Effects of Sulfur Dioxide on Carbon and Sulfur Assimilation, and Ion Uptake by Barley (*Hordeum vulgare*) and Corn (*Zea mays*)

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보리(*Hordeum vulgare*)와 옥수수 (*Zea mays*)의 炭素 및 硫黃同化作用, 無機이온吸收에 미치는 亞黃酸가스의 影響에 關한 硏究

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적 요

아황산가스에 민감한 보리(향천과1호)와 저항성이 큰 것으로 알려진 옥수수(수원19호)를 사용해서 아황산가스의 식물체 지상부에 의한 흡수와 탄소동화작용 및 무기 이온들의 흡수와 전이에 미치는 영향을 광조건과 암조건에서 그리고 15℃의 저온과 25℃의 고온에서 관찰했다. 아황산가스 처리 수준은 0, 3, 10ppm이였고, 방사성 동위원소를 이용한 추적자법으로 수행되었다. 시험결과를 요약하면,

1. 본 조건에서는 SO_2에 의한 가시피해가 두 가지 작물 모두에서 발견되지 않았고, 보리와 옥수수의 SO_2 처리에 따른 엽록소 함량변화도 적었다.
2. 잎의 기공은 광 및 암조건 그리고 온도에 관계없이 SO_2 처리에 의해서 닫혀졌고, 특히 보리는 옥수수보다 훨씬 더 많이 기공이 닫혔다.
3. 2일 간격으로 한 시간씩 SO_2를 처리하면서 3주간 생육시킨 보리 및 옥수수의 건물량과 초장은 모두 SO_2 처리에 의해서 감소되었다.

   이들의 무기물 조성을 보면 보리는 옥수수보다 인산, 망간, 아연의 함량이 많았고, 아황산가스 처리를 받은 보리 및 옥수수에서는 철(Fe) 함량이 현저히 증가되었다.
4. 3ppm의 SO_2로 처리했을 경우 보리는 옥수수 보다 많은 SO_2를 엽면 흡수했으나, 10ppm 농도에서는 오히려 옥수수가 보리보다 더 많은 SO_2를 흡수했었다. 고온(25℃)과 광의 조사는 SO_2 흡수를 촉진시켰다. 지상부로부터 뿌리로의 유황전이는 3ppm의 SO_2 처리가 10ppm에서 보다 컸다.

   암조건은 광조건에 비해서 유황 전이율을 증가시켰다.

   동화된 유황중에서 80% 알콜 추출분획은 15℃에서 보다는 25℃의 SO_2 처리시에 더 많았고, 반대로 수용성분획은 15℃보다 25℃에서 적었다.

   흡수된 SO_2의 아미노산으로의 전환율은 광조건 보다는 암조건에서 더욱 증가되었고 10ppm의 SO_2 처리시에는 3ppm 경우보다 전환율이 낮아졌다.
5. 탄소동화작용 및 동화산물의 뿌리에의 이행은 SO_2 처리에 의해서 현저하게 감소되었다.

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옥수수는 보리보다 광합성능이 큰 것으로 관찰되었고 SO_2에 의한 광합성 저해 정도는 보리에서 더 크게 나타났다.

보리에서는 탄소의 glucose, sucrose 및 fructose로의 전환율이 3ppm 및 10ppm SO_2 처리에 의해서 증가되었다.

아미노산을 주성분으로 하는 양이온 교환수지치환성 분획은 광조건에서 3ppm 및 10ppm SO_2 처리에 의해서 보리나 옥수수의 지상 및 지하부에서 모두 증가되었다.

6. 뿌리를 통한 유황(SO_4^{2-})의 흡수는 SO_2 처리에 의해서 큰 영향을 받지 않았으나, 뿌리에서 지상부로의 전이는 광조건에서 많이 저하되었다. 80% 알콜 추출분획함량(%)은 SO_2 처리에 따라 보리와 옥수수에서 모두 감소되었다.

보리뿌리중의 80% 알콜 추출분획은 옥수수 뿌리에서 보다 더 많았지만 수용성 분획은 그 반대의 경향이었다.

뿌리로 흡수된 유황의 아미노산 전환율은 3ppm 및 10ppm SO_2 처리에 의해서 증가되었고, 보리보다는 옥수수에서 더욱 컸다.

Cysteine은 모든 실험조건에서 methionine보다 더 많이 생성되었다.

7. 보리뿌리에 의한 Fe, K흡수는 3ppm 및 10ppm SO_2 처리에 의해서 촉진된 반면에 H_2O, Cl, P, Cu, Zn의 흡수는 오히려 감소되었다. 보리에서 지상부로의 전이율은 Ca, Fe, K는 SO_2 처리 농도가 높음에 따라 증가되었으나, H_2O, Cu, Mn, Cl, Zn, P는 감소되었다.
8. 옥수수뿌리에 의한 Fe, Cu, Ca, 흡수는 SO_2 처리에 의해서 촉진된 반면에 H_2O, Zn, Mn, Cl, P는 감소되었다. 옥수수에서 지상부로의 전이율은 Ca, Fe의 경우 SO_2 처리에 따라 증가되었으나, Zn, Cl, H_2O, K, Mn, S, Cu는 감소되었다.
9. 이상으로부터 보리는 광합성 능력이 옥수수보다 낮으며 SO_2에 의한 광합성 저해 정도가 더욱 심하였고 무기이온 흡수는 Cl, K를 제외한 다른 원소의 요구도가 옥수수보다 훨씬 컸으며 한편 SO_2에 의한 양분흡수 및 전이의 촉진(Fe, Ca) 또는 감소(H_2O, Cl등) 되는 정도 역시 옥수수보다 더 큼을 알 수 있었다. 이같은 특성이 보리의 SO_2 민감성에 관계된다고 추리된다. 한편 80% ethanol 추출분획중의 유황성분 함량이 옥수수보다 더 큰 반면 함유황 아미노산으로의 전환율은 옥수수가 더 큰 현상이 SO_2에 대한 저항성과 어떠한 관련이 있는지에 대해서 본 실험결과로는 충분히 해석되지 않는다.

Introduction

A. Scope of the present investigation

Few studies (144, 153) seem to have been made on the effect of sulfur dioxide gas on nutrient uptake by higher plants, research being mainly concentrated on visible damage, stomatal action, yield and photosynthesis.

Responses of plants to air pollution are not only primarily dependent on pollutant concentration and exposure time, but also on the amount of pollutant absorbed by the plant per unit of time. The rate of pollutant uptake varies just as much as the species and variety specific resistance. The dose-response relationships, therefore, must be very complex and can only be determined through consideration of an extensive set of conditions. But the present study was carried out under simple conditions to reduce its complexity.

Barley reported as susceptible to sulfur dioxide was compared with corn known to be SO_2 resistant under three levels of SO_2 concentrations (0, 3, 10ppm), in light and dark conditions, and at two different temperatures (15℃ and 25℃).Stomatal opening, chlorophyll contents, carbon assimilation and conversion to sugars, sulfur assimilation and formation of sulfur containing amino acids (cysteine and methionine), growth rate, and uptake of inorganic ions by plants were observed using various kinds of radioactive isotopes.

B. Review of literature

Since sulfur is one of the essential elements for higher plants sulfur dioxide has both harmful and beneficial effects (38, 143). However, the manner in which SO_2 affects the metabolism of the plant is not well understood.

Also interpretation of the results from SO_2 experiments are difficult because the plant responses and photosynthetic responses depend not only on the concentration or duration of exposure but also on several environmental factors such as light, temperature, humidity, nutritional status, and water stress. The evaluation is further complicated by the diverse reactions of different species or different individuals within the same species and the disparate responses of organs at different stages of development or age. However, few experimental investigations have been concerned with combined environmetal effects, such as mineral deficiency, and climatic and water stress.

Although research concerning the damaging effects of SO_2 on plants was initiated in the late nineteenth century and the data accumulated over the past century are voluminous, only selected topics among them will be briefly reviewed for understanding of the factors that determine the rate of SO_2 absorption and the metabolic effects of absorbed components.

1. Visible damage of plants by SO_2

The first visible evidence of SO_2 injury to the plant was recognized in the foliage. The stems, butts, and reproductive parts of plants are visibly more resistant to SO_2 than the foliar. Visible injury, resulting from the rapid absorption of a toxic dose of SO_2, manifests itself as marginal or intercostal necrotic areas which as first have a dull dark green water-soaked appearance, and on drying and bleaching become ivory color in most plant species, but in some browns and reds predominate. According to Linzon's definition (68), visible damage can be grouped to acute injury (macroscopic necrotic injury) and chronic injury (macroscopic chlorotic injury). He also defined that subtle effects were measured by physiological or biochemical changes, and/or reductions in plant growth or yield in the absence of macroscopic injury.

Sulfur dioxide enters leaves mainly through the stomata and is toxic to the metabolic processes taking place in the mesophyll cells. According to Solberg's observation (120), the spongy mesophyll and lower epidermis first collapsed, followed by distortion and chloroplast disruption in palisade cells. When the rice plants were fumigated with 5ppm SO_2 for 100 minutes under suitable conditions, the injury was severe but slight with 1ppm (82). Taniyama (135) observed that visible injury occured when apparent photosynthesis decreased by about 70% in the rice and by approximately 65% in the corn plants, and SO_2 did more suppress apparent photosynthesis than O_3, Cl_2, NO_2, NO, or CO. It is pointed out that younger leaves in the sunflower plant showed more severe leaf injury than those of the older plant and that leaf damage of plants fumigated with 2.0ppm for 6hrs was much more severe than those with 1.0ppm for 12hrs (150). Temple (138) demonstrated that duration of exposure and concentration of the pollutant were of equal importance in producing injury on Chinese elm, but on Norwegian maple and gingko, a concentration of SO_2 was of greater importance than the duration of exposure.

2. Plant resistant to SO_2

Different plant species(39) and varieties (27, 79, 85), and even individuals of the same species, may vary considerably in their sensitivity or tolerance to SO_2. Susceptibility lists have been made by several investigators, but these lists can be used only as guides since variations can occur because of differences in geographical location, climate, and plant stage of growth and maturation. O'gara(84) and Thomas et al. (145) reported that barley was one of the most sensitive plants to SO_2. It was determined by Zimmerman et al. (160) and Dreisinger (28) that corn belonged to one of the most tolerant crops. Kondo (63, 64) suggested the factors influencing SO_2 resistance of plants were stomatal aperture, buffering capacity of protoplast to neutralize H^+, and detoxication ability to oxidize HSO_3^- and SO_3^{2-} into SO_4^{2-} Miller

(82) showed a close relationship between the oxidation rate of sulfite and plant resistance to SO_2.

Setterstom (105) demonstrated that plants grown under heavy shade were more susceptible, young plants were more resistant, and wetting of plant leaf surfaces had little or no effect on susceptibility.

Recently Asada (12) and Tanaka (133, 134)) have investigated the role of superoxidase, catalyzing the disproportionation of the superoxide radicals to hydrogen peroxide and oxygen, in the defense against sulfur dioxide toxicity using leaves of poplar and spinach plants.

It is reported that SO_2 damaging effects resulted partly from reducing pH values of the cytoplasm and that plants had a buffering capacity to recover the lowered pH values in plant cell (126). Thomas et al. (141) observed that SO_2 absorption by alfalfa and beets reduced appreciably the buffer capacity of the leaves. Priebe (92) concluded that polyamines produced by SO_2 exposure took up H^+ and eventually removed H^+. Sugahara et al. (126) measured changes in pH of spinach cytoplasm during SO_2 fumigation and observed the lowered pH tended toward gradual recovery during further fumigation. This result showed that cytoplasm had a buffering capacity.

3. The action of SO_2 on the stomata

The stomata have a variable diffusion resistance between the atmosphere and the interior of the leaf. The stomatal resistance depends on several morphological and physiological factors and is also influenced by numerous environmental factors, of which light and water are the most important. The transfer of gases through the stomata is generally considered to proceed by molecular diffusion, and consequently the resistance is inversely proportional to the molecular diffusion coefficient. The physiological mechanisms regulating stomatal resistance and the action of different substances, including SO_2, or stomata have been reviewed (31, 52, 60, 61, 73, 93, 95, 100, 156).

It is generally accepted that the primary factor controlling SO_2 uptake by plant leaves is the degree of stomatal opening. Therefore the damaging effects of SO_2 depend on the degree of stomatal opening and resistance under a certain condition, regardless of any air pollutant (14, 15, 54, 137, 140).

Since Jones and Mansfield (52) demonstrated that abscisic acid (ABA) treatment supressed the stomatal opening in leaves, roles of ABA in plants have been investigated in relation to ABA contents of plants and transpiration rate. It is generally observed that plants containing rather high amounts of ABA close the stomatal aperture rapidly during SO_2 fumigation (60, 61). Sasamoto et al. (101) reported that K^+ ion and ABA stimulated the Mg^{2+}-activated ATPase activity in interphase fractions between sucrose densities of 1.12 and 1.14 of spinach, but not in those of broad beans and corn. The result indicated that ABA might have a role of controlling the activity of ATPase which is supposed to involve the energy consuming procedure of stomatal action.

4. Reactions of sulfite and sulfate

Sulfite is oxidized to sulfate in the plant leaf, and this ability has been correlated with resistance to toxicity. Since Macleod (71) showed that the sulfite oxidase was localized largely in the microsomal fraction of liver, heart, and kidney, it has been noticed that the predominant site for SO_3^{2-} oxidation was the chloroplast in the plant. Asada and Kiso (9) suggested that a superoxide anion formed by the univalent reduction of oxygen by illuminated chloroplasts was the initiator of sulfite oxidation. Sulfur oxidizing activity in plant leaves was measured (62) by the reduction of cytochrome c in the presence of sulfite and by the decrease of sulfite exogenously applied. Kondo (59) proposed that the entity, catalyzing the sulfite oxidation in leaves, was a dialyzable, low molecular and non proteinous substance, from the result of an experiment in which pronase treatment of the leaf extract (having the highest activity of sulfite oxidation) did not cause the activity to decrease.

Bailey (16) and Cecil (24) reported that disulfite bonds reacted with sulfite according to the equation; $RS-SR + SO_3^- = RS^- + RS-SO_3^-$. It might be the mechanism by which the sulfite reacts with the disulfide proteins to inhibit some

metabolism concerned. Another way of sulfite inhibition is the competition of SO_3^{2-} for the CO_2 or HCO_3^- binding sites (158, 159).

The SO_4^{2-} formed by oxidation of SO_3^{2-} is reduced to the S^{2-} level via the assimilatory pathway of sulfate reduction (13). Asahi (13) suggested that electrons for the reduction of 3-phosphoadenosine 5-phosphosulfate were supplied from the photosynthetic electron-transport system through fraction of disulfide carbon compounds (C-SS) or a substance like C-SS but not from $NADPH_2$. The chloroplast is the demonstrated site for sulfate reduction, and this process appears to be connected with the photosynthetic electron transport that delivers ATP for SO_4^{2-} activation. Schmidt (103) suggested that in chloroplast a sulfate activated by ATP was reduced to sulfite by a sulfhydryl compound and that sulfate was reduced to sulfite by a ferredoxin-dependent sulfite reductase.

It was reported by Baldly et al. (17) that sulfate did not interfere with the mechanism of O_2 evolution, or electron transport, but that by affecting photophosphorylation (10), or ATP utilization (18), it indirectly inhibited the conversion of 3-phosphoglycerate to 1.3-diphosphoglycerate. Although sulfate is less toxic than sulfite (44), sulfate acts as energy transfer inhibitors of photophosphorylation, competing with Pi at its binding site (90).

5. Effects of SO_2 on chlorophylls

About 20% of the total lipid content of chloroplasts consists of chlorophyll, which is an integrated functional brick in the chloroplast membrane. In the living system chlorophyll exists in a highly organized state and may undergo several photochemical reactions such as oxidation, reduction, phaeophytinization, and reversible bleaching (8, 46, 104, 124). Joslyn (53) showed that the conversion of chlorophylls to phaeophytins by acids in 90% aqueous acetone solution was found to occur at a measurable rate at a concentration of 0.0002 to 0.01 N, with about 70% conversion in twenty hours. However, rapid phaeophytin formation has been observed only in studies with high SO_2 concentration and with low pH. These situations rarely exist in the field, and phaeophytin formation may have very little relevance to the decrease of photosynthesis found in plants exposed to SO_2 (4). There are numerous literature references reporting that chlorophylls decomposed after SO_2 fumigation in the field (41, 88, 94, 99). On the other hand Puckett et al. (94) observed that ^{14}C-fixation by the lichen was reduced within 15 minutes of exposure to aqueous sulfur dioxide (75ppm, pH 3.0) but no changes were obtained in the spectra of extracted chlorophyll pigments. Shimazaki (128) proposed that the chlorophyll breakdown was late in time relative to injury of electron transport by fumigating the plants with SO_2 and was mainly due to the bleaching. Sugahara et al. (127) reported that the chlorophyll destruction by sulfite ions under aerobic and illuminated conditions in organic solvent, was not observed for the watersoluble pigmentprotein complex, even if it was 4×10^{-2}M sulfite.

6. Effects of SO_2 on photosynthesis

The inhibition of photosynthesis is often regarded as the first sign of SO_2 action on plants (47, 98). In some species, however, physiological processes such as nitrogen fixation or other processes mediated by sensitive enzymes (40, 131) may be equally or even more rapidly inhibited by SO_2. The degree of SO_2 effects on photosynthesis depends on environmental factors, species or variety of the plants, and growth stage as well as concentration and duration of SO_2 exposure (34, 47, 98, 114, 115, 116, 119, 149). Sugahara (125) noticed that on the exposure of the mixture of SO_2 and O_3 the inhibition of photosystem II reaction was not as significantly enhanced as with SO_2 alone (111), and that photosystem I was not injured by the mixture.

α-hydroxysulfonate which is an enzyme inhibitor of glycolate oxidation, inhibits carbon assimilation and photophosphorylation (11, 12, 19, 100, 130). Also it was reported that α-hydroxysulfonate inhibited PEP carboxylase (83) and that barley leaves fumigated with 5ppm SO_2 were influenced by α-hydroxysulfonate (121). Silvius (117) suggested that sulfur dioxide and sulfur anions were almost equally effective in inhibiting cyclic

and noncyclic photophosphorylation in chloroplast suspensions. Ziegler (80) and Furukawa (33) proposed that SO_4^{2-} competed with CO_3^{2-} in binding the active site of RuDP carboxylase to cause photosynthesis inhibition. This reaction is known to be reversible. It was observed by Thomas et al. (139) that carbon dioxide fixation was reduced by SO_2 as a linear function but the rate of photosynthesis recovered after fumigation unless there was no visible damage on leaves.

7. Effect of SO_2 on photosynthetic electron transport

Shimazaki and Sugahara (112) conducted an experiment of SO_2 effects on the photosynthetic electron transport system by fumigating lettuce plants with SO_2 at 2.0ppm. The result showed that electron flow from water to 2.6-dichloroindophenol (DCIP) was inhibited but electron flow from reduced DCIP to methyl viologen was not affected in chloroplast isolated from SO_2-fumigated leaves. They concluded from this result that SO_2 inactivated the primary electron donor or reaction center. A very similar conclusion was obtained by Shimazaki et al. (113) that the inhibition occurred in photosystem II but not in the enery converting system in chloroplasts isolated from the SO_2-fumigated leaves. Anderson and Avron (1) suggested that photosynthetic electron transport was required for light activation of the enzymes of chloroplast in pea leaf such as NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, NADP dpendent malic dehydrogenase, ribulose-5-phosphate kinase and sedoheptulose-1.7-diphosphate phosphatase, and for inactivation of glucose-6-phosphate dehydrogenase.

8. Factors influencing SO_2 effects

The environmental factors that are conducive to optimum plant growth are usually the same factors that bring about SO_2 injury. These factors are sunlight, moderate temperature, high relative humidity, wind, and adequate soil moisture content. In addition, time of day and season, and plant factors such as genotype, nutrition, stage of growth, and tissue maturation determine the sensitivity of a particular species to SO_2 injury. Majernik and Mansfield (73) reported that, when the relative humidity was greater than 40% at 18℃, SO_2 concentrations from 0.25 to 1.0ppm caused a stimulation in stomatal openings which increased the absorption of SO_2. From 70 to 100% relative humidity there was not much difference in sensitivity, but resistance increased below 70% and became very pronounced below 50%. A temperature over 5℃ is necessary for plant injury to occur. Provided that other factors were not limiting, temperature between 18℃ and 40℃ had little effect on plant response to SO_2 (155).

Daylight is important for stomatal opening. Light over 30,000 foot candles did not have any marked influence on the susceptibility of alfalfa, but below this value decrease of light intensity resulted in less absorption of SO_2, increasing the resistance to SO_2 injury (57).

Materials and Methods

A. Plants used

The experiments were carried out with two species of plant; barley (*Hordeum vulgare L.*, Hyangcheongua-1) sensitive to SO_2 and corn (*Zea mays L.*, Suwon-19) tolerant to SO_2. The seeds of the two species were obtained from Jeju Provincial Office of Rural Development.

To get germination, the seeds were placed in a petri dish, soaked with water, and were incubated at 25℃ for two days. The seedlings after germination were transplanted in sand and allowed to grow for four days, followed by water culture (153) for another four days. The temperature was maintained at 19-22℃ during day time and 14-15℃ at night, adjusted using the fan forced electrical heater.

The sand used was first sieved to pass through 2mm pores, rinsed with diluted HCl for 5hrs and washed with tap water until AgCl precipitation disappeared.

B. Radioactive isotopes used

All the radioactive isotopes used through out

this experiment were beta emitters or/and gamma emitters described in table 1.

Some of them (^{3}H, ^{14}C, ^{35}S, ^{36}Cl, ^{45}Ca and ^{54}Mn) were purchased from NEC (New England Nuclear) and others (^{32}P, ^{42}K, $^{55+59}Fe$, ^{64}Cu, and ^{65}Zn)from KAERI (Korea Advanced Energy Research Institute).

Table 1 Radioactive isotopes used

Elements	Half life	Decay mode	Energy in Mev and emitting %		Chemical forms	*Spec. Act.
			β^-	γ		
$^{3}_{1}H$	12.262y	β^-	0.018—100%		H_2O	0.1uCi/ml
$^{14}_{6}C$	5730y	β^-	0.156—100%		$NaHCO_3(CO_2)$	(2.2uCi/l)
$^{32}_{15}P$	14.28d	β^-	1.71—100%		H_3PO_4	0.1uCi/ml
$^{35}_{16}S$	87.9d	β^-	0.167—100%			1.1uCi/ml
$^{36}_{17}Cl$	3.08×10^{5}y	EC	1.9%		H_2SO_4(or SO_2)	(6.3 or 19uCi/l)
		β^-	0.714—98.1%		HCl soln.	0.013uCi/ml
$^{42}_{19}k$	12.36h	β^-	1.97—18% 3.56—82%	0.32—weak 1.52—18%	K_2CO_3 in H_2O	1.0uCi/ml
$^{45}_{20}Ca$	165d	β^-	0.25—100%		$CaCl_2$ in H_2O	0.2uCi/ml
$^{54}_{25}Mn$	303d	EC	100%	0.835—100%	$MnCl_2$ in HCl	0.1uCi/ml
$^{55+59}_{26}Fe$	^{55}Fe 2.60y	EC	100%	0.0059%		0.04uCi/ml
	^{59}Fe 45.6d		0.273—48% 0.475—51% 1.573—0.3%	0.143—0.8% 0.192—2.8% 1.095—56% 1.292—44%	$FeCl_2$ in H_2O	
$^{64}_{29}Cu$	12.80h	β^- β^+ EC	0.57—38% 0.66—19% 43%	1.34—0.5% 1.34—0.5%	$Cu(NO_3)_2$ in H_2O	1.0uCi/ml
$^{65}_{30}Zn$	245d	β^+ EC	0.327—1.7% 98.3%	1.115—49%	$ZnCl_2$ in HCl	1.0uCi/ml

( ): chemical forms converted to gas and specific activities in the atmosphere.

*: Specific activity of culture solutions.

C. Sulfur dioxide fumigation

1. SO_2 generation

Sulfur dioxide gas was prepared and diluted to 60 ℓ in a vinyl bag to make 3ppm and 10ppm SO_2 (fig.2).

2. Determination of SO_2 concentration

a. Conductimetric method

The principle of this method (3, 89) can be summarized as follows. Sulfur dioxide is collected by aspirating a measured volume of air through a dilute acidified solution of hydrogen peroxide. The sulfur dioxide is oxidized to sulfuric acid by the hydrogen peroxide, yielding two moles of hydrogen ion and one mole of sulfate ion for each mole of sulfur dioxide. The change of conductivity of the solution caused by the increased ionic content is determined. Operational sensitivity ranges from 0 to 20ppm SO_2. To determine the SO_2 concentration of the gas used for fumigating the plants placed in the acryl chamber, 100ml of SO_2 gas was taken up with a gas tight syringe from the vinyl bag and introduced into 300ml absorbing solution consisting of 0.006% H_2O_2 and 10^{-5} N-H_2SO_4. The conductivity of this solution was measured with a digital Ph/mV meter (Philips, Model PW 9409).

The standard solutions were prepared by diluting 31.22ml of 0.1 N-H_2SO_4 to 1000ml solution which is equivalent to 100ug SO_2/ml. This diluted solution was used to make a series of standard solutions ranging from 0.1ug SO_2/ml to 10ug SO_2/ml. From the standard curve (Fig. 1) the concentration of SO_2 gas used for fumigation was determined.

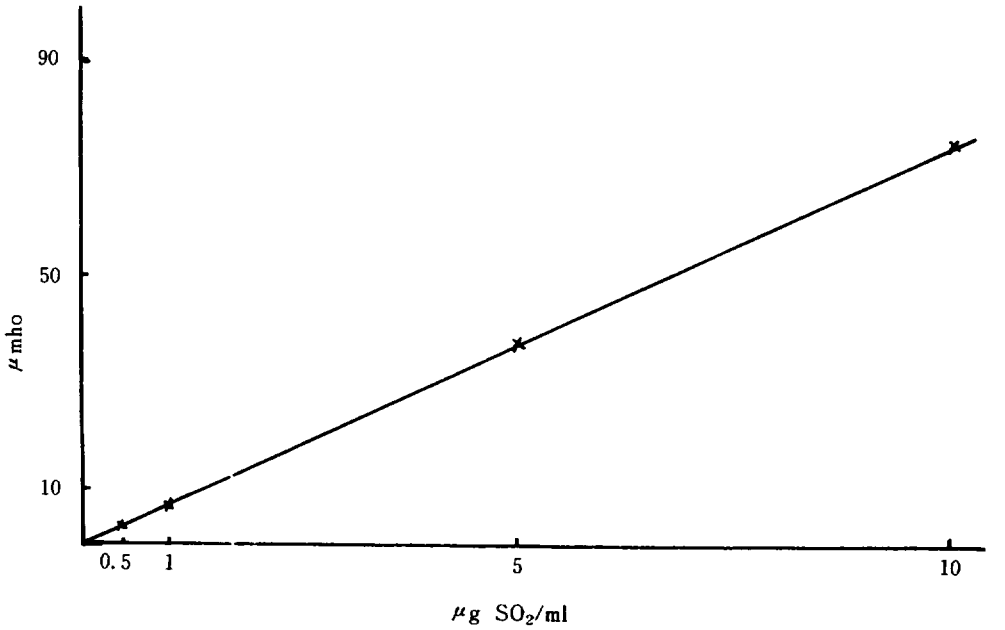

Figure 1. Relationship between electric conductivity and SO_2 concentration of the standard solutions.

b. Gas detector method

The principle of Kitagawa gas detector is one of the dry analyses that makes an application of a chemical reaction and a physical absorption. The sample gas being introduced into a detector tube,

the discolored layer is produced by means of a color changing reaction between gas and the reagent packed in the tube. Since the gas concentration is proportional to the length of discolored layer, the concentration can be easily read off on the top of the discolored layer. The gas detector was calibrated with the conductimetric method and used for checking the concentration of SO_2 gas.

3. Fumigation system

The fumigation system is composed of cylinders (gas containers), acryl fumigation chambers in a growth chamber and SO_2 gas absorbing parts, as shown in Fig. 2. Three levels of SO_2 concentra-

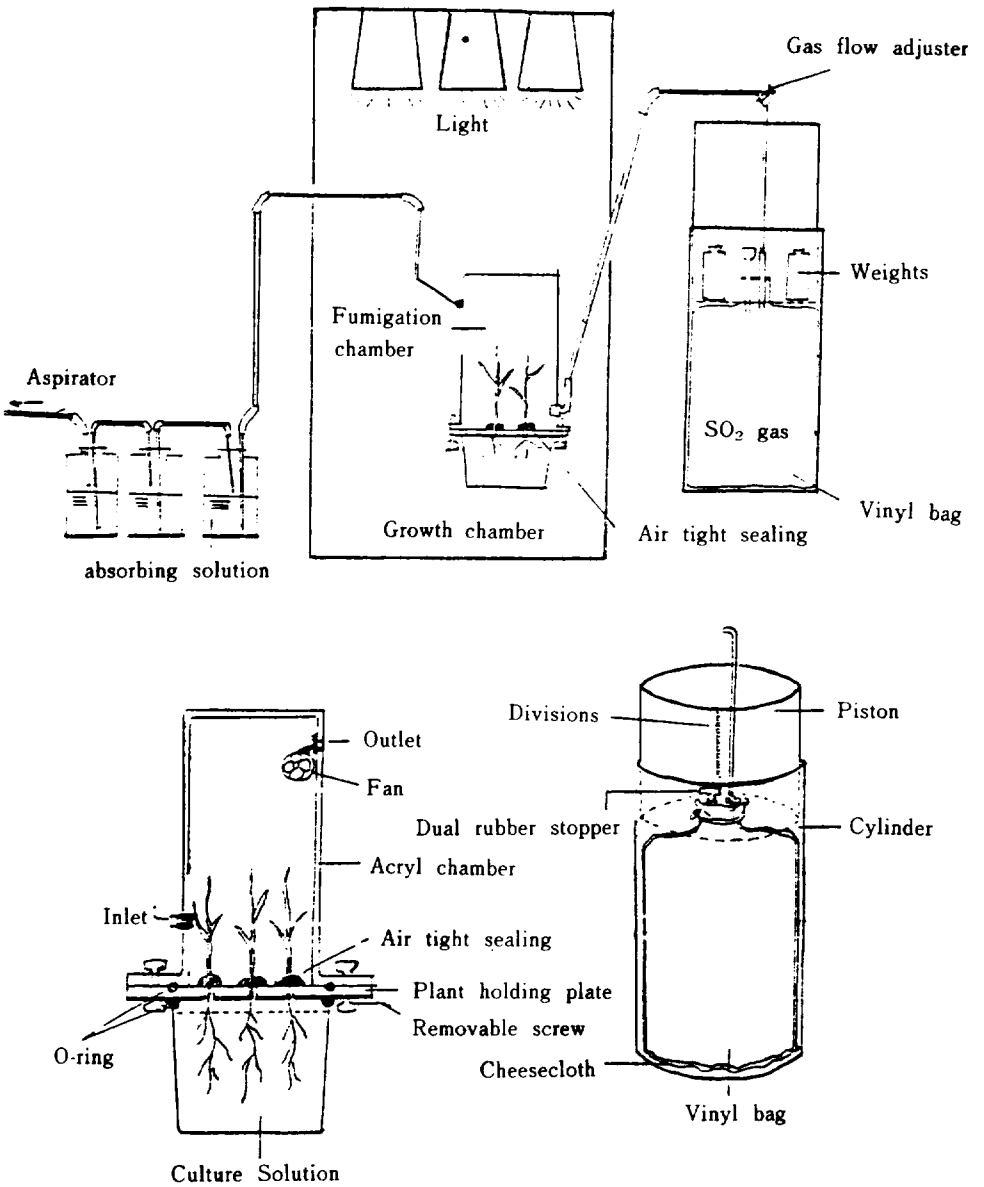

Figure 2. The schematic diagram of SO_2 fumigation system

tion (0, 3, 10ppm) were applied to the plants. The volumes of gas container and fumigation chamber were 60 liters and 10 liters. All the cyclinders were paired with pistons which were freely movable up and down, applying enough pressure on the SO_2 containing vinyl bag when about 2kg of weights were placed on them. The gas pressure obtained by the weights was controlled to get a constant flow rate (about 1ℓ/min.) of SO_2 gas by turning the gas flow adjuster. The SO_2 gas which was not taken by the plants was absorbed in the bottles containing 60ml of the mixed solution of 1% H_2O_2 and 1 N–H_2SO_4. The fumigation chambers were placed inside the growth cabinet which could give 30,000 lux of light intensity and in which room temperature was adjusted from 0℃ to 40℃.

4. Conditions of SO_2 treatment

The SO_2 fumigation was carried out under four different conditions; at 15℃ in the light, at 25℃ in the light, 15℃ in the dark, and at 25℃ in the dark. The light intensity was adjusted to 30,000 lux in the light conditions and there was no light at all in the dark condition. Even for the treatment of 0ppm SO_2, the SO_2-free air flowed through the acryl chamber in which the barley and corn were planted in the same way as the SO_2 treatment.

D. Radioacitvity measurement of the plant samples

1. γ–counting by multi-channel analyser with well-type scintillation detector.

The plant samples labelled with γ-ray emitting elements such as ^{54}Mn, ^{65}Zn, and $^{55+59}Fe$ were put in the vials after measuring dry weights and counted with a well-type scintillation detector Na(Th), type 8SF8/2A–X(Harshaw), combined with a multichannel analyzer MODEL1024 (system BS 27/N of SILENA).

Channel energies of the analyzer were calibrated with the reference sources such as ^{57}Co, ^{60}Co, ^{129}I, ^{133}Ba, and ^{137}Cs. ^{54}Mn and ^{65}Zn, double-labelled to the plant samples at the same time, were counted at the peak of 0.835 Mev(^{54}Mn) and at the peak of 1.15 Mev(^{65}Zn) respectively.

2. Liquid scintillation counting

Radioactivities of all beta emitters were measured with a Berthold's multi-user liquid scintillation counting system, Model LB 5004. The scintillation cocktail devised by Bray (19) was used (table 2). Many references (6, 12, 22, 23, 42, 43, 48, 49, 55, 69, 72, 152) were consulted concerning sample preparation and counting techniques.

Table 2. Composition of Bray's scintillation cocktail

p-dioxane	880 ml	Methanol	100 ml
Ethylene glycol	20 ml	Naphthalene	60 g
PPO	4 g	POPOP	0.02 g

Since the energy level of beta particles from tririum is very low, quenching effect resulted from water content in the counting samples was prevailing. Therefore a quench correction curve was obtained by measuring a series of tritium standard samples provided by Berthold Company. From the quench correction curve the counting efficiency for each sample was determined by the external standard channel ratio method (ESCR). The other elements emitting soft beta were also measured and the counting rates were quench-corrected by the ESCR method.

In case of ^{45}Ca and ^{64}Cu, quench effect caused by acidity was very strong because the plant samples were prepared by acid digestion(^{64}Cu) or dry ashing followed by HCl extraction (^{45}Ca). Especially when the plant samples were extracted with strong acid, much attention had to be paid to get rid of quenching. Neutralization with alkali solution was helpful when the samples contained low salt and were not very acid.

3. Cerenkov counting with liquid scintillation counter

Hard beta sources, ^{32}P and ^{42}K, can be directly counted without adding a scintillation cocktail to the sample solution. This counting method was very useful in saving time and money, but consideration had to be given due to quenching effect when sample solutions were colored or suspended.

Table 3 shows the counting systems and the sample preparation methods for the radioactivity determination of each isotope.

E. Measurement of Chlorophyll content and stomatal aperture influenced by SO_2

1. Measurement of chlorophyll contents

Table 3. Methods of radioactivity counting and sample preparation of the plants labelled with radioactive isotopes.

Isotopes	Counting system	Sample preparation
^{3}H	Liquid scintillation counter (L.S.C)	Distillation
^{14}C	"	Extraction
^{35}S	"	Extraction
^{36}Cl	"	Extraction
^{45}Ca	"	Ashing
^{64}Cu	"	Acid digestion
^{32}P	Cerenkov counting with L.S.C	Ashing
^{42}K	"	Acid digestion
^{54}Mn	γ-spectrometry using NaI (Th) detector	Acid digestion
^{65}Zn	"	Acid digestion
$^{55+59}Fe$	"	Acid digestion

Eight plants each of barley and Corn were fumigated with SO_2 of 0, 3, 10ppm in three fumigation chambers. Contents of chlorophyll a and chlorophyll b were determined by Yoshida's way (157), very similar to Vernon's method (154). The principle of this method is well documented in Stein's handbook of phycological methods (123). Two grams fresh weight of plant samples were cut into pieces, put into a mortar, and crushed thoroughly with a pestle. Acetone was added so that final concentration of acetone became 80%. Enough acetone was added to allow the tissue to be homogenized and the supernatant then decanted thorough a filter paper into a 100ml volumetric flask. The extraction was repeated two or three times more.

2. Stomatal aperture

Just like the observation experiment of chlorophyll change affected by SO_2, the stomatal aperture was observed after SO_2 fumigation. Although various methods of observing stomatal opening had been recommended, Desai (25) suggested the direct visual method and LLoyd's strip method were found to be the best for the study of stomata and the strip method had many advantages over the direct visual method. However it is quite tedious work to peel off the epidermis from the leaves. Therefore a unique method for stomatal observation has been developed throughout this experiment. Bond, a binding material abundant in the market, was diluted with acetone to lessen viscosity, and applied to the slide glass in the form of a thin layer. After two minutes, the plant leaves just out from the fumigation chambers were printed on the slide glass to make direct microscopic observation after they had solidified completely. A micro-meter was used to measure the opening of the stomata aperture of the plant leaves after SO_2

fumigation. More than one hundred stomata were observed for each treatment to calculate the average size of stomatal opening(the longest distance between two guard cells).

F. Chemical composition and growth rate of SO_2 fumigated plants

Barley and corn plants were grown for three weeks with one hour's SO_2 fumigation every two days under different SO_2 concentrations (0, 3, and 10ppm). The plants were grown in the growth cabinet where temperature was adjusted at 20℃ ± 2℃, relative humidity 60–80%, and light intensity 15,000 lux and supplied with Hoagland's culture solution every five days. After harvest, the samples were dried in the oven adjusted at 105℃, weighed, and ground in mill.

One gram of the ground samples was digested with 10ml of the acid solution mixture (H_2SO_4: HNO_3: $HClO_4$=1:5:2) and made up to 50ml volume with distilled water. Phosphorous was determined by the ammonium molybdate method using UV/visible spectrophotometer (Perkin Elmer, Lambda 3). K, Na, Ca, Mg, Fe, Mn, Zn, and Cu were analyzed by atomic absorption spectrophotometry (Perkin Elmer, Model 2380).

Average plant height and weight for 10 plants from three pots per each treatment were measured.

G. Absorption and assimilation of sulfur from SO_2 fumigation

In this experiment, the reaction of SO_2 generation was different from the method used in another experiment; ^{35}S labelled conc. H_2SO_4 and enough copper metal powder were mixed and heated by propane gas burner to produce 3ppm and 10ppm of SO_2 gas in a 60 ℓ vinyl bag. The labelled SO_2 gas was delivered to the fumigation chambers with two levels of SO_2 concentration (3 and 10ppm) for 30 minutes and 60 minutes under four fumigation conditions. Right after the fumigation finished, the fresh weights of samples were taken to follow the procedure Suzuki used (129). Samples were distilled with 25ml of 80% ethanol three times in succession, evaporated to about 3ml in oder to remove alcohol, and then the condensed solution and standard solutions (cysteine and methionine) applied to the TLC plate in which the adsorbent was silica gel with binder. Developer used was a mixed solution of n-butanol: acetic acid: water=4:1:1. After color developing with ninhydrin reagent, the fractions of cysteine and methionine were collected from the TLC plate (5), dissolved in 3ml of alcohol, and added to 9ml of scintillation cocktail to get activity counting. The residues from ethanol extract was boiled with 20ml of H_2O in the water bath for one hour. This filtrate was taken as water soluble and 1ml of it was mixed with 9ml of cocktail for activity counting. The residues from water extract were treated with 4ml of NCS, a tissue solubilizing mixture solution which was provided by the Amersham/Searle Company. After one night 0.1ml to 1ml of this filtrate, according to the degree of color quenching and radioactivity, was added to 9ml of the scintillation cocktail, and counted after cooling for two hours. The procedures of sample preparation mentioned above are summarized and presented schematically in Fig.3.

H. Carbon dioxide assimilation influenced by SO_2 fumigation.

One ml of sodium bicarbonate labelled with ^{14}C (equivalent to 133 uCi) was reacted with 5ml of 1 N–lactic acid in a small vial located inside the fumigation chamber to produce $^{14}CO_2$ gas.

SO_2 fumigation was carried out under the same conditions as other experiments but fumigation

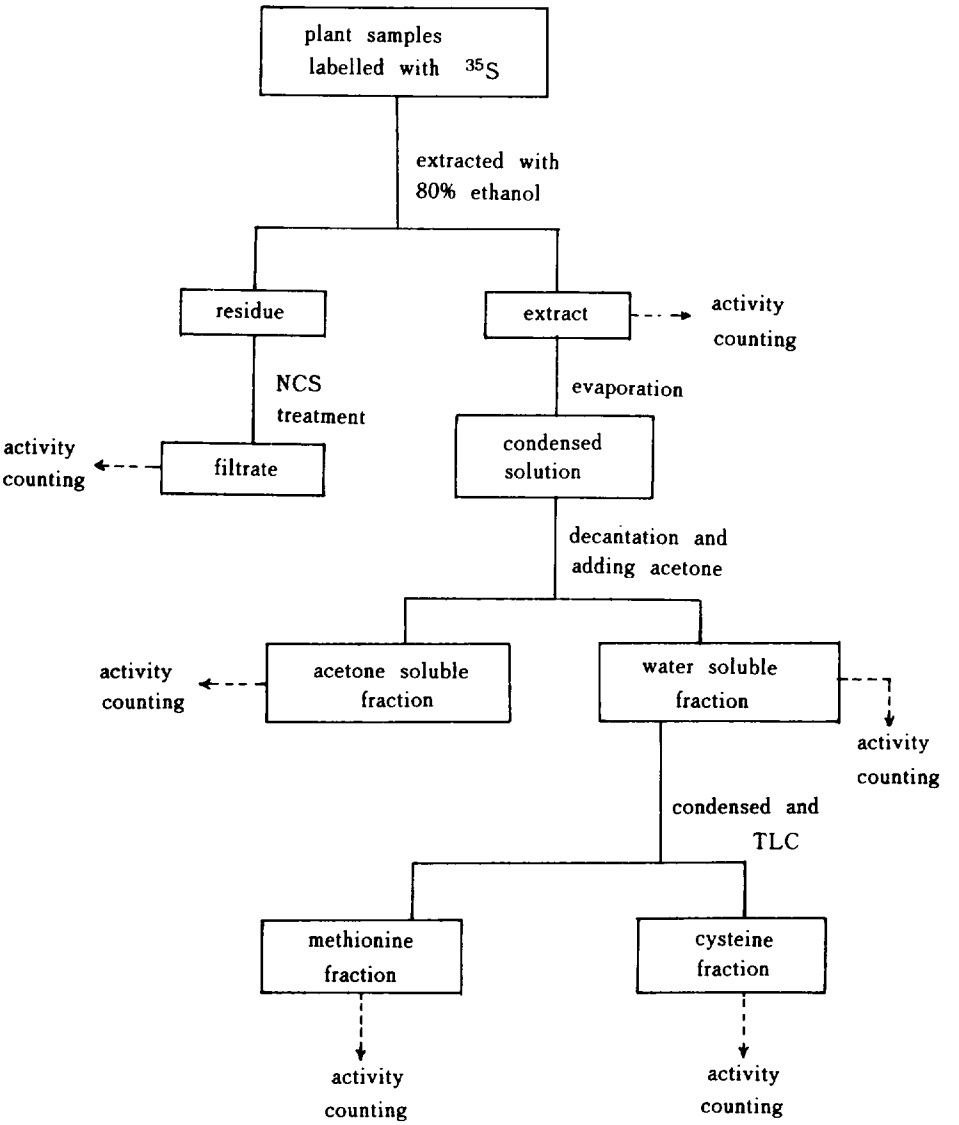

Figure 3. Schematic diagram of the fractionation procedure for the samples labelled with ^{35}S.

time was shortened to 20 minutes from 60 minutes. After 20 minutes of SO_2 fumigation $^{14}CO_2$ gas was generated and treated to the plants immediately for 20 minutes while small fans for air circulation were operated occasionally.

After $^{14}CO_2$ treatments the fractionation of sugars, amino acids and organic acid was followed. The plant samples were weighed and extracted with 25ml of 80% ethanol three times sucessively in 100ml Erlenmeyer flask combined

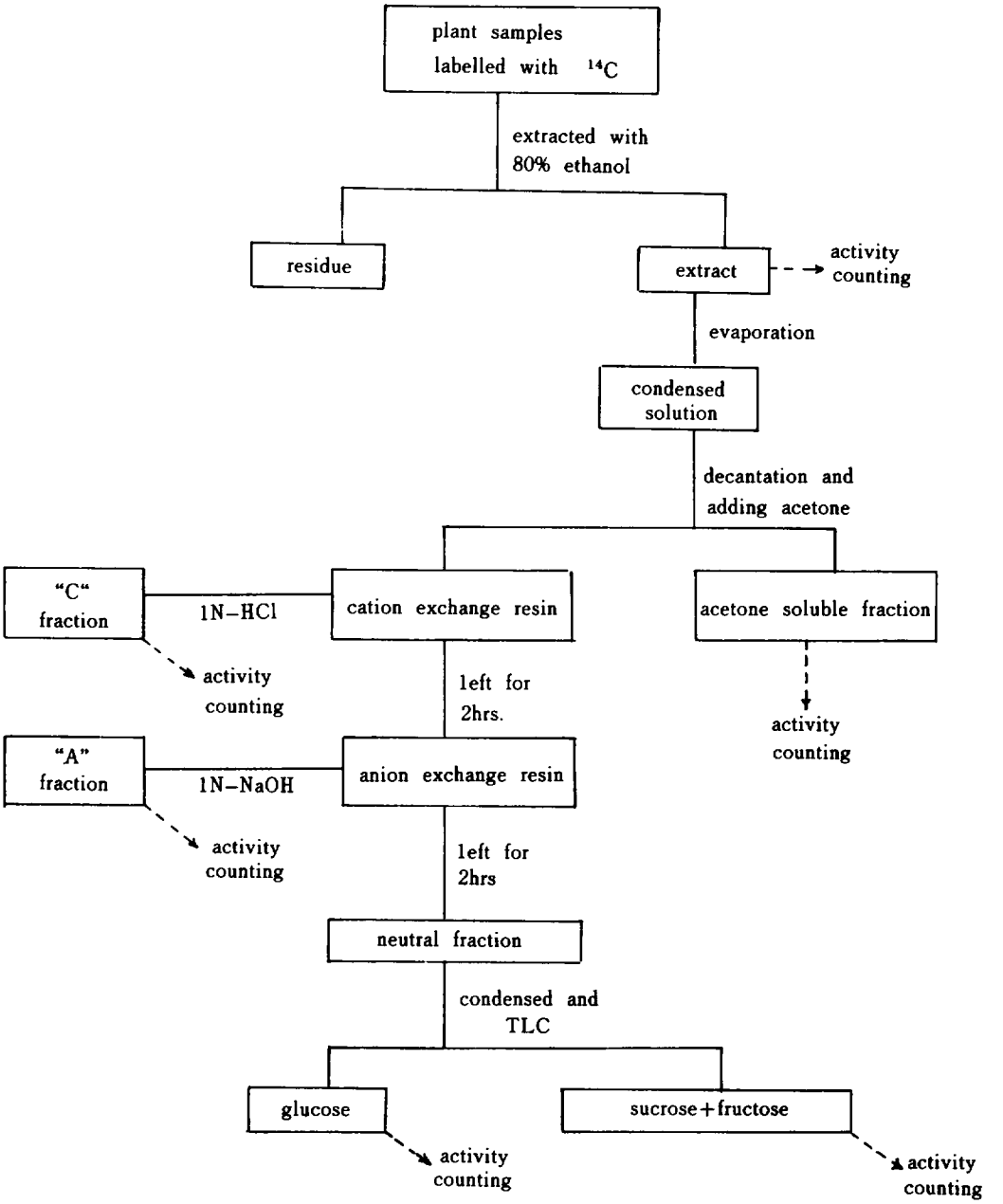

Figure 4. Schematic diagram of the fractionation procedure for the samples labelled with ^{14}C.

with a reflux condenser. The alcohol component was removed from the alcohol extract by rotary evaporator. Then the condensed solution was added to 3g of cation exchange resin (Amberite IR-120) first and left stirring occasionally for 2hrs. Before going to the 3g of anion exchange resin (Dowex A-4) later on. The neutral fraction which was not adsorbed on cation or anion exchange resin was condensed again to make spots on the TLC plate. The pigment fraction which remained on the wall of flask was dissolved with 4ml of acetone, and 0.1ml of the solution was taken for activity counting.

The best separation was obtained with the thin layer chromatograph glass plates prepared by Merck Company when the mixture developer of n-butanol: acetic acid: ethyl ether: water=3:6:8:3 was used. Rf value of glucose was 28 while Rf values of fructose and sucrose were 38 and 39, respectively. The fractions of amino acid (adsorbed by cation exchange resin) and organic acid (adsorbed by anion exchange resin) were taken by treating each 5ml of 1 N-HCl and 1 N-NaOH and counted with LSC. Schematic diagram of the separation procedure is given in Fig. 4.

I. Inorganic ion uptake experiments using radioactive isotopes

All radioactive isotopes used in this experiment were composed of ten elements. Three of them were anions and the rest were cations as presented in table 1.

Half lives, energy distributions, chemical forms, and specific activities are also given in table 1. Each radioactive isotope was labelled to the culture solution ten times diluted from the Hoagland's solution. Radioactive ion uptake by plants was observed after one hour fumigation of SO_2 under the four different conditions.

1. ^{3}H

Barley and corn plants were allowed to absorb water and nutrients in 200ml of the culture solution labelled with $^{3}H_2O$ for one hour under the different SO_2 fumigation conditions. Tritium labelled samples from SO_2 fumigation were washed in running water, weighed, cut into small pieces by scissors, and distilled to extract water from the tissues and the water vapor coming from the flask was condensed using an ice jacket. One ml of the distillate collected in the vials was mixed with 9ml of the scintillation cocktail to get the radioactivity counting.

2. ^{32}P

After SO_2 fumigation and ^{32}P absorption by plants, the samples were ashed in ceramic crucibles at 450℃ for 3hrs, washed with 5ml of HCl (1:1) into the counting vial, made up to 12ml volume with distilled water and counted by Cerenkov counting mode for four minutes.

3. ^{35}S

The stock solution of $H_2{}^{35}SO_4$ (2.2mCi) was added to 2 ℓ of 1/10 strength Hoagland's solution to get 1.1 uCi/ml of spectific activity as shown in Table 1. The plants were allowed to absorb $^{35}SO_4^{2-}$ under different conditions of SO_2 fumigation for one hour. The smples were prepared in the same way as shown in figure 4.

4. ^{36}Cl

After plants absorbed $^{36}Cl^-$ for one hour under different conditions of SO_2 fumigation, they were dried, and shaken in 15ml of 1 N-HNO_3 for 2hrs, and then left for one night. 0.5ml of the extract was taken, mixed with 9ml of cocktail and counted by the external standard channel ratio method.

5. ^{42}K

This nuclide emits very strong beta particles having two maximum energies of 1.97 Mev (18%) and 3.56 Mev(82%). Therefore the counting efficiency by Cerenkov counting method was about 56%. Gamma spectrometry using scintillation detector of NaI (Tl) could only achieve 9% counting efficiency. So all the samples absorbed $^{42}K^{+}$ with SO_2 fumigation were digested with the acid mixture solution in the counting vial and counted for four minutes. All counts obtained from the short half life nuclides such as ^{42}K and ^{64}Cu were corrected for the time lapse caused by the counting order of samples.

6. ^{45}Ca

The dried samples were ashed in a porcelain crucible at a temperature of 550℃ until the ash was nearly white. 10ml of cold distilled water was carefully added after the crucible cooled, followed by the addition of 2ml of 6 N–HCl. A 0.5ml of aliquot was taken into a counting vial containing the scintillation cocktail and counted.

7. $^{55+59}Fe$

Plants were alowed to absorb $^{55+59}Fe$ under different SO_2 fumigation conditons, dried and weighed.

Five ml of an acid mixture (HNO_3: H_2SO_4: $HClO_4$=5 : 1 : 2) was added to the dried plant material in a 25ml of test tube, heated on the hot plate at low heat for several minutes and then increased the temperature until fumes of sulfuric acid evolved. The digested solution was made up to 5ml volume by adding the proper amount of distilled water in order to get a uniform counting-geometry. The test tubes were taken directly to the well type scintillation detector to measure the radioactivity for 5 minutes. Fig.5(a) shows the spectrum of ^{59}Fe.

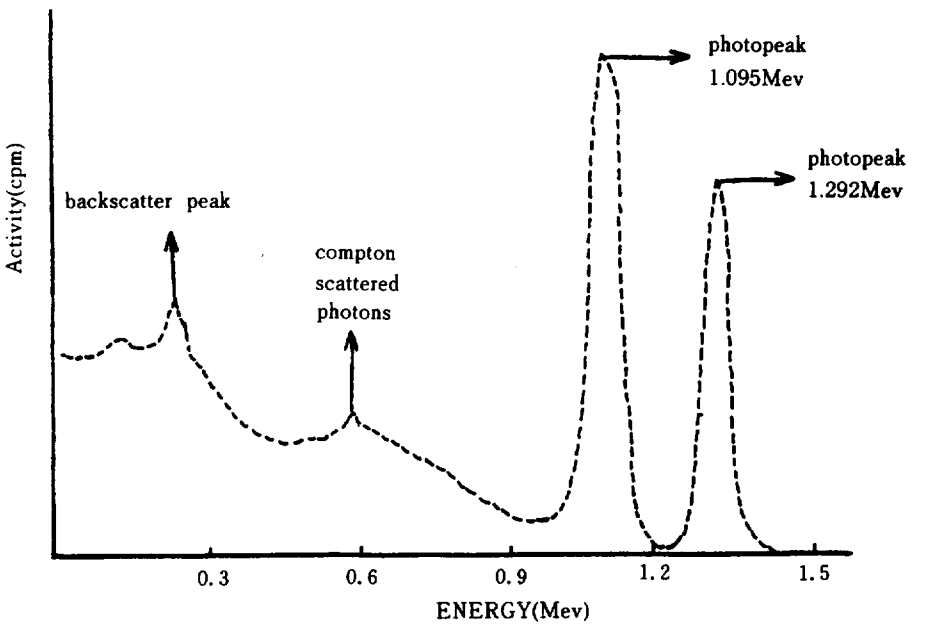

Figure 5(a). Gamma ray spectra for ^{59}Fe

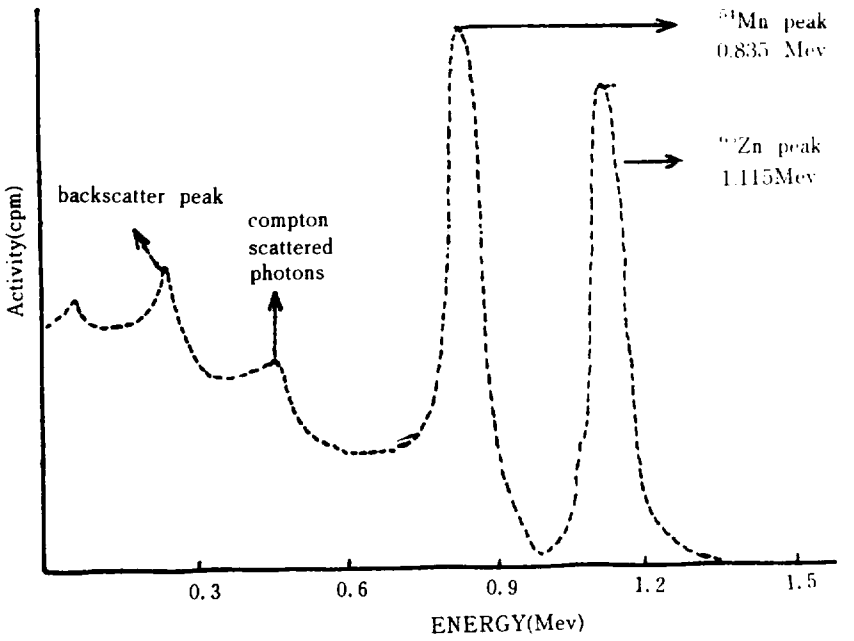

Figure 5(b). Gamma ray spectra for ^{54}Mn and ^{65}Zn

8. ^{64}Cu

After ^{64}Cu absorption by plants with SO_2 fumigation, the acid mixture solution was used to digest the fresh plant samples. There was not enough time to dry the samples because of the short half life time (12.80hr.) of ^{64}Cu. One ml of the diluted solution was, after making up the digested solution to 25ml volume with distilled water, mixed 9ml of cocktail, and counted.

9. ^{54}Mn and ^{65}Zn

As shown in table 1, both of them emit their own specific gamma rays and the energy difference between them is big enough to be resolved by the NaI (Th) detector. A double labelling technique, therefore, was employed in this experiment; ^{54}Mn and ^{65}Zn were labelled to the culture solution altogether and the radio activities of the samples, after the experimental treatment, were measured at the same time. The spectra of ^{54}Mn and ^{65}Zn are given in Fig. 5 (b).

Results and discussion

A. Effect of SO_2 on chlorophyll content and stomatal opening

1. Stomatal actions influenced by SO_2 fumigation

Furukawa and Totsuka (32), and Katz (56) reported that plants exposed to high concentrations of SO_2 gas (1ppm or over), irrespective of whether or not visible injury had occurred, showed a much higher precentage of stomata partly or fully closed than unfumigated check leaves. The results obtained (table 4) seemed to be consistent with Katz's observation; the apertures of stomata narrowed under all fumigation treatments. Average stomatal opening of barley was found to be larger than corn when they were not

Table 4. Stomatal opening as affected by SO_2 fumigation (0, 3, 10ppm) at 15℃ and 25℃ in light and dark conditions.

*	Light		Dark	
	15℃	25℃	15℃	25℃
B0S	6.8	5.7	4.6	4.3
B1S	2.9	2.3	4.2	2.2
B2S	1.7	1.4	2.1	1.0
C0S	5.2	4.1	5.2	3.5
C1S	3.4	3.7	4.9	2.6
C2S	1.3	1.5	4.7	1.6

* Refer to Appendix

fumigated with SO_2 but the stomatal action of barley was much more affected with the SO_2 fumigation than corn, having smaller stomatal aperture. As Willis (156) reported, the stomata of leaves might open in response to light, but close fairly quickly as stress increases. The stomata of barley were observed to be lager under the light condition than under the dark when they were not fumigated but the stomata closed much more under the light condition when fumigated with SO_2. Shimazaki et al. (106) insisted that the guard cell showed high respiratory activity and photosystem Ⅰ and Ⅱ function which might be associated with stomatal opening and consequently respiration. SO_2 fumigation increased respiration rate within 5ppm SO_2 but decreased it at about 10ppm (136), suppressing the photosynthetic O_2 evolution (107).

Table 5 also indicates barley closed the stomata much more than corn during SO_2 fumigation. The reason can be explained provided the fact is accepted that abscisic acid (ABA) plays an important role in stomata closure and corn contains extremely low content of ABA (56).

2. SO_2 effect on chlorophyll content

No visible leaf injury was observed during and after the SO_2 fumigation. The contents of chlorophyll a and b calculated from the sum of absorbances measured at 645nm and 663nm are shown in table 5.

There was no noticeable change of the total chlorphyll content in barley and corn fumigated with SO_2 under the conditions of the present study. Shimazaki (108) pointed out that chlorophyll a and carotenoid began to be destroyed in 2 to 3 hrs. with 2.0ppm SO_2 in the light while chlorphyll b was undamaged after 8hrs. However the result shows that the contents of chlorophyll a and chlorophyll b were not affected by SO_2 fumigation, irrespective of the fumigation conditions such as temperature and light. The reason might be that the fumigation dose rates were not high enough for chlorophyll destruction to take place.

As Shimazaki (110) suggested malondialdehyde (MDA), a product of lipid peroxidation, was formed in SO_2-fumigated leaves. Lipid peroxidation in SO_2-fumigated leaves was due to singlet oxygen produced from O_2. He concluded (109) that the chlorophyll breakdown was mainly due to bleaching. This fact means the chlorphyll destruction is rather an indirect effect of SO_2 than a direct reaction. Malhotra (75) reported that at aqueous concentrations of 100 and 500ppm, SO_2 caused swelling of thylakoid discs and disinte-

Table 5. Chlorophyll content (mg/g fresh weight) in barley and corn fumigated with SO_2 under light and dark conditions at different temperatures.

*	**	Light		Dark	
		15℃	25℃	15℃	25℃
B0S	Ca	0.86	0.91	1.01	1.03
	Cb	0.29	0.28	0.32	0.33
	Tc	1.15	1.19	1.33	1.36
B1S	Ca	0.89	0.97	1.09	1.05
	Cb	0.31	0.33	0.37	0.31
	Tc	1.20	1.30	1.46	1.36
B2S	Ca	0.82	0.90	1.02	1.08
	Cb	0.28	0.30	0.31	0.32
	Tc	1.10	1.20	1.33	1.40
C0S	Ca	1.30	1.31	1.35	1.37
	Cb	0.51	0.35	0.34	0.34
	Tc	1.81	1.66	1.69	1.71
C1S	Ca	1.30	1.34	1.44	1.37
	Cb	0.36	0.38	0.42	0.33
	Tc	1.66	1.72	1.86	1.70
C2S	Ca	1.28	1.32	1.36	1.33
	Cb	0.32	0.38	0.34	0.34
	Tc	1.80	1.70	1.70	1.67

* Refer to Appendix

** Ca: Chlorophyll a, Cb: Chlorophyll b, Tc: total Chlorophylls

grated order intra-chloroplast membranes, resulting in the formation of small vesicles. Although Ricks and Williams (96) suggested that chlorophyll a degradation was higher compared with chlorophyll b the contents of chlorophyll a and b in table 4 were not much changed by SO_2 fumigation.

B. SO_2 effects on the chemical composition and growth rate of plants

1. Dry matter weight and plant height affected by SO_2

Although no visible injury of leaves was observed during and at the end of the growth period, the dry weight and plant height of barley and corn fumigated with 3ppm or 10ppm SO_2 decreased compared to those of the control plants grown under SO_2-free conditions. Kuhn and Faller (65), however, reported that SO_2 fumigation increased shoot growth but decreased root growth. With increasing SO_2 dosage dry matter weight was reported to decrease (50, 151), but there was not noticeable growth reduction between 3ppm treatment and 10ppm treatment (table 6). As Brisley (21) and Katz (53) pointed out, there must be a direct relationship between leaf area destroyed

Table 6. Plant height and dry weight of barley and corn grown for three weeks fumigated with SO_2 for one hour every two days.

		Concentration of SO_2 fumigated					
		0 ppm		3 ppm		10ppm	
		Dry weight (g)	Height (cm)	Dry weight (g)	Height (cm)	Dry weight (g)	Height (cm)
Barley	Shoot	0.7	29.1	0.6	26.5	0.6	27.0
	Root	0.4		0.36		0.35	
	Total	1.1		0.96		0.95	
Corn	Shoot	2.21	34	2.05	32.0	2.0	31.5
	Root	1.81		1.6		1.66	
	Total	4.02		3.65		3.66	

by SO_2 and the resultant plant yield. Also it is suggested (20, 21, 45) that SO_2 fumigation did not reduce the yield unless it produced visible effects. Contradictory to Hill's and Brisley's suggestion (20, 45) but consistent with Matsuoka's (76) the present result showed that dry matter production was reduced by sulfur dioxide fumigation with low concentration for a long term, by which no visible leaf injury could be detected.

2. Inorganic chemical composition of the plants.

Table 7 shows that both crops have different characteristics of inorganic ion uptake pattern which were influenced by SO_2 fumigation. Barley contained a higher amount of phosphorus than corn. Phosphorus content of barley was much reduced with SO_2 fumigation while corn was not. Besides phosphorus, zinc contents of barley were found to decrease with SO_2 fumigation. On the contrary calcium and iron contents of barley seem to increase with SO_2 treatment. A similar tendency was shown in the calcium and iron contents of corn plants.

Matsushima and Harada (80) fumigated two year old satsuma trees with 0 to 5ppm SO_2 for six weeks in November and December and reported that leaves receiving the 5ppm fumigation exhibited a decreased Ca and K content. However when this experiment was repeated in May and June, the Ca content of the leaves was not adversely affected.

Materna (74) enclosed pine limbs in large polyethylene bags which he used as fumigation chambers. Analysis of needles showed that sulfur and calcium increased with age of needles when fumigated vs. control samples. The magnesium also increased in the fumigated tissue, but varied irregularly with the age of the needles. His report indicated that spring fumigation resulted in an increase in potasssium content of the foliage. Another report (78) showed that the changes of inorganic components (N, P, Ca and K) by SO_2 fumigation varied with citrus species; phosphorus content of Citrus Unshu increased with SO_2 fumigation (1 and 5ppm) while Citrus Hassaku decreased. Calcium content in Citrus Unshu, however, decreased with SO_2 and in Citrus Hassaku increased.

As discussed above, the inorganic composition of the plants influenced by SO_2 fumigation was not altered in the same way and dependent on some factors such as fumigation condition and plant species.

Table 7. Mineral composition of barley and corn grown for three weeks fumigated with SO_2 for one hour every two days

*	P %	K %	Ca %	Mg %	Mn ppm	Zn ppm	Cu ppm	Fe ppm
B0S	0.84	4.4	0.21	0.24	122	164	18	80
B1S	0.83	4.0	0.25	0.24	140	160	19	93
B2S	0.81	5.1	0.30	0.24	129	135	18	139
B0R	0.95	3.0	0.20	0.23	900	156	224	450
B1R	0.87	3.2	0.81	0.20	888	171	192	653
B2R	0.79	3.0	0.16	0.23	789	165	121	951
C0S	0.72	3.4	0.37	0.25	119	132	16	189
C1S	0.81	3.5	0.45	0.27	120	146	16	409
C2S	0.74	3.2	0.40	0.24	117	137	11	203
C0R	0.63	2.8	0.30	0.38	647	194	100	508
C1R	0.69	3.2	0.31	0.39	675	209	124	598
C2R	0.68	3.0	0.31	0.43	656	200	108	592

* Refer to Appendix

C. Absorption and assimilation of SO_2

Silvius et al. (118) reported that the labelled sulfur dioxide was found to be extensively absorbed by spinach leaves, indicating that photoreduction of SO_2 had occured. Spedding (122) also observed the uptake of SO_2 by barley leaves at low concentration (365ug SO_2/m^3). According to Olsen (86) healthy cotton plants obtained about 30% of their total sulfur from the atmosphere. An appreciable amount of gas could be absorbed, Thomas and Hill reported (144), without causing any leaf destruction.

In the light, when fumigated with 3ppm SO_2, barley absorbed higher amount of SO_2 than corn. Also Furukawa et al. (35) and Jensen (51) pointed out that plants sensitive to SO_2 absorb a greater amount of SO_2 than the resistant plant species. When fumigated with 10ppm SO_2 in the light, however, corn become superior to barley in the absorption of SO_2 regardless of fumigation time as shown in table 8. The SO_2 absorbed by barley was much greater at both 3 and 10ppm of SO_2 fumigation in the dark than corn. Light accelerated the SO_2 absorption by plants two to three times compared with dark condition. Effectually with 10ppm SO_2 fumigation corn absorbed SO_2 about 12 times more at 15℃ and 17 times more at 25℃ in the light than in the dark as given in figure 6. A close relationship between leaf injury and leaf temperature was noted by Omasa (87). This fact suggests that SO_2 damage to plants can easily occur in the light condition. Tanaka et al. (132) suggested that SO_2 fumigation accumulated H_2O_2, one of active oxygen compounds which are highly reactive to various cell compounds and that the formation of H_2O_2 was dependent on light. As in all biochemical reactions, increasing temperature resulted in higher amount of SO_2 absorption under all conditions. Especially at 10ppm SO_2 and increased of fumiga-

Table 8—1. Distribution of sulfur in barley and corn fumigated with $^{35}SO_2$ under different conditions of temperature (15℃, 25℃) and time(30min, 60min) in the light.

		Fumigation temperatures									
		15℃					25℃				
	*	80 % EtOH soluble (%)	Water soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm × 10^3 g.fr.wt.	80 % EtOH soluble (%)	Water soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm × 10^3 g.fr.wt.
30min	B1S	37.4	8.1	13.8	60.3	64.5	40.4	14.7	10.3	66.0	94.4
	B1R	26.8	6.9	6.0	39.7		16.6	12.0	5.4	34.	
	B2S	44.8	40.4	13.7	99.0	1,951	38.3	48.5	13.1	99.9	16,752
	B2R	0.7	0.2	0.2	1.0		0.05	0.02	0.02	0.09	
	C1S	33.1	13.4	20.3	66.8	53.1	38.4	15.8	9.1	63.4	61.3
	C1R	17.6	10.1	5.5	33.2		20.2	8.4	8.0	36.6	
	C2S	42.6	44.9	11.7	99.2	3,376	44.9	42.3	12.7	99.9	28,159
	C2R	0.4	0.2	0.2	0.7		0.07	0.03	0.02	0.1	
60min	B1S	52.4	22.0	8.5	82.9	101	37.4	32.9	15.5	85.8	123
	B1R	12.0	2.3	2.8	17.1		9.7	2.1	2.3	14.1	
	B2S	46.0	40.9	12.6	99.5	6,752	45.5	36.3	17.9	99.7	21,149
	B2R	0.2	0.09	0.1	0.5		0.1	0.04	0.1	0.3	
	C1S	49.7	29.3	12.7	91.7	76.4	44.7	24.1	16.4	85.2	83.6
	C1R	3.5	1.5	3.3	8.3		6.2	2.5	6.1	14.8	
	C2S	41.8	44.7	13.4	99.9	16,613	51.7	33.9	14.1	99.8	39,852
	C2R	0.03	0.02	0.03	0.07		0.06	0.08	0.08	0.2	

* Refer to Appendix

Table 8—2. Distribution of sulfur in barley and corn fumigated with $^{35}SO_2$ under different conditions of temperature (15℃, 25℃) and time(30min, 60min) in the dark.

		Fumigation temperatures									
		15 ℃					25 ℃				
	*	80% EtOH soluble (%)	Water soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm $\times 10^3$ g.fr.wt.	80% EtOH soluble (%)	Water soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm $\times 10^3$ g.fr.wt.
30min.	B1S	27.0	16.9	11.5	55.3	20.9	37.9	14.6	10.1	62.6	56.9
	B1R	32.3	0.8	11.6	44.7		22.7	5.7	9.0	37.4	
	B2S	48.6	42.4	4.7	95.7	962	58.4	30.3	10.7	99.5	5,256
	B2R	3.7	0.26	0.38	4.3		0.3	0.1	0.1	0.5	
	C1S	29.3	15.0	11.2	55.5	12.6	31.9	11.9	8.7	55.1	32.4
	C1R	38.1	2.0	4.4	44.5		23.7	12.9	10.9	47.5	
	C2S	49.9	30.8	16.4	97.1	344	63.2	27.2	8.5	98.9	1,572
	C2R	1.1	0.05	1.8	2.9		0.6	0.2	0.3	1.1	
60min.	B1S	32.0	15.9	11.3	59.2	30.7	55.2	12.4	8.9	76.6	63.8
	B1R	24.3	5.4	11.1	40.8		15.1	1.8	6.5	23.4	
	B2S	42.1	41.3	15.3	98.6	4,204	67.5	24.1	8.1	99.7	8,674
	B2R	0.3	0.9	0.1	1.4		0.1	0.02	0.1	0.3	
	C1S	33.5	19.5	9.4	62.4	21.9	39.5	13.8	8.2	61.4	47.6
	C1R	13.9	7.1	16.7	37.6		14.5	6.2	17.9	38.6	
	C2S	56.1	40.3	1.2	97.7	1,390	58.1	30.5	10.2	98.8	2,339
	C2R	1.1	0.7	0.6	2.3		0.5	0.3	0.5	1.2	

* Refer to Appendix

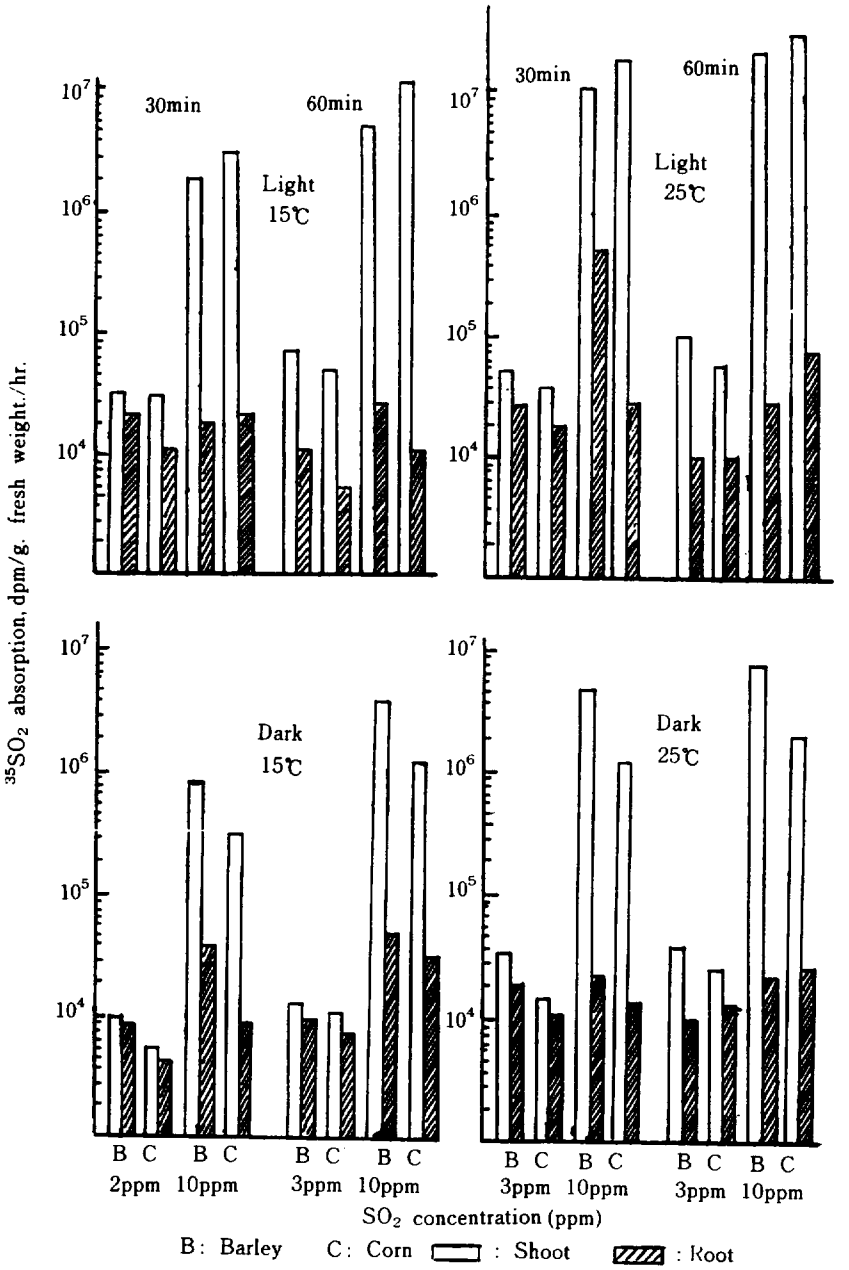

Figure 6. $^{35}SO_2$ absorption and distribution in barley and corn plants fumigated with 3ppm and 10ppm SO_2 for 30 and 60min. and in light and dark conditions.

tion temperature from 15℃ to 25℃ induced a much higher rate of SO_2 absorption by the leaves of barley and corn. Extension of fumigation time from 30 minutes to 60 minutes also increased SO_2 absorption under all the conditions of fumigation.

The translocation rate of sulfur from shoot to root varied with the level of SO_2 fumigation, 3ppm fumigation giving higher rates of sulfur translocation than 10ppm. This fact means that there must be some limiting factors controlling sulfur translocation from shoot to root. This result is well in accord with Roberts' report (97) that the most significant increase in sulfur content was detected in leaf tissue of the white pine seedlings grown under the sulfur dioxide environments. Dark condition increased the sulfur translocation rate compared with light condition as shown in table 8. Matsuoka et al. (77) stated that most of sulfur absorbed by the leaves was accumulated in the state of soluble sulfur compounds. As table 8 indicates the residue fraction, insoluble form, occupied more than 10% of total sulfur in some cases. Water soluble fraction percentage of total SO_2 absorption by plants seemed to vary with the level of SO_2 fumigation; the higher the concentration of SO_2 fumigation, the higher percentage of water soluble fraction. It is found that the water soluble fraction percentage was lower in the dark than in the light with SO_2 fumigation indicating that water soluble sulfate in the leaves was incorporated into the organic compounds in the dark.

Percentage of 80% ethanol soluble fraction at 25℃ of fumigation temperature in the dark was found to be always higher than at 15℃, while percentage of water soluble fraction at 25℃ was lower than that at 15℃ The reason may be that at the higher temperature of fumigation the reaction by which water soluble sulfur compounds can be converted to the ethanol soluble sulfur compound, was more accelerated than at the lower temperature (15℃).

Table 9 shows percentages of methionine and cysteine fraction distributed in the 80% ethanol soluble extract from the plant samples fumigated with $^{35}SO_2$ under the different conditions. Showing a similar tendency of Tisdale's report (148) that higher concentration of sulfate in the nutrition sloution for alfalfa increased cysteine content more than methionine, the plants fumigated with 10ppm SO_2 contained more cysteine fraction than methionine. The reason why enough supply of sulfur, through both the roots and the leaves, brought about a higher ratio of cysteine to methionine can not be explained directly although the pathway of cysteine and methionine synthesis has been well documented (37, 81, 102, 140, 146).

Although the total amount of SO_2 absorption by plants in the dark was lower than in the light, the conversion rates of the absorbed sulfur dioxide to amino acids in the dark were much higher than those in the light. From table 9, it is observed that the incorporation rates of sulfur from SO_2 into the sulfur containing amino acids were much higher at the lower level (3ppm) of SO_2 fumigation than at the higher level (10ppm).

At a fumigation temperature of 25℃ in the light the formation rates of methionine and cysteine were lower than at 15℃. Even in the dark, the formation percentages of methionine at fumigation temperature of 25℃ were lower than at 15℃ although the total methionine produced at 25℃ was much more.

D. Effect of SO_2 on carbon assimilation

It is observed from figure 7 that CO_2-fixation detemined by ^{14}C-trace methodology was reduced under all the different SO_2 fumigation conditions. The reduction of carbon assimilation by SO_2

Table 9. Sulfur distribution in the water soluble fraction from 80% ethanol extraction of the plants fumigated with SO_2 (3 and 10ppm) at different times (30min and 60min) and at different temperatures (15℃ and 25℃) in light and dark conditions.

			Fumigation temperatures					
			15℃			25℃		
			80% EtOH soluble fraction			80% EtOH soluble fraction		
		*	meth. (%)	cyst. (%)	tot. act. dpm $\times 10^3$ g.f.w.	meth. (%)	cyst. (%)	tot. act. dpm $\times 10^3$ g.f.w.
LIGHT	30min	B1S	4.0	21.9	23.9	0.6	14.7	37.8
		B2S	1.4	12.3	874	1.1	9.5	6,416
		C1S	2.8	12.2	17.5	2.3	10.6	23.4
		C2S	0.8	9.5	1,440	0.7	8.4	12,643
	60 min	B1S	1.6	15.6	52.9	1.8	8.0	46.0
		B2S	0.3	9.1	3,105	0.3	3.8	9,622
		C1S	6.2	17.7	37.7	2.3	12.7	35.5
		C2S	0.4	5.9	6,944	0.3	4.4	20,612
DARK	30 min	B1S	15.8	84.0	5.64	14.1	76.4	21.6
		B2S	1.9	50.9	467	1.5	62.9	3,069
		C1S	20.5	79.2	3.51	12.9	66.8	10.2
		C2S	2.4	28.8	171	0.8	34.1	993
	60 min	B1S	14.2	81.3	9.92	7.5	82.8	35.1
		B2S	1.6	51.5	1,769	0.6	35.2	5,856
		C1S	13.6	70.6	7.37	6.0	72.3	19.7
		C2S	0.45	21.6	779	0.7	29.1	1,358

* Refer to Appendix

fumigation was dependent on environmental factors such as temperature, light, and SO_2 concentration. The apparent photosynthesis of barley was much more reduced by SO_2 than that of corn. This result can explain the reason why barley was classified as a SO_2-sensitive plant. The translocation percentage of carbon compounds from shoot to root seemed to be very high in the dark compared with the light condition. Corn, a C_4-plant (58),showed a higher capacity of photosynthesis, measured as total radioactivity of ^{14}C fixed per fresh weight than barley, a C_3 plant. As shown in table 10, glucose and fructose+sucrose produced in barley were found to increase with SO_2 fumigation. C fraction, which was absorbed by cation exchange resin, seemed to increase with SO_2-fumigation. In general C fraction contains mainly amino acids. Sulfur nutrition was suggested to have a close relation with carbon metabolism. Sulfur deficiency reduced the sucrose concentration in the leaves (29) but increased carbohydrate (67).

Although photosynthesis is the principal mode of carbon dioxide fixation by green plants, plants also absorb CO_2 in the dark. Price (91) reviewed a hypothesis that there were two carboxylations; ribulose diphosphate carboxylase as in the Calvin cycle of photosynthesis, and PEP carboxykinase. The hypothesis might account for the carbon

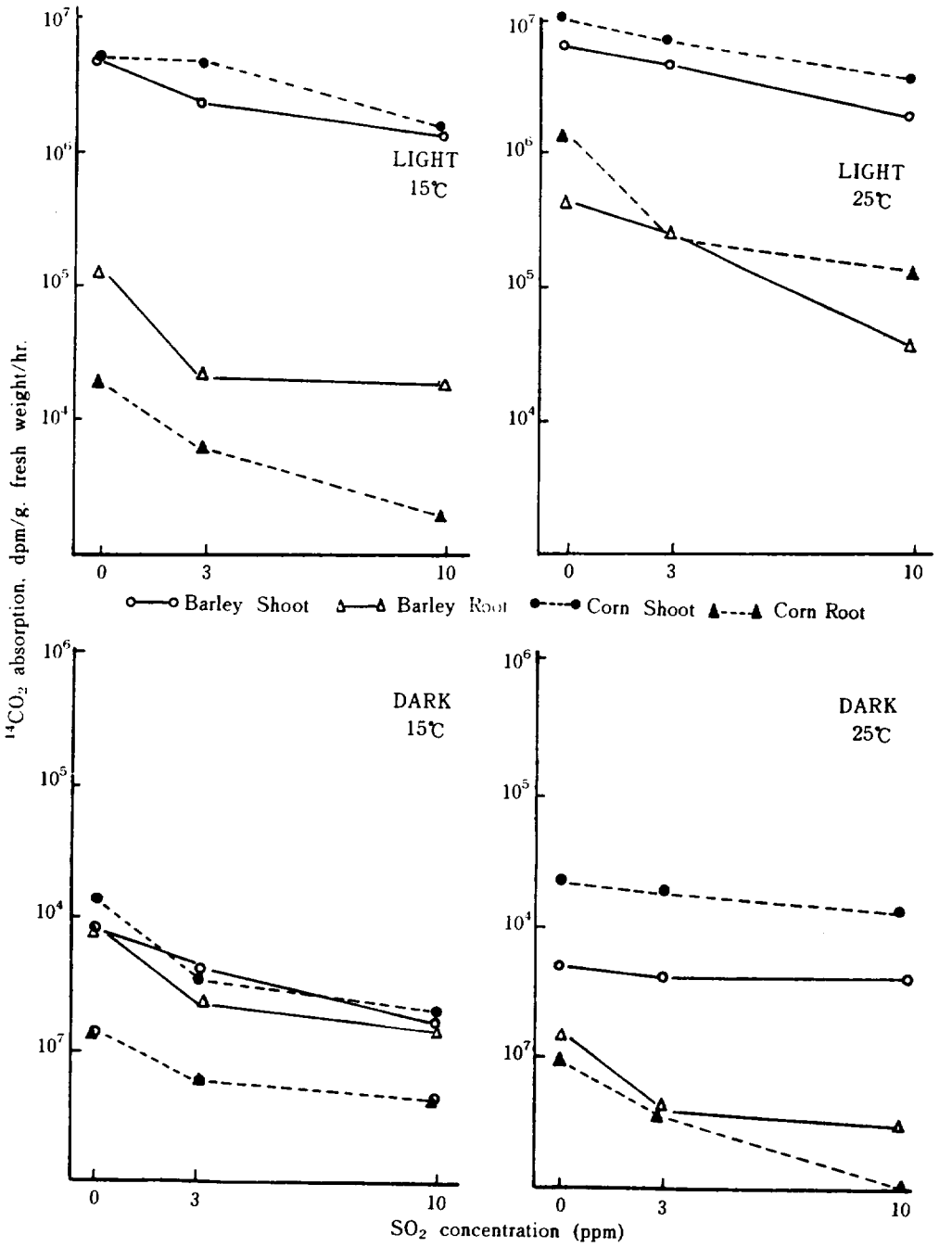

Figure 7. $^{14}CO_2$ absorption by barley and corn plants fumigated with 3ppm and 10ppm SO_2 at 15℃ or 25℃ in light and dark conditions.

Table 10. Distribution of ^{14}C—compounds assimilated in the plants fumigated with SO_2 at 15℃ and 25℃ in the light.

	*	Residue (%)	Pigment (%)	A (%)	C (%)	N (%)	G (%)	F+S (%)	Total act. dpm × 10^3
	B0S	0.03	39.1	10.2	27.7	22.7	8.9	2.3	5,250
	B1S	0.1	21.5	5.7	34.9	36.4	13.2	2.8	2,414
	B2S	0.06	7.8	13.5	49.5	28.3	19.2	5.9	537
	B0R	10.9	26.4	22.0	16.0	24.6			151
	B1R	5.1	45.5	14.1	21.3	13.7			15.7
	B2R	4.9	34.4	12.4	35.2	13.0			10.3
15℃	C0S	0.15	20.4	6.2	12.0	60.9	41.	5.5	5,486
	C1S	0.06	16.7	9.0	22.1	52.2	40.2	4.8	5,124
	C2S	0.08	9.0	12.1	32.0	46.7	38.6	7.4	1,708
	C0R	10.9	15.1	14.5	8.5	49.1			21.0
	C1R	8.8	24.4	16.9	17.5	31.8			5.2
	C2R	11.5	26.9	13.5	23.1	24.3			1.4
	B0S	0.04	22.5	21.7	9.8	45.9	0.3	0.4	6,664
	B1S	0.05	41.7	3.3	22.9	31.7	5.1	5.6	4,951
	B2S	0.06	32.2	36.4	13.0	18.2	4.8	3.2	591
	B0R	10.3	30.9	28.9	9.6	20.2			487
	B1R	9.9	37.1	22.5	10.7	18.1			272
	B2R	2.8	23.	17.7	12.7	44.5			34.9
25℃	C0S	0.06	61.8	6.5	5.0	26.5	18.5	4.0	11,525
	C1S	0.06	27.8	9.4	6.3	56.4	41.0	7.3	10,187
	C2S	0.06	11.0	16.2	14.6	58.0	23.0	16.0	3,723
	C0R	8.3	9.6	20.6	7.5	53.9			143
	C1R	4.6	28.0	11.8	5.7	49.7			166
	C2R	9.8	32.8	14.5	5.4	37.1			136

Residue: 80% ethanol insoluble but $HClO_4$ soluble fraction
Pigment: acetone soluble fraction in 80% ethanol extract
A: fraction asorbed by anion exchange resin
C: fraction adsorbed by cation exchange resin
N: neutral fraction
G: glucose
F+S: fructose+sucrose } a part of neutral fraction
* Refer to Appendix

assimilation in the dark shown in this experiment.

E. SO_2 effects on inorganic ion and water uptake by plants

1. Water uptake as ^3H_2O

Water uptake of barley was much higher than that of corn and more retarded by SO_2 fumigation. Light and higher temperature(25℃) increased the water absorption as given in the figure 8. The translocation of water from root to shoot was accelerated at 25℃ especially in the light; 50% of water absorbed through the root was translocated to the shoot. In the dark the fumigation of 10ppm SO_2 seemed to increase water uptake by plants as compared with 3ppm SO_2 treatment. Water uptake and translocation was more reduced than any other ions, suggesting the reduction of transpiration by SO_2. Measurement of transpiration from this experiment was tried but was not successful probably due to too short fumigation time. However Furukawa et al. (36) stated that fumigation with 2.0ppm SO_2 induced the rapid decline of transpiration rate of rice and tomato.

2. Phosphate uptake as $^{32}PO_4^{3-}$

Phosphate uptake by barley was more decreased by SO_2 treatment than by corn. The reduction of phosphate absorption was more noticeable in the light than in the dark; corn was found to be stimulated by SO_2 and to absorb more phosphate than corn (figure 9) although the mechanism is not well understood at present.

3. Sulfate uptake as $^{35}SO_4^{2-}$

Sulfate absorption by plant roots was not much influenced by SO_2 fumigation but the translocation of sulfate from root to shoot was reduced in the light (figure 10). Corn was very insensitive to SO_2 fumigation in the dark, showing no change in sulfate absorption and translocation. Sulfate absorption of barley roots, calculated as disintegration per minute (dpm) per fresh weight (97) for one hour, was higher than that of corn roots. As table 11 indicates, the sulfate transport from root to shoot decreased with SO_2 fumigation in the light. Ethanol soluble fractions in the shoots of barley and corn were reduced by SO_2 in the light also. Residue fraction which was not dissolved in the water and the 80% ethanol, decreased in both the roots and the shoots by fumigating with SO_2. Sulfate absorption seemed to be light-dependent but not very temperature-dependent. Barley contained a higher amount of 80% ethanol soluble fraction than corn while corn had more water soluble fraction than barley. Table 12 shows that the conversion rates of sulfate absorbed by the roots into methionine and cysteine increased with SO_2 fumigation. The percentages of cysteine were higher than those of methionine under all the experimental conditions. The formation rates of methionine and cysteine were found to be much higher in the corn plants than in the barley.

4. Chlorine uptake as $^{36}Cl^-$

As distinct from other ions described above, corn seemed to demand much more chloride than barley (figure 11). Light induced the increased amount of chloride absorption by the roots of barley and corn, and facilitated the chloride translocation. The temperature increasing from 15℃ to 25℃ had also the same effects as the light did. SO_2 fumigation influenced the plants to reduce their chloride uptake and translocation in the light and dark.

5. Potassium uptake as $^{42}K^+$

Potassium was found to be another favorite nutrient for corn; potassium uptake by corn being higher than by barley as shown in figure 12. No

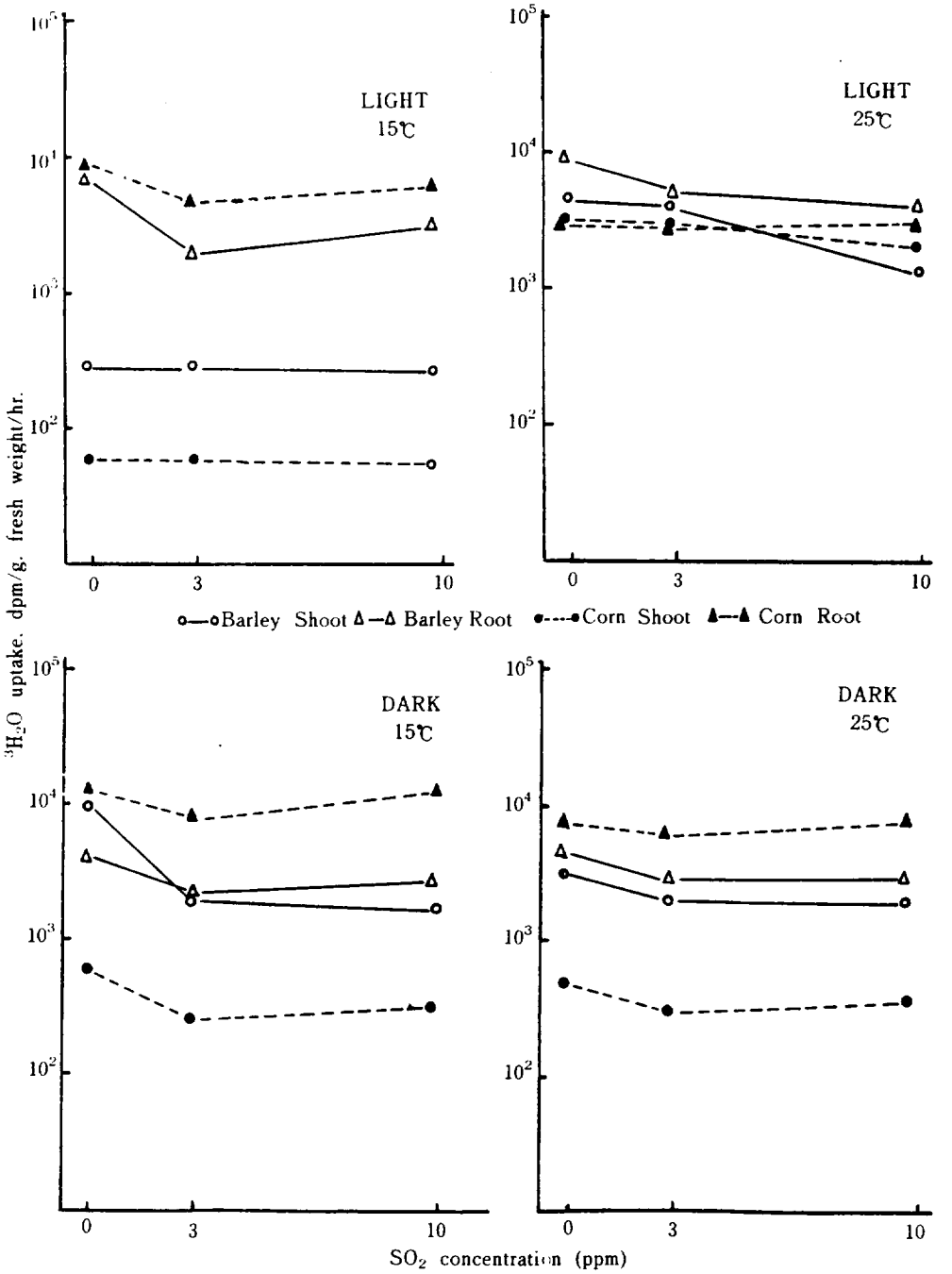

Figure 8. ^3H_2O uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ in light and dark conditions.

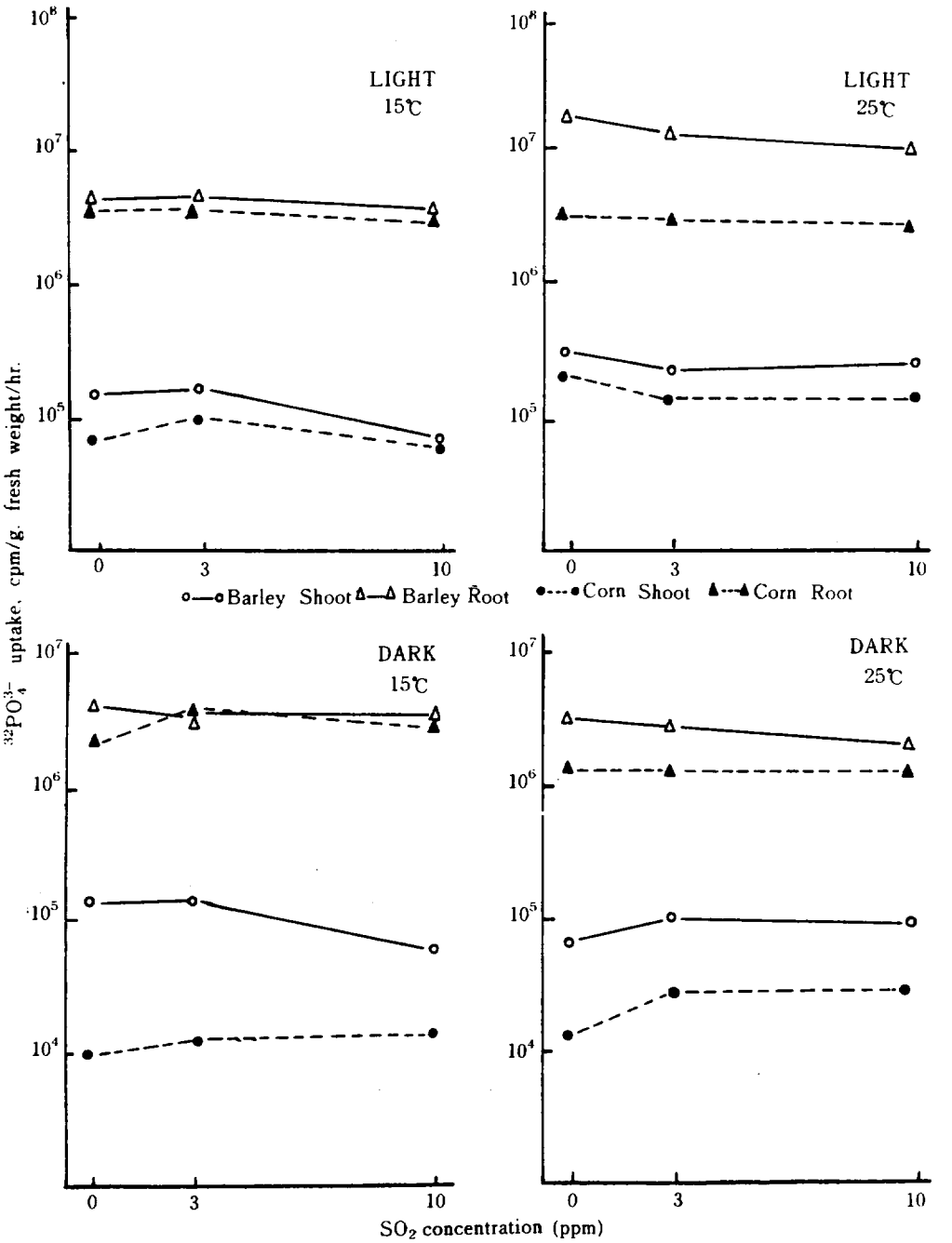

Figure 9. $^{32}PO_4^{3-}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

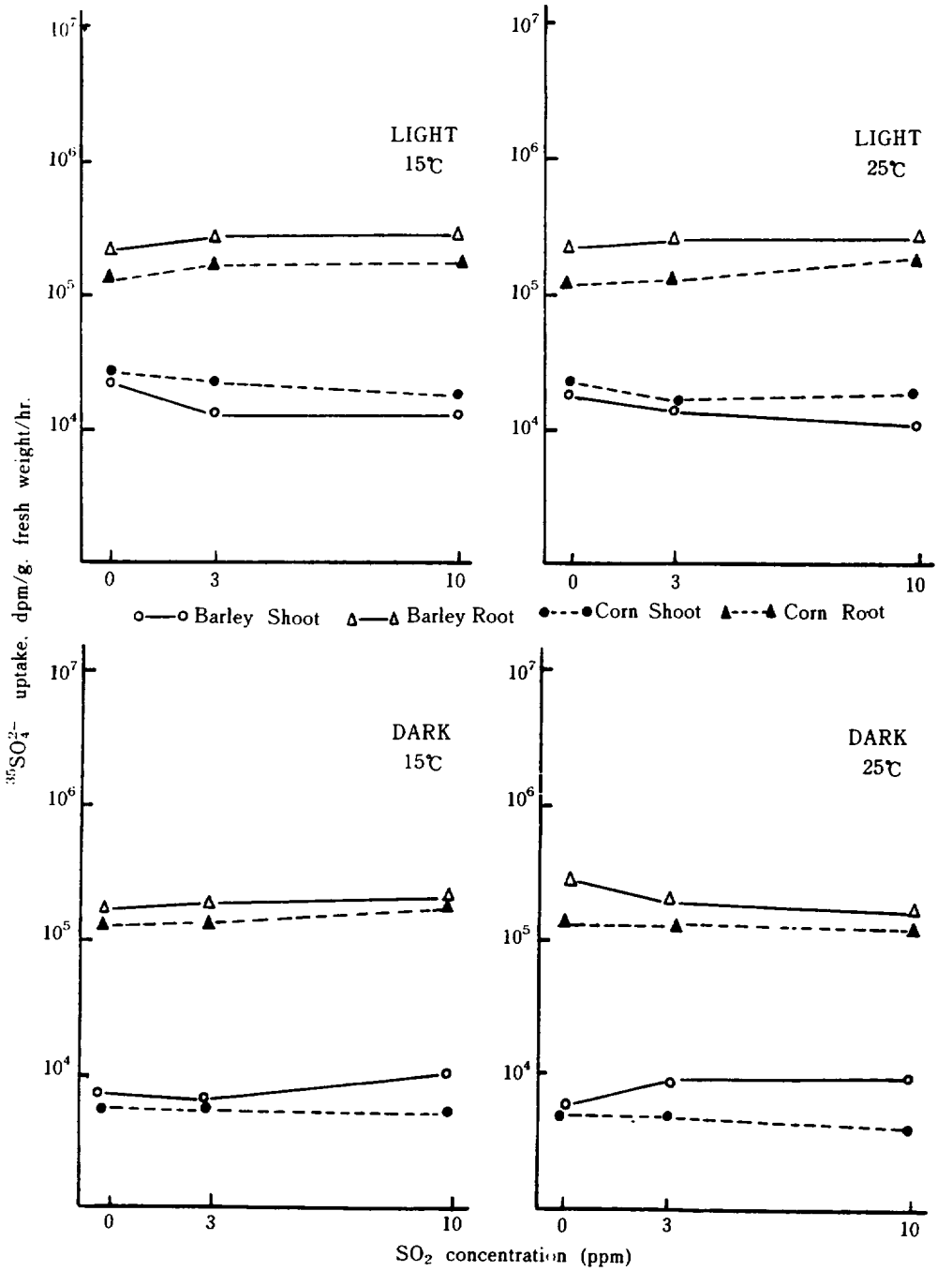

Figure 10. $^{32}SO_4^{2-}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

Table 11. Distribution of sulfur absorbed through the roots as $^{35}SO_4^{2-}$ in barley and corn fumigated with SO_2

		Fumigation temperature									
		15 ℃					25 ℃				
	*	80% EtOH soluble (%)	Water Soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm × 10^3 g.fr.wt.hr.	80% EtOH Soluble (%)	Water soluble (%)	Residue (%)	Shoot or root (%)	Tot. act. absorbed dpm × 10^3 g.fr.wt.hr.
LIGHT	B0S	3.5	3.8	1.9	9.2	283	3.9	2.5	0.7	7.2	284
	B0R	63.9	9.3	17.4	90.7		76.4	6.7	9.8	92.8	
	B1S	2.5	1.6	0.8	5.0	317	3.4	1.9	0.6	5.9	283
	B1R	78.4	3.2	13.4	95.0		77.3	6.4	10.3	94.1	
	B2S	2.0	1.9	0.9	4.8	338	2.6	1.0	0.4	4.0	299
	B2R	77.7	5.1	12.4	95.2		81.7	6.1	8.2	96.0	
	C0S	5.1	6.8	2.5	14.4	197	6.3	6.5	2.3	15.0	202
	C0R	36.8	35.0	13.8	85.6		47.1	28.8	9.1	85.0	
	C1S	4.3	5.3	2.3	11.9	220	4.3	4.8	1.7	10.8	205
	C1R	42.9	34.6	10.1	87.7		55.0	25.7	8.5	89.2	
	C2S	3.5	4.4	1.7	9.5	218	4.5	4.1	1.4	10.0	229
	C2R	34.1	47.1	9.2	90.4		55.2	28.0	6.7	89.9	
DARK	B0S	2.2	1.2	0.4	3.9	187	0.9	0.9	0.2	2.0	268
	B0R	75.4	11.3	9.3	96.1		80.9	7.8	9.3	98.0	
	B1S	0.7	0.7	0.4	1.8	215	2.6	1.3	0.5	4.4	235
	B1R	77.6	12.0	8.7	98.2		76.6	10.2	8.8	95.6	
	B2S	2.1	1.5	0.4	4.0	257	2.6	1.7	0.5	4.8	202
	B2R	80.2	8.7	7.1	96.0		78.6	7.8	8.7	95.2	
	C0S	1.5	1.6	0.5	3.6	174	1.4	1.4	0.5	3.4	170
	C0R	49.4	36.5	10.5	96.3		41.6	46.0	9.0	96.6	
	C1S	1.3	1.8	0.8	3.8	159	1.2	1.4	0.7	3.3	173
	C1R	34.5	51.9	9.6	96.1		43.1	44.5	9.1	96.7	
	C2S	1.1	1.3	0.4	2.8	217	1.2	1.2	0.6	2.9	157
	C2R	41.1	47.0	9.0	97.2		40.0	48.9	8.2	97.1	

* Rafer to Appendix

Table 12. Conversion of $^{35}SO_4^{2-}$ into methionine and cysteine in barley and corn plants fumigated with SO_2 under different conditions.

		Fumigation temperatures					
		15 ℃			25 ℃		
	*	80% EtOH Soluble fraction			80% EtOH Soluble fraction		
		meth. (%)	cyst. (%)	tot. act. $\frac{dpm \times 10^3}{g.fr.wt.}$	meth. (%)	cyst. (%)	tot. act. $\frac{dpm \times 10^3}{g.fr.wt.}$
	B0S	4.2	6.1	10.2	5.8	8 6	11.08
	B1S	4.4	8.9	7.93	6.9	12.5	9.72
	B2S	12.5	13.2	6.91	6.7	9.1	7.88
	B0R	0.13	1.1	181	0.18	0.75	217
	B1R	0.36	1.6	261	0.32	0.8	219
	B2R	0.31	2.6	263	0.23	0.83	244
LIGHT							
	C0S	5.3	13.6	9.95	5.6	8.6	12.7
	C1S	5.6	13.9	9.56	6.4	12.4	8.81
	C2S	8.5	18.2	7.70	8.4	10.6	10.3
	C0R	0.80	3.3	72.5	0.46	2.3	95.1
	C1R	0.86	3.9	94.4	0.57	2.4	113
	C2R	0.92	4.0	94.4	0.30	2.1	126
	B0S	6.1	6.9	4.16	5.2	6.1	5.86
	B1S	7.1	8.2	3.54	6.1	6.1	6.17
	B2S	6.9	9.1	3.01	6.9	6.3	5.33
	B0R	0.11	0.23	141	0.10	0.30	161
	B1R	0.14	0.25	167	0.13	0.40	180
	B2R	0.17	0.28	206	0.95	0.51	159
DARK							
	C0S	3.8	16.4	2.96	11.5	16.7	2.47
	C1S	5.6	21.7	2.41	13.6	16.7	2.09
	C2S	13.7	28.0	2.32	29.5	33.4	1.82
	C0R	0.29	1.03	86.0	0.40	2.03	70.9
	C1R	0.51	2.0	74.9	0.46	2.18	74.5
	C2R	1.1	2.4	89.2	0.59	2.7	58.1

* Refer to Appendix

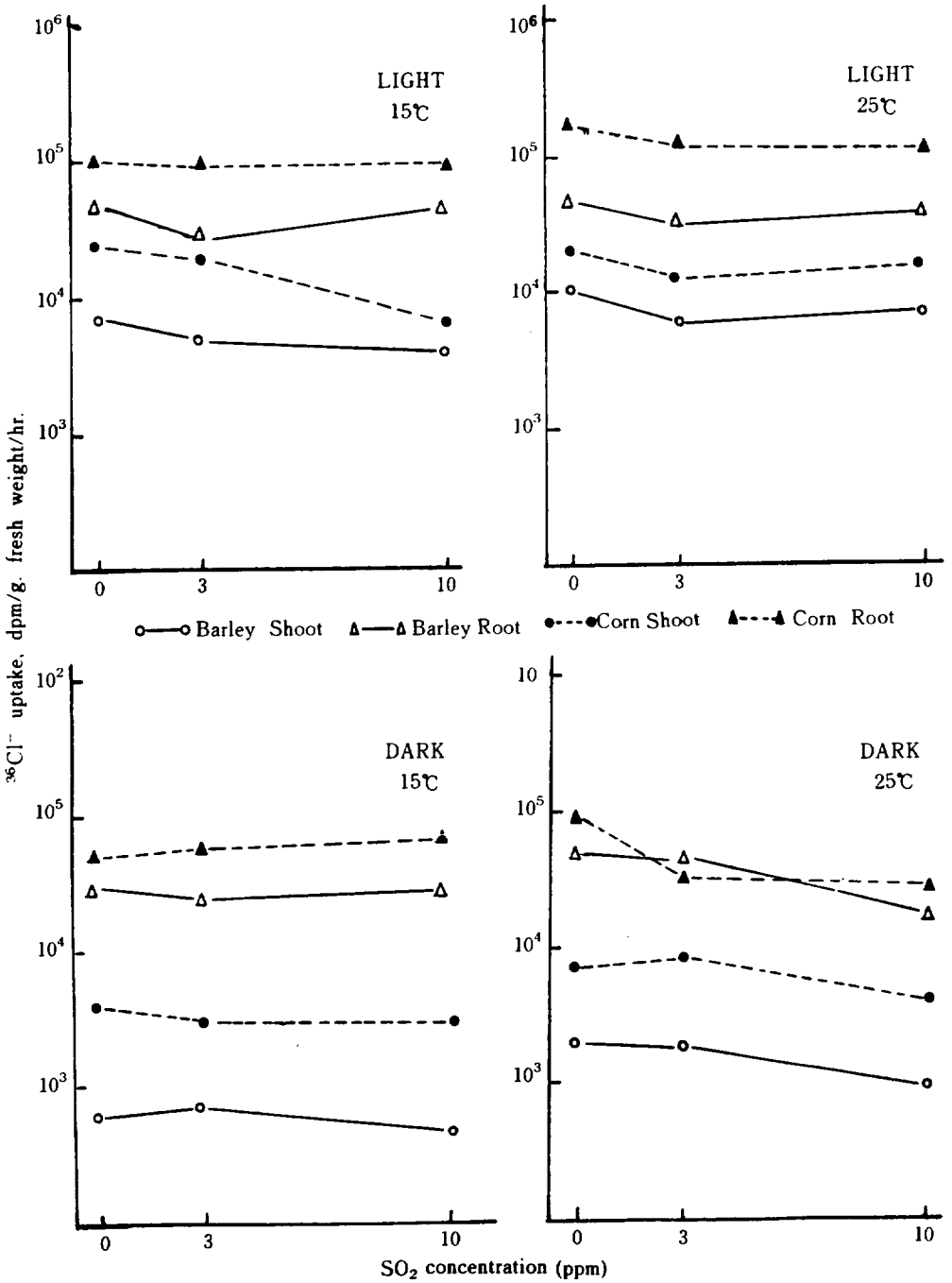

Figure 11. $^{36}Cl^-$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

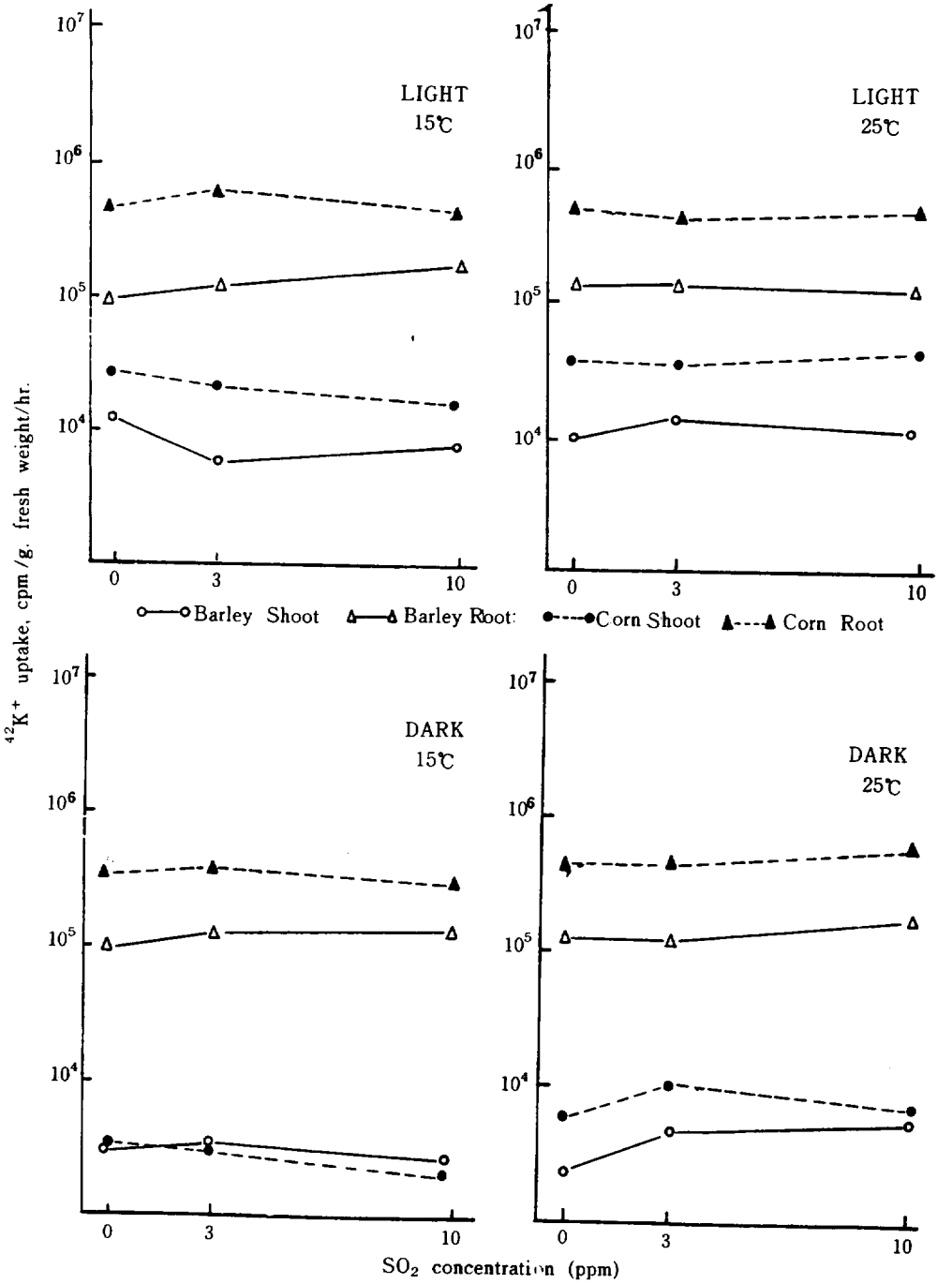

Figure 12. $^{42}K^+$uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

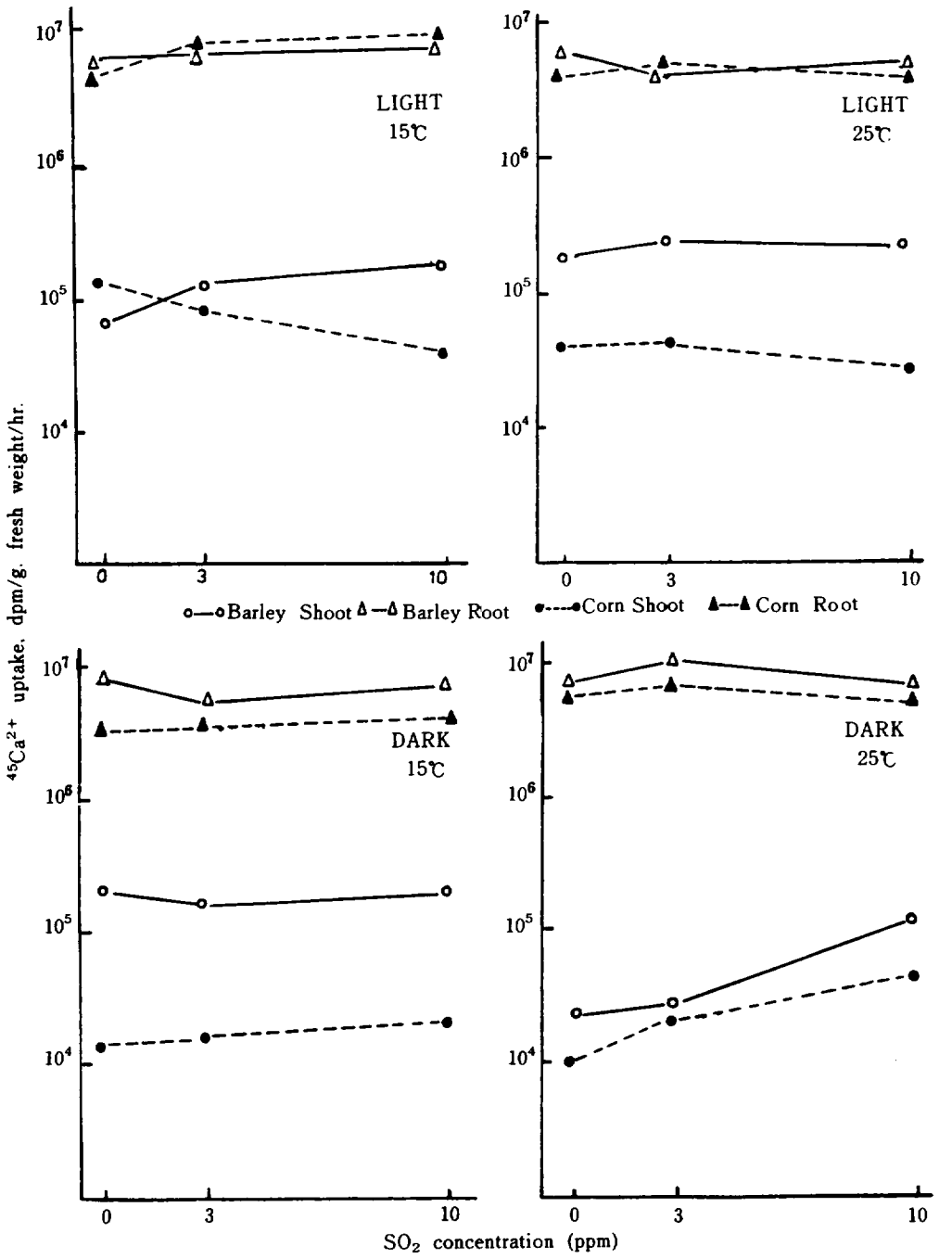

Figure 13. $^{45}Ca^{2+}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

effect of SO_2 fumigation on K^+ absorption by plant roots was observed irrespective of temperature and light conditions. Potassium uptake by plant roots was not greatly influenced by the light but light considerably increased the potassium translocation. This is, however, hardly correlated with the fact (30) that the stomatal aperture under various conditions was much involved in K^+ uptake.

6. Calcium uptake as $^{45}Ca^{2+}$

Calcium absorption and translocation of barley were noticeably increased with SO_2 fumigation in the light and dark regardless of temperature conditions (figure 13). Corn showed an increased calcium absorption by roots with a higher translocation of calcium absorption by roots with a higher translocation of calcium in the dark. Barley, in general, was found to have a higher ability to absorb calcium than corn. The increase of calcium uptake with SO_2 fumigation might be correlated with the fact that cations exist in the cell sap, giving a buffering capacity to the SO_2 fumigated plants.

7. Manganese uptake as $^{54}Mn^{2+}$

There was a tendency that manganese uptake and translocation decreased with SO_2 fumigation (figure 14). Barley absorbed more manganese in all conditions with the exception of 25℃ and dark condition than corn did. Light and higher temperature brought about an increased uptake of manganese.

8. Iron uptake as $^{55+59}Fe^{2+}$

Both barley and corn, as figure 15 indicates, were found to absorb more iron with SO_2 fumigation in all the fumigation conditions except the treatment of SO_2 at 25℃ in the dark.

Average increasing rates of iron absorption by the roots of barley and corn plants fumigated with SO_2 were over 200 percent and average increasing rates of iron translocation to shoot were about 130 percent compared with the control plants which did not receive SO_2. This fact is very interesting although the mechanism by which the SO_2 fumigation stimulated iron absorption and translocation in plants is not fully understood at present. Iron probably might be associated with some biochemical reactions which can be stimulated by SO_2 fumigation. It appeared that light helped the plants to absorb much more iron while temperature rising did not.

9. Copper uptake as $^{64}Cu^{2+}$

Arnon (7) reported that a copper enzyme was localized in the chloroplast of spinach beet and capable of participating in oxidation-reduction reactions. As shown in the figure 16, SO_2 fumigation retarded the copper translocation of barley and corn from root to shoot in the light or in the dark. The copper absoption by barley was reduced more with SO_2 treatment than by corn. In the light the roots of barley and corn could take up much more copper than in the dark.

Barley seemed to have a stronger ability to absorb $^{64}Cu^{2+}$ than corn. The rates of copper transport from root were found to be very low compared with another cations such as Ca^{2+} and K^+

10. Zinc uptake as $^{65}Zn^{2+}$

The amounts of Zn translocated to the barley shoot were higher than that of corn. Under the light condition, Zn absorption by plant roots was not affected but the Zn transport from root to shoot was increased. Zn transport of corn increased by raising the temperature from 15℃ to 25℃. SO_2 fumigation gave the plants a negative effect on Zn absorption and transport (fig. 17).

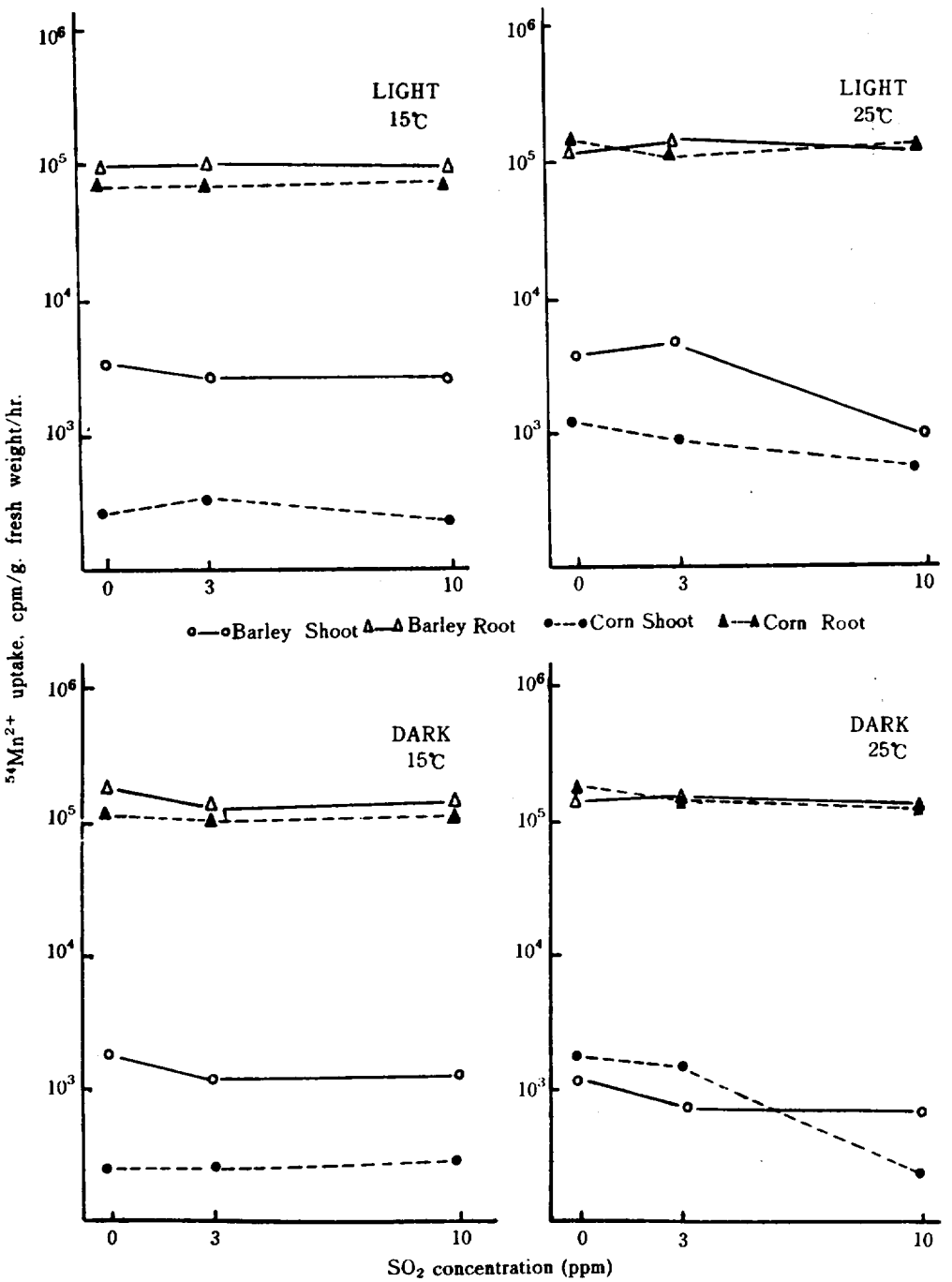

Figure 14. $^{54}Mn^{2+}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions

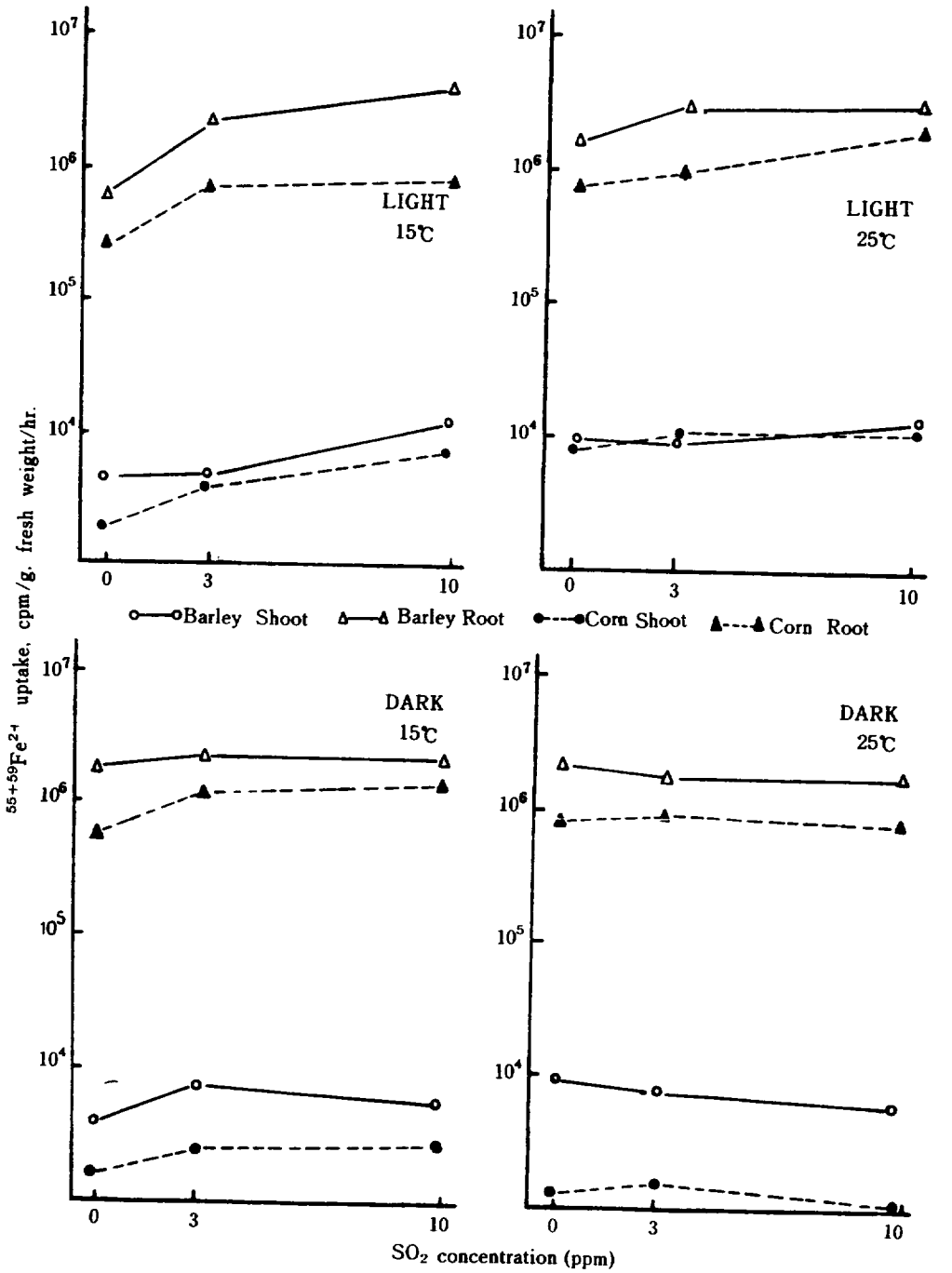

Figure 15. $^{55+59}Fe^{2+}$ **uptake by barley and corn plants influenced by** SO_2 **fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.**

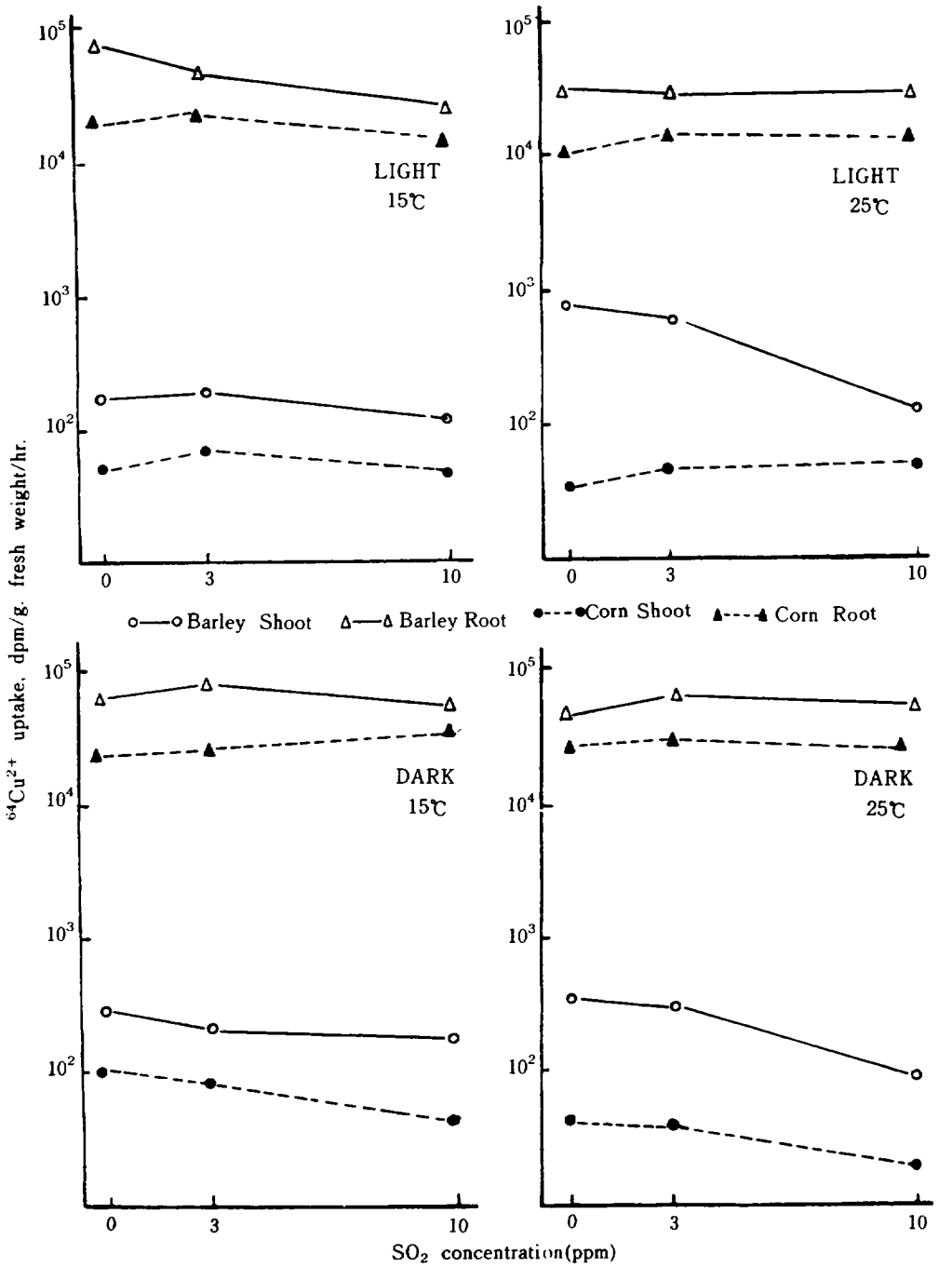

Figure 16. $^{64}Cu^{2+}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

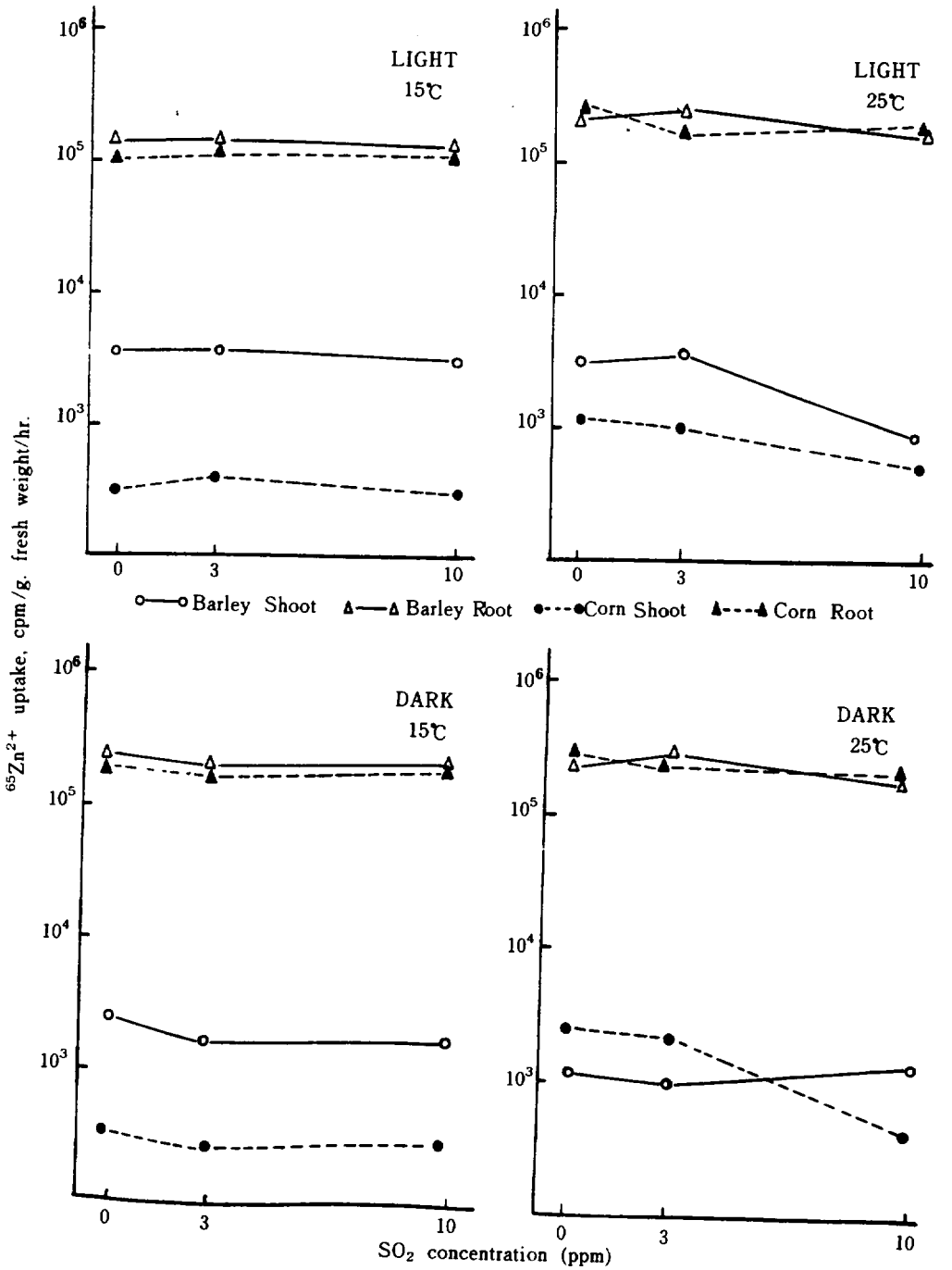

Figure 17. $^{65}Zn^{2+}$ uptake by barley and corn plants influenced by SO_2 fumigation of 3ppm and 10ppm at 15℃ or 25℃ in light and dark conditions.

As discussed above, some ions such as calcium and iron were positively affected by SO_2 fumigation, consequently giving a higher degree of absorption and translocation when compared with the control treatment. However, other ions were negatively affected by SO_2 and their absorption and transport were retarded.

In spite of numerous reviews (2, 26, 65, 69, 117) on inorganic ion transport in the higher plants, no complete picture of ion uptake can be obtained yet. Figure 18 shows the results summarized; root absorption and transport to shoot of the tested ions are presented as a percentage index compared with the control (100%).

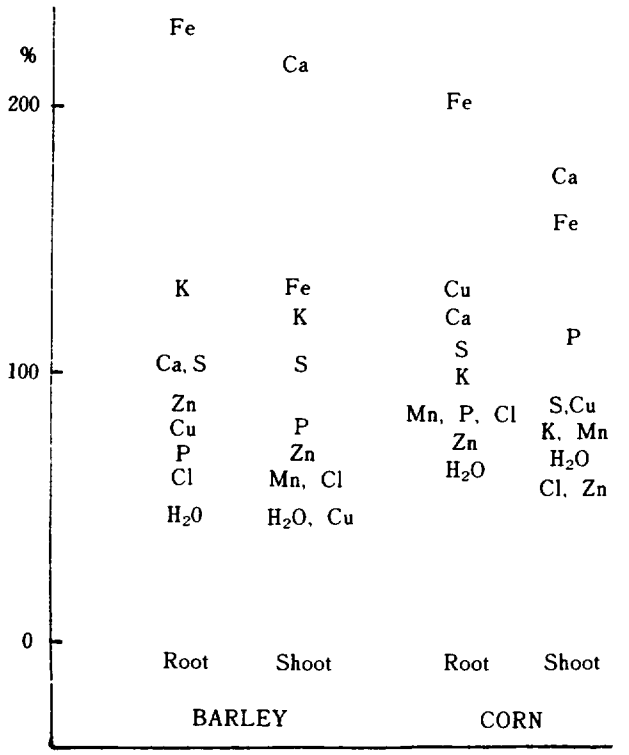

Figure 18. The degree of acceleration or inhibition, presented as percentage index, of inorganic ion uptake by barley and corn fumigated with SO_2.

Summary

The effects of sulfur dioxide (SO_2) on carbon and sulfur assimilation, and on absorption and translocation of various kinds of inorganic ions and water were studied introducing barley (*Hordeum vulgare L.*, Hyangcheongua-1) susceptible to SO_2 and corn (*Zea mays L.*, Suwon-19) resistant species. Three levels (0, 3, and 10ppm v/v) of SO_2 fumigation were carried out in light or dark condition, and at two different temperatures (15℃ and 25℃). Radioisotope techniques were employed to investigate the conversion percentage of inorganic sulfate, SO_2 and CO_2 into the organic compounds and to observe the degree of inhibition or acceleration of water and ion uptake by SO_2 treatment. The results obtained are summarized as follows;

1. Under the conditions of the present study no visible symptoms of the effect of SO_2 on the plants were found. Little change of total chlorophyll content was observed from the barley and corn fumigated with SO_2.
2. Average stomatal opening was clearly influenced by fumigation of SO_2 regardless of light and temperature conditions. Barley closed the stomata much more than corn.
3. Dry matter weight and plant height were measured for the barley and corn grown for three weeks fumigated with SO_2 every day. Measurments showed that both barley and corn were affected by SO_2, losing their dry matter weight and plant height according to the fumigation dose. Concentrations of P, Mn and Zn in barley were higher than those of corn. Iron content increased with the fumigation dose of SO_2 in barley and corn.
4. Barley absorbed much more SO_2 than corn when fumigated with 3ppm SO_2 but corn took up a higher amount of SO_2 than barley at 10ppm SO_2. High temperature and light condition accelerated SO_2 absorption by plants.

   The translocation rate of sulfur from shoot to root at 3ppm of SO_2 fumigation was much higher than that of 10ppm. Dark condition increased the translocation rate when compared with light condition.

   The percentages of ethanol soluble fraction at 25℃ of fumigation in the dark was found to be higher than that of 15℃ while the percentages of water soluble fraction of 25℃ fumigation was lower than that of 15℃.

   The conversion rates of the absorbed sulfur dioxide to amino acids (methionine and cysteine) increased more in the dark than in the light. Fumigation of 10ppm SO_2 reduced the conversion rate compared with 3ppm.
5. The fixation of carbon dioxide as well as the translocation of ^{14}C-compound from shoot to root was inhibited by SO_2 fumigation.

   Corn had a higher capability of photosynthesis than barley which was severely affected by SO_2.

   Glucose formation as well as fructose plus sucrose in the barley was found to increase with SO_2 fumigation.

   The fraction absorbed by cation exchange resin, composed mainly of amino acids, increased in the shoots and roots of barley and corn according to SO_2 fumigation (3ppm and 10ppm) in the light.
6. Sulfate absorption by plant roots was not influenced by SO_2 fumigation but the translocation of sulfate from root to shoot was reduced in the light.

   The 80% ethanol soluble fraction in the shoots of barley and corn decreased with SO_2 fumigation in the light. The percentages of 80% ethanol soluble fraction in the barley roots were much higher than those in the corn roots while the water soluble fraction had the opposite tendency.

   The conversion rates of sulfate absorbed through the roots into methionine and cysteine increased with

SO_2 fumigation and were much higher in the corn plants than in the barley. There was always more cysteine produced than methionine.

7. Fe and K uptake by the roots of barley was accelerated but H_2O, Cl, P, Cu, and Zn were reduced by SO_2 fumigation.

   Translocation of Ca, Fe and K in the barley shoot was increased by SO_2 while H_2O,Cu, Mn, Cl, Zn, and P were translocated less by SO_2 fumigation.

8. Fe, Cu, and Ca uptake by the roots of corn was accelerated but H_2O, Zn, Mn, P and Cl were reduced by SO_2 fumigation.

   Translocation of Ca and Fe in the corn shoot increased by SO_2 fumigation while Cl, Zn H_2O, K, Mn, S and Cu were translocated less by SO_2 fumigation.

9. It can be concluded that SO_2 sensitivity of barley resulted from its low photosynthesis which was much more inhibited by SO_2 fumigation than that of corn. Barley absorbed more ions like PO_4^{3-} SO_4^{2-} Ca^{2+} Fe^{2+}, Cu^{2+} and Mn^{2+} with the exception of K^+ and Cl^- than corn while the degree of inhibition and stimulation in ion uptake by barley was more severe than that of corn. However the result can not correlate SO_2 sensitivity of the plants with the fact that corn contained more sulfur in the 80% ethanol soluble fraction than barley while the rate of conversion into the sulfur containing amino acids was higher in corn than in barley.

Appendix. Description of the code used in table.

BOS Shoots of the barley fumigated with 0ppm SO_2
BOR Roots of the barley fumigated with 0ppm SO_2
B1S Shoots of the barley fumigated with 3ppm SO_2
B1R Roots of the barley fumigated with 3ppm SO_2
B2S Shoots of the barley fumigated with 10ppm SO_2
B2R Roots of the barley fumigated with 10ppm SO_2

COS Shoots of the corn fumigated with 0ppm SO_2
COR Roots of the corn fumigated with 0ppm SO_2
C1S Shoots of the corn fumigated with 3ppm SO_2
C1R Roots of the corn fumigated with 3ppm SO_2
C2S Shoots of the corn fumigated with 10ppm SO_2
C2R Roots of the corn fumigated with 10ppm SO_2

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