, so less than 10% of the sulfur was lost, presumably by absorption onto the walls of the chamber.

When a plant was in the chamber, the SO_2 concentration increased after acidification of the reservoir, at the same rate as in the absence of a plant, but reached a peak after 10 min if illuminated or 25 min if in darkness, and then declined due to the rapid absorption of the SO_2 by the plants (Fig. 1). It was clear from the absorption and metabolism of $[^{35}S]SO_2$ in darkness that there was no absolute light requirement for these processes.

The distribution of ^{35}S in compounds extractable from leaves fumigated with $[^{35}S]SO_2$ was investigated in resistant and sensitive cucurbit cultivars, and in young and mature leaves of each cultivar, in the hope of finding a biochemical clue to resistance differences. In general, the differences in distribution of ^{35}S in internal compounds were too small to provide a clear indication of a metabolic correlate of resistance (the details of these studies will be reported elsewhere). To complete the survey of metabolites, emitted volatile sulfur was then examined.

Emission of volatile sulfur from individual detached leaves was measured, after the fumigation with SO_2 for 30 min, by means of a leaf chamber coupled to a flame photometric sulfur analyzer. Young and mature leaves emitted volatile sulfur, and the emissions were light dependent (Fig. 2). When the light was turned off, the emission decreased rapidly to less than 10% of the light-dependent emission rate. When the light was turned on after a brief dark period, the emission rate returned to the rate that would have been observed had the light not been turned off. In these respects, the emission of volatile sulfur in response to gaseous SO_2 closely resembles the emissions in response to a solution of SO_4^{2-} or HSO_3^- (28). Another cucumber cultivar, National Pickling, and two cultivars of *Cucurbita pepo*, Prolific Straight neck squash and Small Sugar pumpkin, which were used in our earlier studies (4, 5, 28) each exhibited light-dependent H_2S emission following fumigation with SO_2 (data not shown).

The emission rates of young leaves increased to much higher levels than those of mature leaves, and the young leaves emitted volatile sulfur for a far longer time than did mature leaves. The maximal emission rate of young leaves occurred 30 to 90 min after beginning of monitoring the sulfur emission, whereas that of mature leaves occurred between 15 and 60 min. A typical maximal rate of emission in the light (Fig. 2), in nmol S min^{-1} $leaf^{-1}$, was 7.9 for a young leaf and 2.0 for a mature leaf. The young leaf typically had approximately half the area of the mature leaf which was obtained three nodes below the young leaf. The integral of the emission rate curves over time, *i.e.* the total emissions, differed by a factor of approximately 10 on a per leaf basis, and 20 on a per cm^2 basis, in this particular experiment. Maximal rates of emission and the relative rates for young and mature leaves varied from plant to plant, but young leaves always emitted at least 10

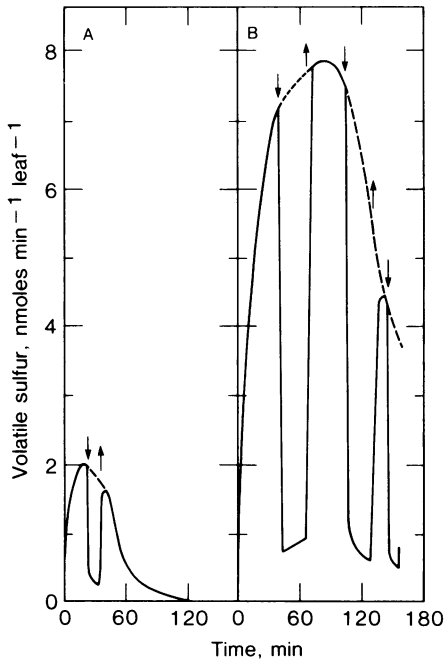

FIG. 2. Emission of volatile sulfur from cucumber leaves after fumigation with SO_2. Immediately after fumigation with SO_2 for 30 min in light, young and mature leaves, third and sixth leaves from top, were detached and each was placed in a separate leaf chamber. Emission of volatile sulfur was monitored at 28°C by sulfur analyzer in light (2 mw cm^{-2}) or in dark. A, Emission of volatile sulfur from mature leaf (leaf area, 109 cm^2); B, emission from a young leaf (58 cm^2). ↑ and ↓ indicate 'light on' and 'light off', respectively.

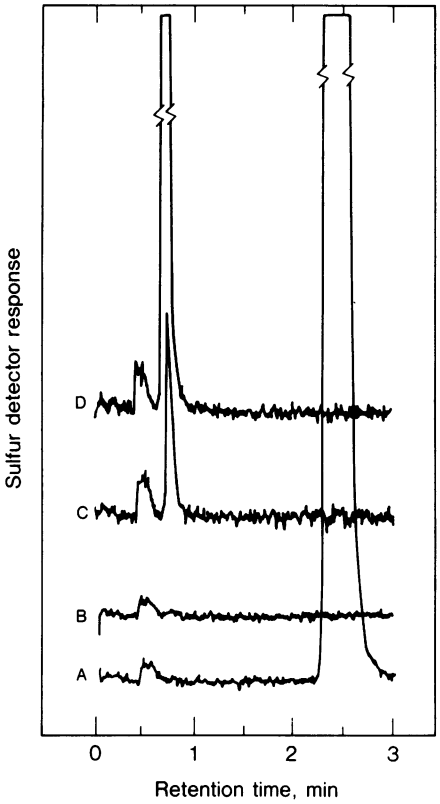

FIG. 3. Gas chromatograms of volatile sulfur. Conditions for fumigation with SO_2 and emission of volatile sulfur were the same as those in Figure 1. Air sample (2 ml) was taken from SO_2 fumigation chamber or leaf chamber containing a young leaf by syringe and analyzed by gas chromatography on a column of Chromosil 330 packed in a Teflon tube (1.828 m × 0.32 cm) mounted in Varian 3700 GC equipped with flame photometric detector. Column temperature was 40°C and carrier gas (N_2) flow rate was 15 ml min^{-1}. Detector output, set at attenuation 1 and range 10^{-9} A mv^{-1}, was in direct mode, so response is proportioned to $[S]^2$. A, Air from SO_2 fumigation chamber at end of fumigation. B, Air from leaf chamber at 6 min; C, at 14 min; D, at 30 min. Retention times of authentic compounds were 0.75 (H_2S), 1.55 (CH_3SH), 2.45 (SO_2), 3.0 (CH_3SCH_3), and 18.0 min (CH_3SSCH_3). Small peak at 0.55 min was also produced by injection with an empty plastic syringe, but not with a glass syringe.

times as much volatile sulfur as mature leaves. In extreme cases, the emission ratio exceeded 100. Illuminated attached leaves also emitted volatile sulfur after fumigation of the intact plant with SO_2. The emission patterns of attached leaves were quite similar to those of detached leaves. Even after fumigation for 1 h with SO_2 in air at 2.5 μl/L, which is a combination of concentration and time insufficient to cause injury, emission of volatile sulfur was observed at a low rate, and the rate of emission by young leaves was greater than that of mature leaves.

The chemical nature of the volatile sulfur was determined by gas chromatography (Fig. 3). The volatile sulfur in the air of the fumigation chamber at the end of the fumigation was overwhelmingly SO_2 (Fig. 3A). Six min after transfer of the leaves from a 40-L fumigation chamber containing SO_2 to a 0.4-L leaf chamber with SO_2 free air, a small peak of H_2S appeared (Fig. 3B). The peak of H_2S increased with time until 30 min (Fig. 3, B–D) and then decreased, but at no time were peaks of SO_2, CH_3SH, CH_3SCH_3, or CH_3SSCH_3 found. H_2S was not detectable in the fumigation chamber at the end of the fumigation (Fig. 3A) because the dilution was 100-fold greater than occurred in the leaf chamber.

The sulfur emission was confirmed to be solely H_2S by experiments with $[^{35}S]SO_2$. Young leaves always absorbed a few times more $[^{35}S]SO_2$ than did mature leaves, per unit of fresh weight, which is proportional to leaf area (Table I). Volatile sulfur emitted from leaves after fumigation with $[^{35}S]SO_2$ was trapped partially by NEM and the remainder was trapped by a second trap containing zinc ion. NEM reacts with H_2S,SO_2 as SO_3^{2-} and sulfhydryl compounds, but too slowly to be 100% effective at trapping sulfide at the rate the air was bubbled through the trap. It therefore provided a qualitative indication of the chemical species containing volatile sulfur, whereas the zinc trap provided a quantitative estimate of H_2S in the volatile sulfur. The ^{35}S trapped by NEM ran as a single compound with a mobility of 0.9 cm toward the cathode during electrophoresis on cellulose plates with 0.1 M sodium acetate buffer (pH 4.0), and it had a R_F value of 0.92 by chromatography on cellulose plates developed with *tert*-butyl alcohol:88% formic acid:water (14:3:3, v/v). When the values were compared with those of authentic compounds, NEM-$[^{35}S]SO_3^-$ (2.9 cm to anode; $R_F = 0.54$), NEM-$[^{35}S]$sulfide (0.9 cm to cathode; $R_F = 0.93$), and $[^{35}C]$NEM-SCH_3 (0.9 cm cathode; $R_F = 0.92$), the NEM derivative of the volatile ^{35}S compound had the same electrophoretic and chromatographic mobilities as NEM-sulfide and NEM-SCH_3. However, we concluded that H_2S was the only sulfur compound emitted from leaves because (*a*) analysis by gas chromatography had shown H_2S but not CH_3SH to be present; (*b*) zinc ion will not trap CH_3SH while NaOH will, but no ^{35}S was found in an additional NaOH trap after the zinc trap, and the radioactivity in the zinc trap was in a precipitate, as expected for the sulfide salt; and (*c*) the odor of the volatile sulfur compound was that of H_2S, not that of CH_3SH. An unknown compound (peak 6) reported by Weigl and Ziegler (27) is probably NEM-sulfide, judging from chromatographic behavior.

The ^{35}S remaining in the leaves at the end of the emission period was extracted and analyzed. More than 50% of the radioactivity remaining in the leaves was found as $[^{35}S]SO_4^{2-}$, the rest being in SO_3^{2-}, sulfide, cysteine, glutathione, and at least one unknown compound. The extent of conversion of ^{35}S in $[^{35}S]SO_2$ to ^{35}S in emitted $[^{35}S]H_2S$ was approximately 13% in young leaves and 0.2% in mature leaves in a typical experiment (Table I). The radioactivity released into the petiole solution was only 0.1% to 0.2% of total ^{35}S absorbed by young or mature leaves. Young cucumber leaves, which were preloaded with $[^{35}S]$ Na_2SO_4 through the roots of intact plants, emitted less than 0.2% of absorbed ^{35}S as $[^{35}S]H_2S$ when the plant was fumigated with unlabeled SO_2. Approximately 85% of the absorbed ^{35}S was still $[^{35}S]SO_4^{2-}$ at the end of the experiment. Therefore, H_2S formation in response to SO_2 appears to be the result of a relatively direct light-dependent reduction of SO_2 (HSO_3^-/SO_3^{2-}) to sulfide, perhaps catalyzed by sulfite reductase (13, 17, 20, 23). H_2S does not appear to arise through oxidation of SO_2 to SO_4^{2-} followed by light-dependent reduction of SO_4^{2-} to sulfide.

The results presented in this paper clearly demonstrate that H_2S was the only sulfur compound emitted after potentially injurious acute exposure of cucumber plants to SO_2; that young leaves which possess developmentally determined resistance to injury by SO_2 emitted many (10 to 100) times more H_2S than sensitive leaves; and that approximately 10% of absorbed SO_2 can be emitted as H_2S by resistant leaves. This is the first clearcut difference to be found between SO_2 metabolisms in sensitive and resistant leaves. The large variation between experiments in the rate of H_2S emission by young *versus* mature leaves appears to reflect a physiological difference between young leaves on young plants and young leaves on older plants (H. Rennenberg, unpublished observations).

Rapid metabolism of HSO_3^-/SO_3^{2-} derived from SO_2 is believed to be the principal biochemical mechanism for resistance to SO_2 (16). However, we and Garsed *et al.* (11) have observed little difference in the abilities of resistant and sensitive leaves to oxidize SO_2 to SO_4^{2-}. Therefore, the oxidation of SO_3^{2-} to SO_4^{2-} does not

appear to determine the resistance to SO_2, although the oxidation is a major path for removal of SO_3^{2-}. A second possible detoxification path has been demonstrated here: the light-dependent reduction of SO_2 to sulfide, coupled with the release of much of the sulfide from leaf tissue as H_2S. The dramatically greater H_2S emitting activity of leaves with developmentally determined resistance, compared with that of leaves with developmentally determined sensitivity, is a strong indication that reduction of SO_2 to H_2S may indeed by a biochemically significant process contributing to resistance to SO_2.

The fact that most of the SO_2 can be oxidized to SO_4^{2-} without conveying resistance, while reduction of a lesser portion of the SO_2 to H_2S is associated with resistance, raises the possibility that it is not SO_2, but rather a product of the reduction of SO_2, which is the main cause of injury. If this hypothetical reduction product is detoxified by further reduction to H_2S, or if the sulfur of SO_2 is diverted to H_2S instead of being used to form the hypothetical toxic reduction product, then the anomaly of the correlation of resistance to SO_2 with the metabolic fate of a small portion of total SO_2, would be explained.

The conversion of 10% or more of absorbed SO_2 to emitted H_2S by resistant leaves also raises the possibility that, under certain circumstances, actively growing plants may be a source as well as a sink for atmospheric sulfur.

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