# Accumulation of Sulfur in Soybean Leaves Exposed to Sulfur Dioxide

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## ABSTRACT

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Leaves of soybean plants fumigated with sulfur dioxide in a closed chamber were used to analyze the total sulfur content. The leaf sulfur content of plants exposed to 800 ppb sulfur dioxide for 4 days, 8 hr/day, increased from 0.12-0.19% of untreated control to 1.2-1.45% of dry weight, an increase of approximately 6-9 times. There were no significant difference in sulfur contents among varieties showing different sensitivities to sulfur dioxide. Increase of sulfur content was more apparent in leaves fumigated with higher concentration of sulfur dioxide with short duration, i.e. three days of treatment. By prolonging fumigation duration to 6 days, sulfur content was not significantly different among leaves exposed to different concentrations of sulfur dioxide. Sulfur content was generally higher in leaves fumigated with low concentration for a long duration as compared to those fumigated with high concentration for a short duration. Leaves without macroscopic symptoms had a less sulfur content than those with symptoms. However, no significant difference of sulfur content was detected among leaves with different degree of injuries by sulfur dioxide. The application of carbon dioxide not only affected the sensitivity of soybean to sulfur dioxide, but also affected sulfur contents in leaves. The degree of injury was reduced significantly when elevated carbon dioxide level (800 ppm) was applied; the increased amount of carbon dioxide was accompanied with a lowered sulfur contents in treated leaves.

Key words: soybean, sulfur dioxide, carbon dioxide, sulfur content, air pollution.

## INTRODUCTION

Sulfur is an essential element for plant growth and the deficiency of this element may result in a reduction of yield and poor quality of the products (11,31). The sulfur contents of plants are generally between 0.1 to 1.5% of dry weight (11,20). Plants growing in soil with high sulfur compounds may increase their sulfur contents to certain extents. The sulfur contents of soils all over the world are mostly insufficient and have to be applied artificially in order to obtain an appropriate yield (38). In Taiwan, ammonium sulfate is frequently used as a main nitrogen fertilizer for the field crops and therefore sulfur deficiency of the cultivated lands is uncommon.

Plants can also absorb sulfur from air by the forms of sulfur dioxide and hydrogen sulfide. In the areas where active volcano and sulfur springs present, the amount of sulfur in the air is very high (21,27,30). In Western Europe, approximately 23% of total lands obtained 38 kg/ha/year of sulfur dioxide from air; and in England an estimate of about 72 kg/ha/year sulfur dioxide reached agricultural areas (3). In Taiwan, the total emission of sulfur oxides was estimated to be 588,474 tons in 1991 (13). Field crops generally require 10-50 kg/ha of sulfur for adequate growth. It was reported that in the areas where plants were grown in soils containing low sulfur could benefit from the uptake of sulfur dioxide from air (3,7,9,10,12,15,23,28, 37). However, plants could also be injuried and result in a yield reduction if too much sulfur dioxide was absorbed (4,5,15,22,25,26,39).

It was known from our earier reports that sensitivity of soybean to sulfur dioxide was greatly affected by varietal differences, durations and concentrations of sulfur dioxide treatments and environmental conditions where soybeans were grown and treated (34). These differences may be resulted from the absorption and accumulation of sulfur dioxide by treated plants. The objective of this study was to reveal the relation between the degree of injury and the amount of sulfur dioxide uptake of the plants through the aid of total sulfur content analysis.

## MATERIALS AND METHODS

The closed glass chambers with natural light were used in this study. In the glass chambers the concentrations of sulfur dioxide and carbon dioxide, relative humidity, temperature and air velocity were controllable as described in the previous reports (34). Preparation of test plants and evaluation of degree of injury by sulfur dioxide also followed the same methodology in the report (34). Two soybean (*Glycine max* (L.) Merr.) varieties for each of sensitive (Palmetto and Hybrid 2217), moderately tolerant (Kaohsiung sel. 10 and Yellamel) and highly tolerant (Acadianex deragen and Hualen 2) varieties were used in these studies.

### Analysis of sulfur contents

Soybean leaves on the whole test plant were dried in an oven at 85 C for 48 hrs. The dried leaves were cut into small pieces and ground into powder by a coffee grinder. The leaf powder was further dried in an oven at 105 C for 2 hrs. After resumed to room temperature, 3 ml of acid solution (equal volume of $HClO_4$ and $HNO_3$) were added to 0.2 g tissue powder and mixed thoroughly. This mixture was first heated on stove at 80 C for 1 hr and then raised to 160 C for another 2 hrs for processing of nitrification. After nitrification, the temperature was lowered down to 140 C and an additional 3 ml of 3 N HCl were added. The mixture was maintained at 140 C for 10–30 min, then cooled to room temperature and brought to 100 ml using deionized distilled water. After subsequent addition of 1 ml of 25% HCl the suspension was heated to boiling (about 200 C) on a hot plate and then 10% of $BaCl_2$ was pipetted in drop by drop until the precipitation appeared. After resumed to room temperature, the solution was filtered through an ashless filter paper (Whatman 41). The retaining materials on filter paper were washed with deionized distilled water until chlorine ions became non-detectable by 0.1 N $AgNO_3$, dried in an oven of 70–80 C for 30 min, and then kept in a desiccator and weighed. The weight of this final product, $BaSO_4$, was used to calculate sulfur content of the leaf samples (31).

## RESULTS

### Effect of different exposure durations and concentrations of sulfur dioxide on the sulfur content of leaves

Two experiments were conducted under this catagory.

(1) Soybean varieties Palmetto, Kaohsiung sel. 10, and Hualen 2 at 28 days after sowing were treated with 400, 800 and 1600 ppb of sulfur dioxide for 11, 5 and 3 days, respectively. All leaves were sampled and analyzed for total sulfur content. Sulfur content of the leaves treated with long duration and low concentration of sulfur dioxide were higher as compared to those treated with short duration and high concentration of sulfur dioxide (Table 1). This phenomenon appeared to be true for all three tested varieties, regardless of their differences in sensitivities to sulfur dioxide. Under acute dosage 1,600 ppb of sulfur dioxide exposure, foliage sulfur content of three tested varieties were almost the same; however, under lower concentrations (800 and 400 ppb) of sulfur dioxide exposures, a higher sulfur content was detected in sensitive variety as compared to that in tolerant ones.

(2) Palmetto and Kaohsiung sel. 10 at 40 days after sowing were treated with 400, 800 and 1600 ppb of sulfur dioxide, respectively, for 3 or 6 days. After fumigation treatment, all leaves were sampled and analyzed for total sulfur content. Under short duration (3 days) of treatment, sulfur content of soybean leaves

TABLE 1. The sulfur contents in soybean leaves fumigated with different concentration of $SO_2$[1]

| Varieties | Concentration of $SO_2$ (ppb) | | | CK |
|---|---|---|---|---|
| | 1600 | 800 | 400 | |
| Palmetto | 0.89 (342.3) | 1.47 (565.3) | 1.72 (661.5)[2] | 0.26[2] |
| Kaohsiung sel. 10 | 0.89 (278.1) | 1.24 (387.5) | 1.60 (500) | 0.32 |
| Hualen 2 | 0.91 (379.2) | 1.17 (487.5) | 1.40 (583.3) | 0.24 |

[1] Twenty eight-day-old plants were treated for 3, 5, or 11 days (8 hr/day) respectively, with 1600, 800 and 400 ppb of $SO_2$.

[2] Percent of sulfur content (dry weight), numbers in parenthesis are percent of the check.

TABLE 2. The sulfur contents in soybean leaves fumigated with different concentrations and durations of $SO_2$[1]

| Varieties[3] | Duration of treatment (day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | | | | 6 | | | |
| | Concentration of sulfur dioxide (ppb) | | | | | | | |
| | 1600 | 800 | 400 | CK | 1600 | 800 | 400 | CK |
| PMT | 0.87 (1242.9) | 0.64 (914.2) | 0.69 (985.7) | 0.07 | 1.17 (1063.6) | 1.09 (990.9) | 1.12 (1018.2)[2] | 0.11[2] |
| KHS 10 | 0.91 (433.5) | 0.75 (357.1) | 0.61 (290.5) | 0.21 | 1.12 (746.7) | 0.93 (620) | 1.00 (666.7) | 0.15 |

[1] Forty-day-old plants were treated for 3 and 6 days (8 hr/day) respectively with 1600, 800 and 400 ppb of $SO_2$.
[2] Percent of sulfur contents (dry weight), numbers in parenthesis are percent of the check.
[3] PMT=Palmetto, KHS=Kaohsiung sel. 10.

TABLE 3. The sulfur contents in leaves of different sensitive soybean fumigated with $SO_2$[1]

| Varieties | Sulfur content (%) | | Degree of injury (%) |
|---|---|---|---|
| | Treated | CK | |
| Palmetto | 1.30 (753.5)[2] | 0.17 | 89.7 |
| Hybrid 2217 | 1.30 (678.7) | 0.19 | 93.3 |
| Kaohsiung sel. 10 | 1.45 (785.9) | 0.19 | 20.1 |
| Acadianex dcragen | 1.20 (975.6) | 0.12 | 3.2 |
| Yellamel | 1.31 (829.1) | 0.16 | 30.2 |

[1] Thirty-day-old plants were treated for 7 days (8 hr/day) with 800 ppb $SO_2$.
[2] Percent of sulfur contents (dry weight), numbers in parenthesis are percent of the check.

TABLE 4. The sulfur content in soybean leaves with different degrees of injuries fumigated with $SO_2$[1]

| Varieties[2] | Class of injury | | | | | | | CK |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | |
| H 2217 | 0.58 (252) | 1.50 (652) | 1.34 (583) | 1.56 (678) | 1.60 (696) | 1.60 (696) | 1.83 (796) | 0.23[3] |
| YLML | 0.87 (458) | 1.70 (895) | 1.56 (821) | — | — | — | — | 0.19 |

[1] Thirty-day-old plants were treated for 7 days (8 hr/day) with 800 ppb $SO_2$.
[2] H 2217=Hybrid 2217, YLML=Yellamel.
[3] Percent of sulfur contents (dry weight), — means no leaves in this category, and numbers in parenthesis are percent of the check.

increased and the increment paralleled with the concentrations of sulfur dioxide applied (Table 2). The amount of increase was greater in sensitive variety Palmetto than in tolerant variety Kaohsiung sel. 10. When the duration of treatment was prolonged to 6 days, the sulfur content increased further to about 10 times of controls in Palmetto and about 6–7 times in Kaohsiung sel. 10. However, this increase was not in accordance to the exposure concentrations; no significant difference in sulfur content was detected among leaves treated with different concentrations of sulfur dioxide for longer duration.

**Foliage sulfur content of soybean plants with different sensitivities to sulfur dioxide**

Five soybean varieties, Palmetto, Hybrid 2217, Kaohsiung sel. 10, Acadianex dcragen, and Yellamela at 30 days after sowing were fumigated with 800 ppb sulfur dioxide for 7 days, 8 hrs/day. At the end of treatment, all leaves were examined for the degree of injuries and analyzed for total sulfur content. Although five tested varieties differed greatly in degree of injuries by sulfur dioxide, about 90% on highly sensitive varieties (Palmetto and Hybrid 2217), 20 to 30 % on moderately tolerant varieties (Kaohsiung sel. 10 and Yellamela) and less than 5% on highly tolerant variety (Acadianex dcragen), their increments of sulfur contents in leaves were not significantly different (Table 3).

**Sulfur contents of leaves with different degree of injuries by sulfur dioxide**

Two soybean varieties, Hybrid 2217 and Yellamela at 30 days after sowing were treated with 800 ppb sulfur dioxide for 7 days, 8 hr/day. At the end of treatment, all leaves were sampled and separated into different classes based on degree of injuries and then subjected for total sulfur content analysis.

The sulfur contents of symptomless leaves of sensitive variety Hybrid 2217 and tolerant variety Yellamela increased 2.5 and 4.6 times, respectively, of that of the checks (Table 4). Leaves of Hybrid 2217

TABLE 5. Effect of $CO_2$ on the degree of injury of soybean plants fumigated with $SO_2$[1]

| Varieties | Concentration of $CO_2$ (ppm) | | |
|---|---|---|---|
| | 800 | 400 | 280–340[2] |
| Palmetto | 6.59 c | 16.60 b | 42.42 a[3] |
| Hybrid 2217 | 15.47 c | 29.70 b | 51.10 a |
| Kaohsiung sel. 10 | 2.12 c | 11.71 b | 28.31 a |
| Acadianex deragen | 0.84 b | 4.73 b | 16.57 a |
| Yellamel | 0.70 c | 4.40 b | 13.56 a |

[1] Thirty one-day-old plants were treated for 4 days (8 hr/day) with 800 ppb $SO_2$ and different concentrations of $CO_2$.

[2] The ranges of $CO_2$ in this chamber were 280–340 ppm.

[3] Degree of injury, each data was the mean value of eight replicates. Data in each row followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

TABLE 6. The sulfur content in leaves of soybean plants fumigated with $SO_2$ in the presence of different concentration of $CO_2$[1]

| Varieties | Concentration of $CO_2$ (ppm) | | |
|---|---|---|---|
| | 800 | 400 | 280–340 |
| Palmetto | 0.370 (49.1) | 0.631 (83.7) | 0.754[2] |
| Kaohsiung sel. 10 | 0.302 (51.2) | 0.446 (75.6) | 0.590 |

[1] Thirty one-day-old plants were treated for 4 days (8 hr/day) with 800 ppb $SO_2$ and different concentrations of $CO_2$.

[2] Percent of sulfur contents (dry weight), numbers in parenthesis are percent of the check.

with sulfur dioxide injury symptoms increased 6–8 times of sulfur contents of the check. However, there were no significant differences in sulfur content among leaves which show different degree of injuries. Leaves of Yellamel showed only 1st to 2nd classes of injuries. Their leaves, however, had about 8–9 times of sulfur content as compared to that of the untreated control (Table 4).

**Effect of carbon dioxide on the sulfur contents of soybean leaves fumigated with sulfur dioxide**

Palmetto and Kaohsiung sel. 10 plants at 31 days after sowing were treated with 800 ppb sulfur dioxide which were mixed with 280–340, 400, and 800 ppm carbon dioxide, respectively, for 4 days, 8 hr/day. After treatment, all leaves were examined for degree of injuries and then sampled for total sulfur content analysis.

For all varieties tested, the degree of injury by sulfur dioxide was significantly reduced as the concentration of applied carbon dioxide increased (Table 5). There was almost no injury caused by 800 ppb sulfur dioxide in tolerant varieties Acadianex deragen and Yellamel when carbon dioxide was maintained at 800 ppm in fumigation chamber (Table 5). The total leaf sulfur content decreased accordingly with the increasing concentration of carbon dioxide in sulfur dioxide fumigation chamber. The amount of sulfur in leaves fumigated with the presence of 800 ppm carbon dioxide was approximately half that of fumigated with ambient level of carbon dioxide (Table 6).

## DISCUSSION

Plants can absorb air pollutants directly by leaf tissues and serve as sinks. Under sunlight, sulfur dioxide is mainly absorbed through stomata and only a very small fraction may infiltrate through cuticle layers into plants (14,16,17,24,29,35,36). From our studies it was obviously that within a fairly wide range of concentrations in air, the rate of absorption of sulfur dioxide increased lineally with increased duration of exposure and concentration of the pollutant. The result was similar to the report of Taylor and Tingey (35).

It was noted that the actual sulfur contents in leaves of varieties at different level of sensitivity were not significantly different when exposed to tested doses of 400 to 1600 ppb. It is thus clear that the mechanism of the sensitivities of soybean to sulfur dioxide was unlikely due to the different rate of uptake of the pollutant. The sulfur contents of untreated control leaves varied widely. The acutal sulfur contents may represent better the real uptake of the pollutant than that by calculating from untreated controls to reveal the percent increases.

Increment of sulfur content in plants fumigated with sulfur dioxide was reported on many field crops. It was thought that this phenomenon might be used as indication of sulfur dioxide injury of the plants (1,2,8,18, 19). However this proposition was questioned by the facts that plants themselves contain a large amount of sulfur compounds which per se might be greatly affected by cultivation environments. Therefore it is generally agreed that sulfur contents of plants could not be used as a sole tool for diagnosis of sulfur dioxide injury (6).

Concentration of carbon dioxide affected significantly the stomatal conductance of the test plants; the higher carbon dioxide generally lead to a lowered stomatal conductance (Hsieh, et al. unpublished data) and thereby influencing the amount of sulfur dioxide uptake and the degree of injury. With the presence of 800 ppm carbon dioxide, both the sulfur content and degree of injury of soybean by sulfur dioxide lessened apparently. In fact, any condition that alters stomatal conductance may result in change of responses to sulfur dioxide. Rist and Davis (33) reported that stomatal conductance and sulfur content in pinto bean leaves exposed to sulfur dioxide at low temperature range were less than those exposed at high temperature range.

By comparisons of sulfur contents of sulfur dioxide treated soybean leaves, it was found that there were no significant differences among leaves with different degrees of injuries. The leaves without macroscopic symptoms had significantly increased sulfur contents as compared to those without sulfur dioxide treatment, however, the amount increment was less than those with symptoms. It is apparent that soybean plants can absorb and accumulate pollutants to certain levels before symptoms appear. When plants absorb a less than tolerable levels of sulfur dioxide, symptoms are unlikely to show up. Similar reports have appeared on symptomless peanut, sweet potato, water convolvulus, cabbage and banana after they were fumigated with 300–600 ppb of sulfur dioxide for 30 days, 8 hr/day (19).

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## 摘　要

謝式坪鈺[1]、蕭榮福[2]、呂明長[3]、陳煜焜[1]. 1994. 大豆葉片燻蒸二氧化硫後硫的累積. 植病會刊3:101-106. (1. 台中市　國立中興大學植物病理學研究所，2. 南投縣　臺灣省政府水土保持局，3. 南投縣　臺灣省政府農林廳)

在可控制二氧化硫濃度、二氧化碳濃度及相對溼度之密閉燻蒸室中，利用自然光狀況下，以二氧化硫燻蒸大豆處理800 ppb四天後，葉片含硫量由0.12–0.19%增加到1.2–1.45%，增加率約6–9倍，不同敏感品種間葉片含硫量並無顯著差異。短時間(3天)處理高濃度二氧化硫植物體內含硫量，比處理低濃度有顯著增加，延長處理時間至六天，則大豆葉片內含硫量不受處理濃度之影響，其增加量無明顯差異。低濃度長時間處理葉片含硫量比高濃度短時間處理高，處理後葉片受害程度及其含硫量除外觀無病徵者較低外，其他各受害等級間並無明顯差別。二氧化碳濃度不但影響大豆受二氧化硫傷害程度，也影響大豆葉片之含硫量，二氧化碳濃度愈高傷害愈低，含硫量也愈少。

關鍵詞：大豆、二氧化硫、二氧化碳、空氣污染、硫。