Distribution and Reaction of Sulfur Dioxide After Absorption by Plants

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It is a well-known fact that there are many phytotoxic air-pollutants such as sulfur oxides, nitrogen oxides, chlorine, fluorides, oxidants (ozone, PAN and its homologues) and ethylene. Among them sulfur dioxide is the most serious pollutant in Japan because it is emitted abundantly to the atmosphere during the combustion of many fuels such as petroleum and coal, and during the roasting of sulfide ores in smelting plants.

Though different species of plants vary widely in their sensitivity to sulfur dioxide, they generally show the injurious symptom on leaves when exposed to gas in the concentration higher than 0.5 ppm for several hours.

Elucidation of mechanism of its injury to plant is an important problem to be solved for the protection of agricultural environment. Therefore distribution and reaction of sulfur dioxide after absorption by plants were experimented with fumigation chambers[1].

Continuous fumigation in gas of low concentration

Paddy, Chinse rape, mustard and soybean grown in pots were placed in glass chamber of contents of about 3.8 m^3 (1.5 m in length ×1.5 m in width ×1.7 m in height), and fumigated at the constant concentration of sulfur dioxide 5 to 10 hours in daytime for several days at 25±2°C in temperature and about 60 per cent in humidity until the injurious symptom appears on leaves. In a few days after fumigation, the leaves were sampled for chemical analysis together with the control.

It is presumed from the reports by Yonemaru[2], Thomas[3], Tanaka[4] et al. that sulfur absorbed through stomata as sulfur dioxide is incorporated less than that absorbed through root as sulfate ion, and is mostly water-soluble and it converted to mainly sulfuric acid and lesser amount to organic compounds such as sulfonic acid. Therefore, both total and water-soluble sulfurs were determined by the routine gravimetric method, and the solubility and the sulfur absorbed as sulfur dioxide were measured as shown in Table 1. Analytical procedures are as follows:

1) Total sulfur: Place 4 g of air-dried sample accurately in a platinum dish, add 20 ml of 4 per cent lime suspension, mix well, dry at 120–130°C in oven, and incinerate at about 500°C. Dissolve ash with diluted hydrochloric acid, oxidize with a small quantity of nitric acid, add hydrochloric acid and heat to dryness on water bath. Dissolve it with 50 ml of 0.3 N hydrochloric acid, filter, wash with hot water, boil the filtrate, add a little excess of 10 per cent barium chloride solution, heat on water bath, filter, wash with hot water, dry the precipitate, incinerate, weigh as barium sulfate and calculate percentage of sulfur in sample.

2) Water-soluble sulfur: Place 4 g of air-dried sample accurately in a 250 ml volumetric flask, add about 200 ml of water at 30°C, shake it for an hour at 30 to 40

Table 1. Sulfur in leaves (% on dry basis)

Sample No.	Plant	Part	SO_2 fumigation	Total S [A]	H_2O-soluble S [B]	Solubility of S [100B/A]	Absorbed as SO_2 Total S [A']	H_2O-soluble S [B']	Solubility of S [100B'/A']
1	Paddy at tillering (*Oriza sativa*)	Leafblade	Control	0.177	0.122	68.9	—	—	—
			0.25ppm, 54hr	0.480	0.355	74.0	0.303	0.233	76.9
2	Paddy at flowering (*Oriza sativa*)	Terminal leaf	Control	0.140	0.112	80.0	—	—	—
			0.25ppm, 54hr	0.332	0.308	92.8	0.192	0.196	102.1
3	Chinese rape at growing (*Brassica campestris var. Komatsuna*)	Leaf	Control	0.784	0.647	82.5	—	—	—
			0.5ppm, 10hr	1.453	1.241	85.4	0.669	0.594	88.8
4	Mustard at flowering (*Brassica cernua*)	Leaf	Control	0.668	0.585	87.6	—	—	—
			0.3ppm, 46hr	1.721	1.602	93.1	1.053	1.017	96.6
5	Soybean at flowering (*Glycine max*)	Leaf	Control	0.118	0.038	32.2	—	—	—
			0.8ppm, 36hr	0.482	0.411	85.3	0.364	0.373	102.5

revolutions/minute, while keeping at 30°C during extraction, cool promptly to the ordinary temperature, dilute to the mark with water and filter through a dry filter. Pipet 150 to 200 ml aliquot of the filtrate into a 200 ml beaker, add a few ml of nitric acid, evaporate to dryness on water bath, add about 30 ml of 0.3 N hydrochloric acid, filter and wash with hot water. Then determine sulfur in the filtrate by the above mentioned gravimetric barium sulfate method.

Table 1 shows that the ratio of water-soluble sulfur to the total is higher in all fumigated leaves than in the control, and the sulfur solubility absorbed as sulfur dioxide is 75 to 100 per cent. Accordingly, watr-soluble sulfur will be more correlated with the grade of air pollution by sulfur oxides, and the analysis of both total and water-soluble sulfurs is recommended as a more reliable criterion of the kind of air-pollutant.

Sulfur absorbed through root as sulfate ion is a nutrient or constituent element for plants and assimilated into physiologically important compounds such as amino acids, protein and vitamin B_1, allyl sulfide in onion, or sinigrine in mustard. In order to investigate the difference between sulfur absorbed through

Table 2. Amino acid containing sulfur in leaf (millimol per 100 g on dry basis)

Sample No.	Part of plant	SO_2 fumigation	Free amino acids Cystine*	Free amino acids Methionine	Hydrolyzed amino acids Cystine*	Hydrolyzed amino acids Methionine	Hydrolyzed amino acids Total	Absorbed as SO_2 Total S [A]	Protein S [B]	Incorporated S (%) [100B/A]
1	Leafblade of paddy	—	tr	0.01	2.86	3.54	6.40	—	—	—
		+	tr	0.02	2.90	4.14	7.04	9.45	0.64	6.8
2	Terminal leaf of paddy	—	tr	0.24	1.08	1.40	2.48	—	—	—
		+	tr	0.20	1.21	1.42	2.63	5.99	0.15	2.5
3	Leaf of Chinese rape	—	tr	0.04	2.07	4.79	6.86	—	—	—
		+	tr	0.04	4.04	5.22	9.26	20.87	2.40	11.5
4	Leaf of mustard	—	0.04	0.01	3.13	3.97	7.10	—	—	—
		+	0.06	0.03	4.07	4.88	8.95	32.84	1.85	5.6
5	Leaf of soybean	—	0.03	0.06	1.23	2.66	3.89	—	—	—
		+	0.03	0.06	1.29	2.82	4.11	11.35	0.22	1.9

* As half molecule

stomata on leaf surface and that absorbed through root, amino acids containing sulfur, in leaves cystine and methionine, were determined as shown in Table 2. The outline of the analytical procedures is as follows:

1) Free amino acid: Place 5 g of cut fresh leaves accurately in an Erlenmeyer flask, extract 3 times with each 80 ml of 75 vol. per cent, evaporate to dryness in vacuum, and dissolve with water. Extract pigment with dichloromethane, evaporate the water phase to dryness in vacuum, and dissolve with accurately 25 ml of citric buffer solution (pH 2.2). Pipet 1 ml of the solution accurately into column of amino acid analyzer (Hitachi KLA-3B) and determine amino acids automatically.

2) Hydrolyzed amino acid: Place 1 g of cut fresh leaves accurately in flask, exidize with 20 ml of performic acid (9 vol. of 85 per cent formic acid plus 1 vol. of 30 per cent hydrogen peroxide)[5] at 0°C overnight, add 3 ml of 47 per cent hydrobromic acid, evaporate to dryness in vacuum, transfer with 40 ml of 6 N hydrochloric acid into ampoule, seal in vacuum, and hydrolyze protein at 110°C for 21 hours. Evaporate to dryness in vacuum, neutralize with sodium hydroxide solution, evaporate again, and dissolve with accurately 20 ml of citric buffer solution (pH 2.2). Pipet 0.5 ml of the solution accurately into column of amino acid analyzer and determine amino acids as in 1.

Table 2 shows that the quantities of free amino acids are very little except methionine in the terminal leaf of paddy which is less in fumigated leaves than in the control. However, the quantities of amino acids hydrolyzed from protein are more in all fumigated leaves than in the control. The rates of incorporated sulfur calculated from Tables 1 and 2 are 11.5 per cent in Chinese rape, 1.9 per cent in soybean and only a few per cent in average.

Accordingly, when plant is exposed to sulfur dioxide in low concentration for a long time, it will absorb a large amount of gas through stomata, but such a poisonous sulfur will be rather difficult to be assimilated to protein and likely to become mainly sulfuric acid and a small amount of organic acids such as sulfonic acid, both of the acids being soluble in water.

Acute fumigation using radioisotope

Tomato, cucumber, cineraria, soybean, zelkova and pine grown in pot were placed in a transparent plastic chamber with the capacity of about 82 L (49 cm in length ×34 cm in width ×49 cm in height), and fumigated for 5 hours with about 0.5 ppm (in average) of radioactive sulfur dioxide [$^{35}SO_2$] produced from sodium sulfite labeled with ^{35}S and sulfuric acid.

Autoradiographs of fumigated samples were taken by the routine method as shown in Figs. 1 and 2, and radioactivity of ^{35}S in leaves were counted as shown in Table 3.

Photos show that the distribution of sulfur

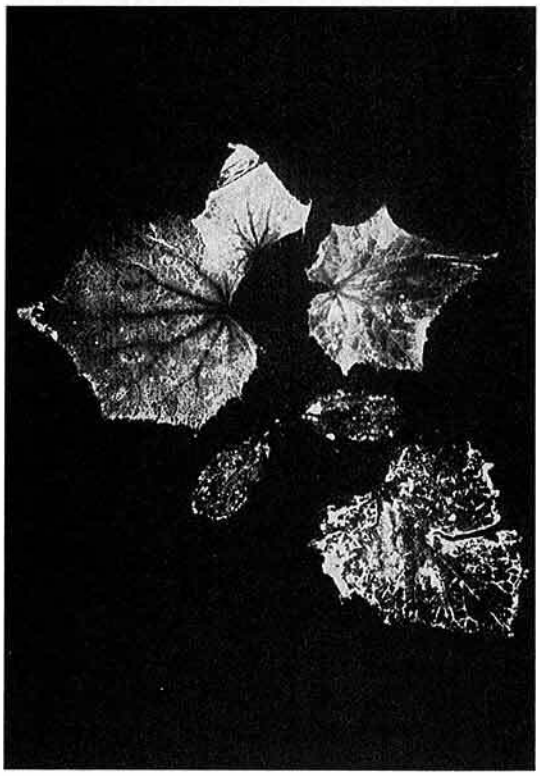

Fig. 1. Autoradiogram of cucumber fumigated by $^{35}SO_2$

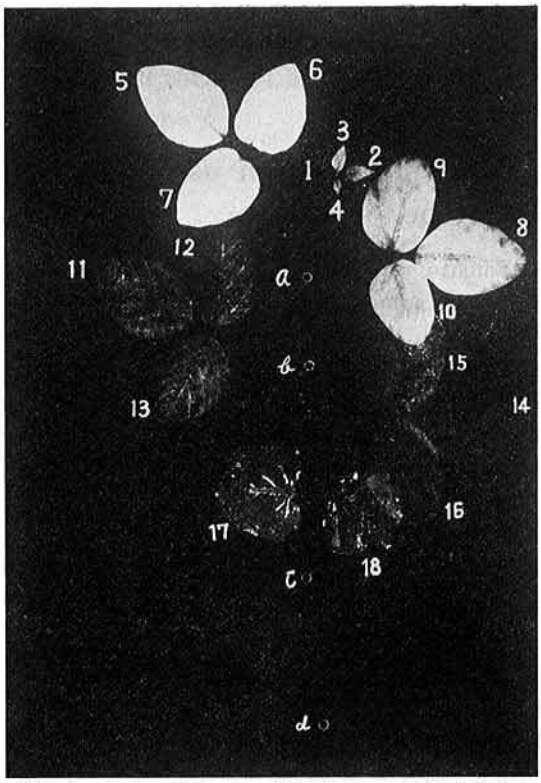

Fig. 2. Autoradiogram of soybean fumigated by $^{35}SO_2$

as air-pollutant is rich in growing leaves, especially in the parts enclosed between their veins, and ranged in the following order:

leafblade>leafstalk>stem>root

Table 3 shows that the percentage of water-soluble sulfur is more than 78 per cent in the same way as in Table 1. These results confirm that sulfur absorbed through stomata as sulfur dioxide is mostly water-soluble in leaves.

With fumigated soybean leaves, the distribution of absorbed ^{35}S at the different parts or positions of leaf was counted as shown in Table 4.

In this Table, ^{35}S is distributed almost in leaves, especially in the 5th, 6th and 7th positions where assimilation is very active. Accordingly, it is supported that sulfur dioxide is absorbed mainly through stomata with carbon dioxide and disturbs the assimilation in plant tissue or cell.

Summary

Since sulfur dioxide is the most serious air-pollutant in Japan, the elucidation of mechanism of its injury to plant is an important problem to be solved for the protection of agricultural environment. Therefore, the distribution and the reaction of sulfur dioxide after absorption by plants were experimented with fumigation chambrs. The results obtained are summarized as follows:

1) The greater part of sulfur absorbed as sulfur dioxide from the stomata on leaf surface was easily soluble in water. Accordingly, the determination of water-soluble sulfur was more reliable than that of the total, and the former value had higher correlation with the grade of air pollution. And it was presumed that water-soluble sulfur compounds originated from sulfur dioxide were mainly sulfuric acid with some sulfonic acid, both of them react as harmful substances to plant tissues.

Table 3. Radiochemical analysis of ^{35}S absorbed as $^{35}SO_2$ by crops

Sample (leaf)	^{35}S		
	Total (cpm) [A]	H_2O-soluble fraction (cpm) [B]	Solubility (%) [100B/A]
Tomato (*Lycopersicon esculentum*)	12,223	9,549	78.1
Cucumber (*Cucumis sativa*)	1,042	816	78.3
Cineraria (*Senecio cruentus*)	4,545	3,841	84.5
Soybean (*Glycine max*)	1,351	1,137	84.2
Zelkova (*Zelkova serrata*)	6,218	5,204	83.7
Pine (*Pinus densiflora*)	8,480	7,586	89.5

Table 4. Distribution of ^{35}S absorbed by soybean

Part and position (*cf.* Fig. 2)		Absorption/cm² (cpm)	Absorption ratio/cm²*	Absorption rate in each leaf** (%)	Absorption rate in each part** (%)
Leaf	1	130	34.5	0.47	96.2
	2	154	40.8	0.74	
	3	192	50.9	0.81	
	4	175	46.4	0.32	
	5	377	100.0	13.48	
	6	348	92.3	10.63	
	7	354	93.9	10.96	
	8	235	62.3	10.18	
	9	200	53.0	7.33	
	10	230	61.0	6.29	
	11	120	31.8	5.77	
	12	156	41.4	6.01	
	13	122	32.4	4.69	
	14	78	20.7	4.26	
	15	92	24.4	4.41	
	16	92	24.4	4.21	
	17	136	36.1	5.83	
	18	88	23.3	3.61	
Stem	a	68	18.0		3.7
	b	52	13.8		
	c	5	1.3		
Root	d	5	1.3		0.1

* Ratio to 100.0 in the 5th position of leaf
** Calculated from (absorption ratio/cm² × weight)

2) The distribution of sulfur as air-pollutant was confirmed with autoradiogram of ^{35}S to be rich in growing leaves, especially in the parts enclosed between their veins, and to be ranged in the following order:

leafblade>leafstalk>stem>root

3) Incorporation of sulfur dioxide to protein composed of cystine or methionine was less that of nutritive sulfate through roots. The assimilation of sulfur as sulfur dioxide was not much, ranging 1.9 to 11.5 per cent in leaves of a few crops after fumigation for several days.

4) The fractionation of water-soluble sulfur compounds or the elucidation of such a harmful organic compound should be investigated further.

References

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