国立公害研究所研究報告　第66号
Research Report from the National Institute for Environmental Studies, Japan. No. 66, 1984.

Studies on Effects of Air Pollutant Mixtures on Plants

Part 2

環境庁　国立公害研究所
THE NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES

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Research Report from the National Institute for Environmental Studies No. 66

Studies on Effects of Air Pollutant Mixtures on Plants Part 2

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Preface

The toxic effects of sulfur dioxide, nitrogen dioxide and ozone on plants have been extensively studied at the intitute by conducting a special research program since 1976. The results of the first three years program were published in the Research Report No. 11 (1981) entitled "Studies on the Effects of Air Pollutants on Plants and Mechanisms of Phytotoxicity".

In the first program, most studies were concerned in the effects of the single air pollutant. However, plants are usually exposed to the mixed air pollutants in the urban area and few results have been reported on the effects of mixed air pollutants on plants. For clear understanding of the effects of the mixed pollutants, the second three years research program "Studies on Effects of Air Pollutant Mixtures on Plants" have been conducted from 1979 to 1982.

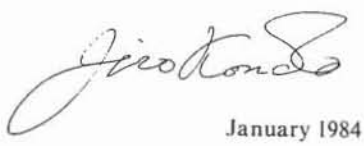
Mixed gas showed either additive, synergistic or antagonistic effect of the single gases. The sensitivity of plants to mixed pollutants was changed by species and by combination of the pollutants. The mechanism of phytotoxicity was studied from physiological, biochemical and micrometeorological standpoints. These results are collected in this report. The detailed description of the facilities in which the experiments are conducted is also included. The extensive studies should be continued to reach the complete understanding of the mechanism of phytotoxicity.

The previous report No. 11 (1981) seems to call attention among biologists as well as environmental scientists. We appreciate that the useful suggestions and discussion are given to the report.

It is hoped that this report is also of some use for scientists who are interested in the toxic effects of atmospheric pollutants.

Jiro Kondo, Eng. D.

Director of the National Institute
for Environmental Studies



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Effects of NO_2 and O_3 Alone or in Combination or Kidney Bean Plants. I. Growth, Partitioning of Assimilates and Root Activities

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Ten-day old kidney bean plants (*Phaseolus vulgaris* L. cv. Sin-edogawa) were exposed to 2.0 and 4.0 ppm NO_2, and 0.1, 0.2, and 0.4 ppm O_3 alone or in combination for 2, 4 and 7 days. The effects of those air pollutants were examined with respect to the growth, partitioning of assimilates, nitrogen uptake, soluble sugar content, and respiration by focusing our attention especially on root activities.

The reduction in dry matter production was observed in all treatments except NO_2 at 0.2 and O_3 at 0.1 ppm where the increase in dry weight of the plants at early stage of the exposure was nearly equal with control plants. The exposure to mixture of both gases at low concentration produced more than an additive effect on the growth at 2 to 4 days after the start of the exposure. The percent of nitrogen in whole plants was increased by all treatments. The higher percent of nitrogen found in O_3 exposure will result from the growth retardation which pushed up the concentration of nitrogen in the plants as the absorption of nitrogen by roots was unaffected by O_3 exposure. The coexistence of O_3 with NO_2 significantly reduced the incorporation and assimilation of NO_2 by the plants.

The concentration of soluble sugars in roots was thinned by the gas treatments. The high correlation was obtained between soluble sugar content and dry weight in roots. In spite of the decrease in soluble sugar level, root respiration was unchanged until 5 days after the start of the exposure. It is suggested from those results that, although biomass and soluble sugars translocated from leaves are reduced by the exposure to NO_2 and O_3, root activities relating with nitrogen absorption and respiration are maintained at almost the same level as control plants.

Key words: NO_2—O_3—Kidney bean—Growth—Sugars—Root respiration.

Since air pollution was recognized to cause serious problems on human activities and biomass production of natural and agricultural ecosystems, many extensive works have been done in regard to the complex effect of air pollutant mixture. The focus of the researches ranged widely from the biochemical view points in order to solve the action mechanism of the pollutants to the chronical influences of long-term gas exposures on foliage and dry weight growth of plants. A number of review paper has covered those subjects (Heath 1980, Heggestad and Heck 1971, Horsman and Wellburn 1976) where there are sometimes crucial contradictions

among precisely documented data. For ecample, in several reports, ozone generally acted as an inhibitor to plant growth (Heagle et al 1972, Oshima et al 1975, Tingey et al 1971. Tingey et al 1973), while in other reports ozone stimulated the growth (Bennett 1977). Those facts suggest that a more analytical research has to be performed.

In the series of our studies, we intended to reexamine the effect of air pollutants, especially NO_2 and O_3 alone or in combination on photosynthetic CO_2 assimilation, the translocation of photosynthates, and the growth in dry weight. The present report will deal with the effect of the pollutants on the growth of whole plants, the partitioning of assimilates among each plant part and root activities in kidney been plants.

Materials and Methods

Two seeds of kidney bean plants (*Phaseolus vulgaris* L. cv. Shin-edogawa) were sown on a plastic pot (7 cm in diameter and 11 cm in depth) which contained an artificial soil; vermiculite, peat moss, perlite and fine gravel (2:2:1:1 V/V). The basal fertilization consisting of 1 g of Magamp K ($N:P_2O_5:K_2O$ = 6:40:5 from W. R. Grace Co., Tennessee, U.S.A.) and 3 g of magnesia lime was carried out. No additional fertilizer was applied during the course of the experiment. The plants were germinated and grown in an artificially lit growth cabinet.

The environmental conditions in the cabinet were set up at 25 Klux, 14-10 hr day and night cycle, 25° C and 70% R.H. One of plants in a pot was thinned 7 days after sowing to equalize the visible plant size in the community which usually consists of more than 90 pots in one set of the experiment. The exposures of plants to NO_2 and O_3 alone or in combination were started on the 10th day and ended on the 17th day after sowing. NO_2 was exposed at 2.0 and 4.0 ppm, and O_3 at 0.1, 0.2, 0.4 ppm. In the mixed gas treatments, the combinations of NO_2 and O_3 concentrations were 2.0 and 0.1, 2.0 and 0.2, 2.0 and 0.4, and 4.0 and 0.2 ppm.

Twelve plants were harvested in the morning (usually from 10 to 11 am) of 12, 14 and 17 days after sowing. One half of the plants were dried at 80° C for a few day to measure their dry weight. The other half were divided into two and were frozen in a freezer at around −20° C for sugar analysis after measuring the fresh weight. The dried samples of each plant part were divided into two and ground to fine powder with a vibrating sample mill (TI-100, Seiko manufactory Ltd., Tokyo) for the determination of nitrogen content by a Yanaco CN-corder (MT-500, Yanagimoto Co., Ltd., Kyoto). The frozen smaples of the trifoliate leaf, primary leaf and root were homogenized with 80% ethanol by a Polytron (type 10/35, Kinematica, Switzerland). Ethanol extract was evaporated nearly to dryness and then centrifuged at 10,000 rpm for 10 min to remove chlorophyll insoluble to water. A dried aliguot of alcohol-water soluble fraction was warmed at 70° C for 20 min with TMS (trimethylsilane) to synthesize the volatile derivatives of sugars for the gas chromatographic analysis. A silicon OV-17 at 2% on chromosorb W (AW-DMSC 60/80 mesh) was packed in a glass column (5 mm in inside diameter and 2 m in length) through which nitrogen was flown at 60 ml/min as a carrier gas. The sugars were determined by a gas chromatograph analyzer (Shimazu Co., Ltd., GC-5A) with a flame ionization detector. Phenyl-β-glucoside was selected as an internal standard.

The root respiration was measured in the separate experiment where NO_2 and O_3 was exposed singly at 2.0 and 0.2 ppm, respectively, to the plants under the exactly same conditions as descrived above. The roots of threee plants were harvested 1, 3, 5 and 7 days after the start of gas exposure. The roots were thoroughly washed with tap water and cut into small pieces. The root segments (5-10 mg dry weight) were introduced into a cell with 3 ml of distilled water and their O_3 uptakes was determined by a Clark-type oxygen electrode.

Results

Growth of a whole plant and each plant part of kidney bean unexposed to air pollutants are illustrated in Fig. 1 with the logarithmic scale in a vertical line. The whole plant shows a nearly straight curve which means the constant relative growth rate (0.15 g/g·day ± 0.056 in the average of five determinations with standard deviations) throughout the experiment ranging from 8 to 20 days after sowing. The primary leaf grew relatively slowly, stopped the growth 2 weeks after sowing and then decreased the dry weight gradually. It is thought that the primary leaf has almost matured during the gas exposure period (10-17 days after sowing). The trifoliate leaf which commenced to unfold just before the start of gas exposure, grew exponentially, and then its growth rate declined at the middle of gas exposure. The root growth seems to keep pace with the growth of a whole plant as it showed a similar growth rate with that of whole plant.

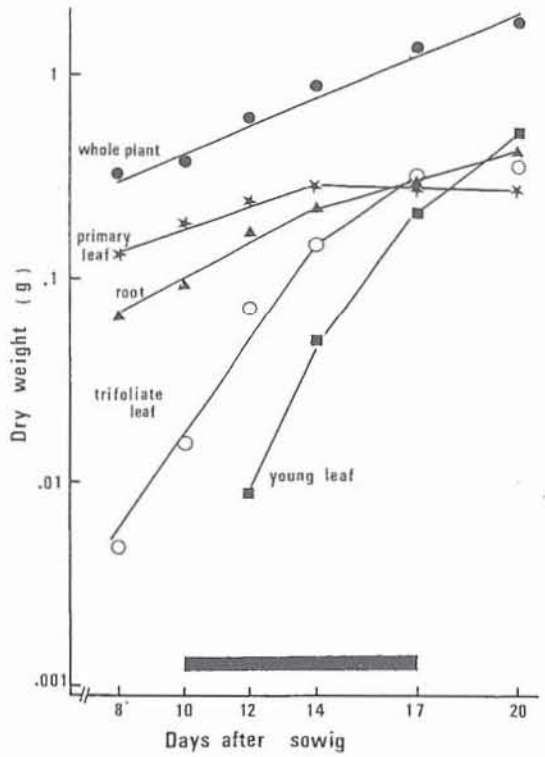

Fig. 1 Growth curves of the whole plant and each organ of kidney bean from 8 to 20 days after sowing. The treatments of gas exposure to NO_2 and O_3 were carried out 10 to 17 days after sowing (indicated with a black bar in the figure).

Changes in dry weight growth of the whole plant during the gas exposure period are shown in Fig. 2. The exposures to NO_2 at 2.0 ppm or O_3 at 0.1 ppm resulted in the insignificant decrease in dry weight growth for the first 4 days. No visible injury was observed in both treatments during this period. The leaves exposed to NO_2 at 2.0 ppm became apparently dark green, which suggests an activation of chlorophyll synthesis by the assimilation of nitrogen from NO_2. The

significant decrease in dry weight growth occurred when the plants were kept in gas mixture 2.0 ppm NO_2 and 0.1 ppm O_3 for 7 days. NO_2 treatment at 4.0 ppm caused typical symptoms of the visible damage especially on the primary leaf and considerable reduction in dry weight growth of a whole plant. Waxy and water-soaking appearances developed along the margin of the leaf for the first 1-2 days and then the collapsed, irregular-shaped discoloration between the vein spread over the leaf as the exposure period was prolonged. In the case of exposures to O_3 at 0.2 and 0.4 ppm, the typical symptom of O_3 injury so called silvery flecks and reddish purple stipple was observed all over the leaves from the early phase of gas exposures. The dry matter production was deteriorated more heavily than other gas exposures when treated singly, especially with 0.4 ppm O_3 where dry weight of the plants increased only slightly during the 7 days incubation. The mixed exposure of NO_2 and O_3 at 2.0 and 0.1 ppm and at 2.0 and 0.2 ppm resulted in a more drastic decrease in dry weight growth than expected from an aditive effects of the exposure to each gas singly for the first 4 days, but it became less than additive effects at 7 days after the start of exposure. The combination of 2.0 ppm NO_2 and 0.4 ppm O_3 showed an almost similar pattern to that in the case of the exosure to O_3 singly, which implies the growth in that combination is determined by the high concentration of O_3 above the threshold. Visible injuries found in the mixed gas exposures were mostly an O_3-dominant type.

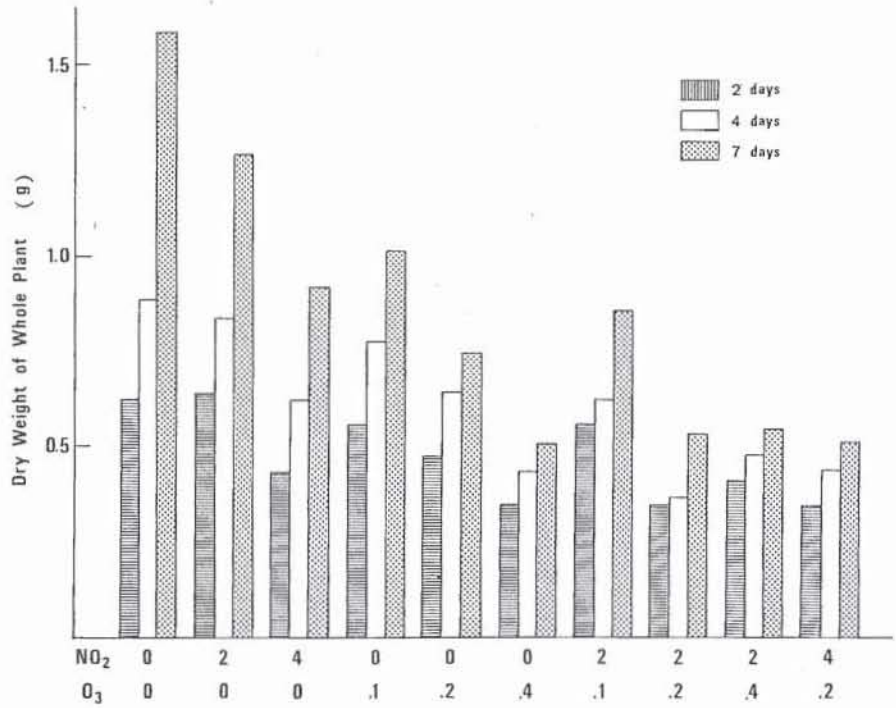

Fig. 2 Effect of the exposures to NO_2 and O_3 alone or in combination on dry weight of the whole plant of kidney bean plants.

It is anticipated that the exposure to NO_2 and O_3 singly or in combination should affect the distribution of photoassimilated carbon to each plant organ. The percent of dry weight in each part among whole plant are shown in Fig. 3. As for control plant, the percent of primary leaf, which nearly reached maximum development at the start of gas exposure (Fig. 1), decreased with time during the gas exposure. In contrast, percent of trifoliate leaf and stem including newly formed young leaves increased with time, while that of root stayed quite constant. The plants exposed to NO_2 at 2.0 ppm or O_3 at 0.1 ppm possessed the distribution patterns almost identical to the contorl plant. In the plants treated with other gas concentrations and compositions there was a general trend in the distribution pattern that the percents of root and trifoliate leaf become lower and that of primary leaf is kept higher until the end of gas exposure than the percent in the control plant.

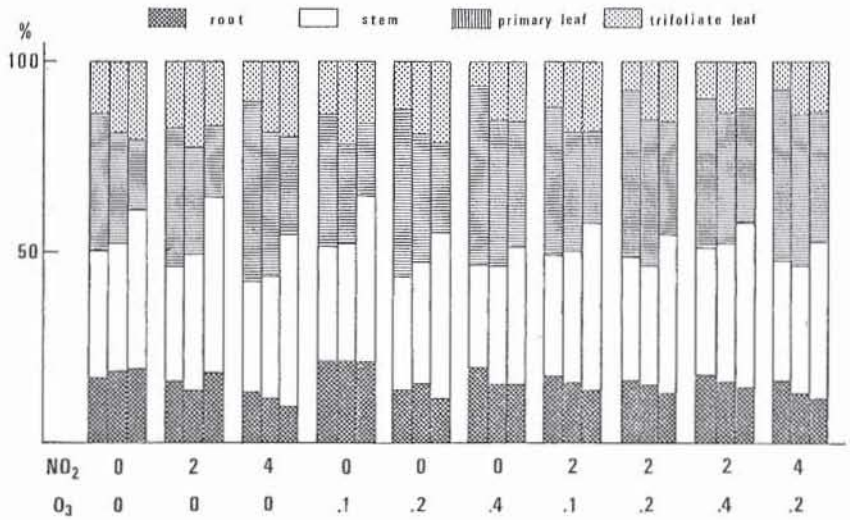

Fig. 3 Effect of the exposures to NO_2 and O_3 alone of in combination on the relative dry weight of each organ in kidney bean plants.

The result in Fig. 3 arouse the thought that the ratio of root over shoot should be changed by most of gas treatments. The calculated root/shoot ratio, shown in Table 1, fell into the range from 0.20 to 0.24 in the control plant. The eposure to 0.1 ppm O_3 gave slightly higher values than the control. Little difference was observed in NO_2 at 2.0 ppm. In other treatments, however, there was a tendency that the values went down with time and were considerably lower than the control. The lowered value of root/shoot ratio suggest that the exposures to NO_2 and/or O_3 at concentrations and combinations applied in the esperiment could weaken the translocation of photosynthates of leaves to roots.

Table 1 Root/shoot ratio of kidney bean plants exposed to NO_2 and/or O_3 at various concentrations

NO_2 (ppm)	0	2	4	0	0	0	2	2	2	4
O_3 (ppm)	0	0	0	.1	.2	.4	.1	.2	.4	.2
2 days	.20	.19	.15	.27	.16	.24	.21	.19	.21	.19
4 days	.23	.16	.13	.28	.18	.18	.18	.18	.19	.15
7 days	.24	.22	.11	.27	.13	.18	.16	.14	.16	.13

The total nitrogen percent in a whole plant of the control has a decreasing tendency with time (Fig. 4). The percent at the end of the exposure period was almost a half of the value at the start of exposure. This suggests that under the culture condition in the present experiment the supply of nitrogen from the artificial soil in pots should become limited as the plants grow up. The growth of plants was supported by the basal nitrogen fertilizer and no additional application of nitrogen was carried out until the end of experiment. The exposure to NO_2 acted to push up the level of nitrogen percent in the plants as expected, suggesting that the plants incorporate and utilize nitrogen from NO_2. The plants in the control obviously have a lower nitrogen percent than the plants exposed to O_3 (except 0.1 ppm) which fully depend on nitrogen absorbed by root unlike in the case of NO_2 exposure. The increased nitrogen percent by O_3 exposure implied either the stimulation of nitrogen uptake by root or the concentration of nitrogen due to the decrease in the biomass production. The same phenomenon was also observed in the mixed treatments.

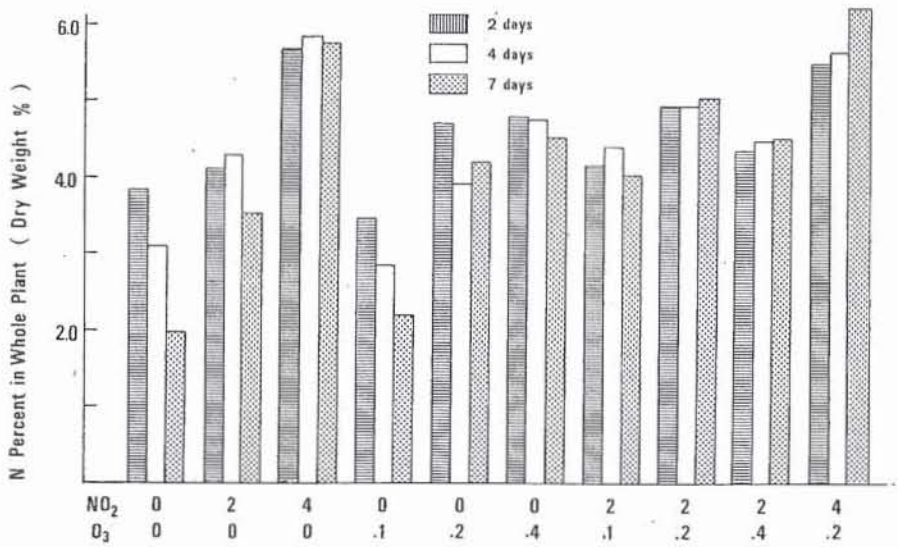

Fig. 4 Percent of nitrogen based on dry weight in the whole plant of kidney bean plants exposed to NO_2 and O_3 alone or in combination.

The differences in the amount of nitrogen in a whole plant between 0 to 2, 2 to 4 and 4 to 7 days after the start of the exposure show how much nitrogen is incorporated into the plants during those periods. The nitrogen uptake rate is calculated from the average of three individual values obtained form three intervals and is expressed by mg/ whole plant.days (Fig. 5). Since the plants exposed to NO_2 have the sources of nitrogen, one from atmospheric NO_2 and the other from nutrient in the artificial soil, if the absorption of nitrogen by the root is unaffected by NO_2 exposure, nitrogen above the level of the control can be considered to come from atmospheric NO_2. The incorporation of NO_2 by leaves was performed at a considerably higher rate than the absorption of medium nitrogen by roots in both 2.0 and 4.0 ppm NO_2 treatments as shown in Fig. 5. The exposure to O_3 seems to be unaffective to nitrogen uptake by roots. This is a rather contradictory result because O_3 exposure lowered the percentage in dry weight occupied by roots among a whole plant, which makes us conjecture the decrease in absorption activity of

nutrients by the root of the plants exposed to O_3. Supposing that O_3 does not change nitrogen uptake rate even in mixed treatments with NO_2, the excess nitrogen over the control is considered to originate from atmospheric NO_2. When NO_2 was mixed with O_3, its absorption by leaves was greatly suppressed. In the combinations of NO_2 and O_3 at 2.0 and 0.2, and 2.0 and 0.4 ppm, little incorpotation of NO_2 was observed. Actually, for the result to be convincible, the experiment with an isotopic nitrogen should be conducted to distinguish between the two sources of nitrogen.

Soluble sugar content was determined as a major indicator relating with metabolic activities in the plants. Four species of sugars, ie. glucose, fructose, inositol and sucrose were detected on a gas chromatogram. One more peak occaisonally appeared as a minor component among sugars. This peak could not be identified. The primary and trifoliate leaves did not show any consistent changes in sugar content and composition with the treatments of gas exposures, therefore, data are not shown. On the other hand, in the root, the percent of glucose among soluble sugars tended to decline as the damages from gas exposures increased (Fig. 6). Glucose was undetectable in the exposure to 0.4 ppm O_3, and 4.0 ppm NO_2 and 0.2 ppm O_3 for 7 days. The percent of fructose stadyed quite constant regardless of gas exposures.

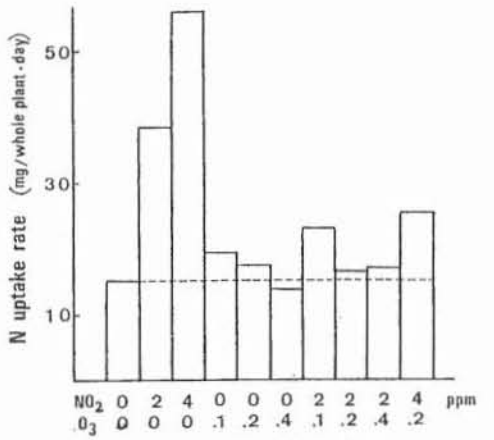

Fig. 5 Nitrogen uptake rate of kidney bean plants exposed to NO_2 and O_3 alone or in combination. The rates were calculated from the difference in nitrogen content of the whole plant at three consecutive sampling points.

The content of soluble sugars including four species of individual sugar was decreased in roots of plants exposed to air pollutants. The dry weight of roots 7 days after the start of the exposure was plotted against soluble sugar content at that time. As shown in Fig. 7, a relatively high correlation was obtained between the two. This result suggest that the amount of soluble sugar in roots has some relationship with the dry matter production of roots.

Since the absorption of major nutrients like nitrogen is an energy-dependent process, root respiration may partly reflect the activity of the nutrient uptake affected by the gas exposure. The root-respiration was measured by an oxygen electrode with excised roots of potted plants exposed to NO_2 at 2.0 ppm or O_3 at 0.2 ppm. In this experiment the mixed gas exposure to NO_2 and O_3 was omitted. As shown in Fig. 8, no significant decrease in O_3 uptake was observed in either of the treatments until 5 days after the start of the exposure. The root respiration became considerably lower than the control at 7 days, and especially O_3 induced a more drastic decrease than NO_2.

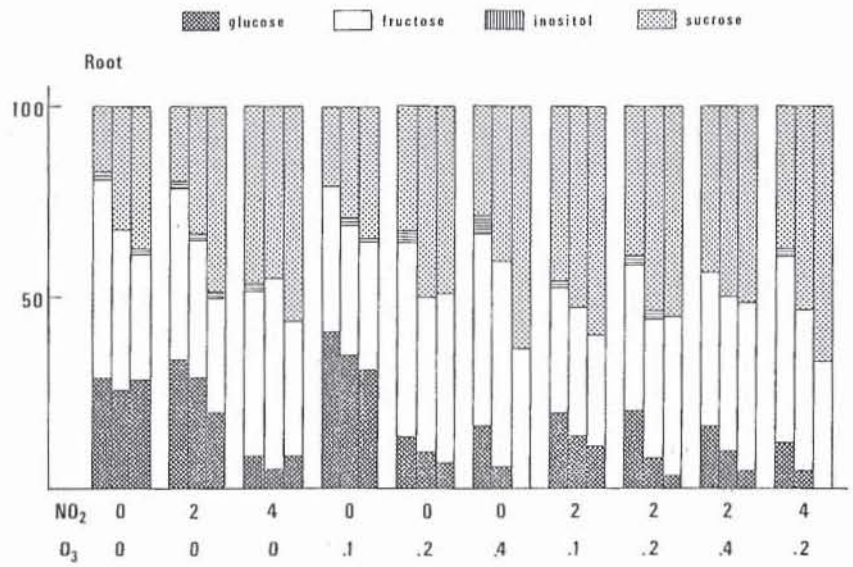

Fig. 6 Distribution of glucose, fructose, inositol and sucrose in soluble sugars extracted from roots of kidney bean plants expose to NO_2 and O_3 alone or in combination

Discussion

Effect on growth and partitioning of dry matter in the plants

In the present experiment the visible symtoms of injuries tended to first appear and develop most severely in the almost mature primary leaf which has been exposed to air pollutants for the longest period. The apeparance of the symptoms on the first trifoliate leaf just at a developing stage was observed in the case of the prolonged exposure to high concentration of air pollutants.

The concentration of NO_2 at which the plant growth is suppressed seems to be higher than that of O_3. The exposure of kidney bean plants to NO_2 at 0.1 ppm for 10 to 15 days caused no significant decrease in dry weight (Totsuka et al. 1978a). The growth of the plants was even stimulated when the fumigation was carried out during nighttime at 0.3 ppm for 2 weeks (Yoneyama et al. 1980). When the concentration was raised to higher levels than those values, the growth suppression was reported to become apparent. The pinto bean plants exposed to 0.5 ppm NO_2 for 10 to 19 days decreased the dry weight by 6 to 22% over the control (Taylor and Eaton 1966). The fumigation to 1 ppm NO_2 for 2 weeks resulted in 15% inhibition in dry matter production of kidney bean (Totsuka et al 1978b). Two ppm NO_2 used in the present experiment as the lowest concentration had little effect on dry matter production during 2 to 4 days exposure, while the significant reduction was observed at 7 days (Fig. 2). Four ppm NO_2 was too high for the plants to maintain the normal growth from the first 2 days.

The considerable reduction in the dry matter production was associated with O_3 exposure at all range of concentrations used in this experiment as expected from the results reported by many researchers (Heagle et al. 1972, Oshima et al. 1975, Tingey et al. 1971, Tingey et al. 1973). In this experiment the plant growth was already affected by the exposure to 0.1 ppm O_3 for only 2 days. Bennet et al (1974) confirmed that the growth of kidney bean plants was apparently stimulated by the exposure to 0.03 ppm O_3 for 12 days. Considering both results, the threshold

may fall into somewhere between 0.03 and 0.1 ppm in the plants.

In our result the combinations of NO_2 and O_3 at the low concentraiton, i.e. 2.0 and 0.1 ppm, or 2.0 and 0.2 ppm, produced more than the additive effect on the inhibition of growth at the early stage of the exposure. In other combinations, the plants were severely damaged by the exposure to either gas alone, thus it is considered futile to discuss on the combined effect.

Fig. 3 and Table 1 clearly demonstrate that the root growth is impaired most by gas exposures except in the case of the low concentrations of NO_2 and O_3 alone. The decrease in the root/shoot ratio by O_3 expousre has already been reported by several research workers (Bennet and Runeckles 1977, Horsman et al. 1980, Ogata and Maas 1973, Oshima et al. 1978, Reinert and Weber 1980). The specific inhibition of root growth implies that the translocation of photosynthates from the source leaf with the biggest contribution to the roots is affected by the gas exposure. In the kidney bean plants used here, such an organ is considered to be the primary leaf. Okano et al. (1984a and b) substantiated using ^{13}C as a tracer that the translocation rate of photosynthetically assimilated carbon from the primary leaf to the root is heavily slowed down by the exposure to NO_2 and O_3, alone or in combinaiton. The plant may have an inherited nature to put the priority on the shoot growth under the stressed conditions even though the root growth is sacrificed (Wardlaw et al 1968). Whitmore and Mansfield (1983), however, reported with several grasses exposed to 0.1 ppm NO_2 that the root/shoot ratio was unaffected even when the plant growth was signicantly retarded on the basis of dry weight.

Effect on nitrogen input

Since the plant has an ability to incorporate atmospheric NO_2 into its body constituents (Matsumaru et al 1979), it is quite understandable that NO_2 exposure should push up the level of nitrogen in the plant as seen in Fig. 4. The atmospheric NO_2 at 2.0 and 4.0 ppm contributed more to total nitrogen economy in kidney bean plants than medium nitrogen absorbed by roots did (Fig. 5). The percent of nitrogen in the whole plant was also significantly elevated by O_3 exposure which unaffected the absorption rate of nitrogen from roots. The higher percent of nitrogen caused by O_3 exposure will be the result of the growth retardation of the plants which uptake medium nitrogen at the similar rate as the control plants do. A ladino clover exposed to 0.3 and 0.6 ppm O_3 for 2 hr a day for a week possessed a higher percent of nitrogen than the control (Letchworth and Bulm 1977).

Effect on soluble sugars and root respiration

The alternation in the composition of soluble sugars occurred in wheat leaves exposed to NO_2 at 1 ppm (Prasad and Rao, 1980). Fructose became detectable, which was abscent in the control plants. In the case of O_3 treatment, Banes (1972) and Dugger et al (1966) reported the increase in reducing sugars with pine and lemon seedlings respectively. In contrast, three-year old ponderosa pine needle exposed to 0.3 ppm O_3 reduced 80% ethanol soluble sugars (Miller et al. 1969). In our experiment, soluble sugar contents in leaves fluctuated unexpectedly and no clear-cut trend was produced by gas exposures.

The amount of soluble sugars in the roots was consistently decreased by both NO_2 and O_3 exposures. Among soluble sugars the percent of glucose declined remarkably with increasing concentration of either gas alone or in cimbinaiton (Fig. 6). High correlation was found between sugar content and dry weight of roots (Fig. 7). Those results confirm that the reduced translocation of sugars from leaves to roots should cause the reduction in dry matter production of roots. The O_3 fumigation significantly leveled down the content of starch, sucrose and reducing sugar in the roots of green ash seedlings (Jensen 1981).

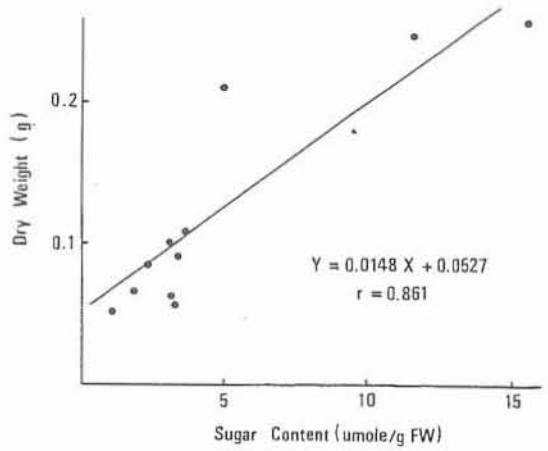

Fig. 7 Correlation between the dry weight and the sugar content in kidney bean roots sampled 7 days after the start of gas exposures.

Since soluble sugars in plant organs are utilized as substrates for the respiration, it is expected that the reduction in their contents would suppress the root respiration. Contrary to this anticipation, the effect of both NO_2 and O_3 appeared very slowly and did not become significant until 7 days after the exposure (Fig. 8). The pools of soluble sugars for the root respiration and the synthesis of body consitituents might be separated, and the gas exposure might only affect the pool for the latter. The result that medium nitrogen uptake by roots, which is thought tobe energy-dependent processes,was not altered by O_3 exposure may support the above interpretation. On the other hand, Hofstra et al. (1981) reported that the exposure of bean plant to O_3 turned root tips brown and reduced the respiratory activities measured by both triphenyl tetrazolium cloride staining and CO_2 evolution. Those phenomena became evident well before the visible injury appeared on the leaves. Further investigations should be conduced to clarify the reasons producing a discrepancy discussed above.

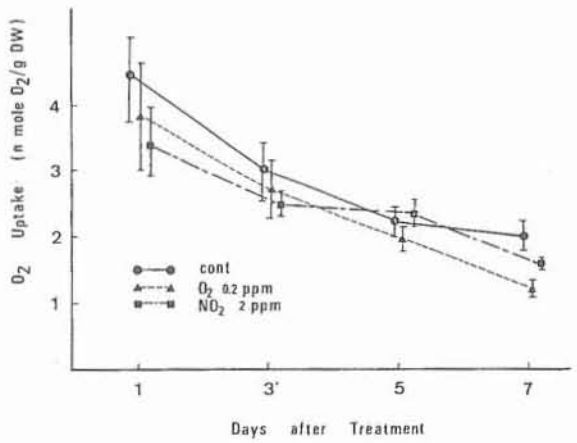

Fig. 8 Effect of the exposures of NO_2 and O_3 alone on respiration in excised roots of kidney bean plants. The respiration was measured by an oxygen electrode.

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インゲンマメに対する二酸化窒素とオゾンの単独及び複合暴露の影響
I　生長，同化産物の分配と根の活性

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播種後10日目のインゲンマメ（Phaseolus vulgaris L. cv. Sin-edogawa）が二酸化窒素2.0と4.0ppm，オゾン0.1，0.2と0.4ppm単独及びそれ等の複合で2，4と7日間暴露された。汚染ガスの影響は，生長，同化産物の分配，窒素の吸収，可溶性糖含量と根の活性，特に呼吸に注目して調べられた。

乾物生産の減少はほとんどすべての区において認められたが，二酸化窒素2.0ppmとオゾン0.2ppm区では暴露初期においては対照区とほぼ同様な生長を示した。2種のガスを低い濃度で混合した場合には暴露開始後2日目と4日目において生長を相乗的に抑制することが観察された。全乾物あたりの窒素濃度はすべての区において高まった。オゾン暴露において窒素濃度が高まるのは，根による窒素の吸収は影響されずに生長が抑制されるため一種の濃縮現象が起きたためと考えられる。オゾンが共存することにより植物による二酸化窒素の取り込みと同化は著しく減少した。

根の可溶性糖濃度は汚染ガス処理により減少した。根の可溶性糖濃度と乾物重との間には高い相関が認められた。可溶性糖量が減少するにもかかわらず，根の呼吸は暴露開始後5日目まで対照区との間に有意な差は得られなかった。以上本実験により，二酸化窒素とオゾンを暴露することにより乾物生産と葉から根へ転流される可溶性糖量は減少するが，根の活性に関係する窒素吸収や呼吸は対照区とあまり変らないことが示された。

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Effects of NO_2 and O_3 Alone or in Combination on Kidney Bean Plants. II. Amino Acid Pool Size and Composition

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The effects of NO_2 and O_3 exposures alone or in combination were investigated with respect to amino acid pool size and composition. The short-term exposures to NO_2 at 4.0 ppm alone or in combination with O_3 at 0.4 ppm induced rapid increase in total amino acids among which glutamine occupied most of the part. Total amino acids were also increased by O_3 exposure after 2 hours lag period. The accumulation of glutamine was predominant among amino acids with increased amounts.

When the exposure period was extended to 2 to 7 days, increase in total amino acids was observed in all the three treatments, though most remarkable in 4.0 ppm NO_2. In the root, the exposure to O_3 resulted in the highest concentration of total amino acids. Asparagine, in place of glutamine, became a major amino acid. The percent of asparagine was especially increased by the mixed exposure to NO_2 and O_3. In contrast, a decreasing trend was found in glutamate. Those results indicate that glutamine which accumulates extensively in an early phase of the gas exposure seems to be gradually converted into other amino acids, mainly asparagine. Summarizing the relationship between the changes in each amino acid brought about by the exposure to air pollutants, the high correlation was obtained among amino acids belonging to the serine family such as glycine, serine and cysteine.

Key words: NO_2—O_3—Kidney bean—Amino acids.

Metabolic activities in plants are reported to be disturbed by the exposure to air pollutants. Numbers of enzymes and body constituents such as sugars, amino acids, organic acids and so on have been tested in this regard (Wellburn et al. 1980). In the previous paper, the single and mixed exposures of NO_2 and O_3 were found to decrease sugar content in the root of kidney bean plants (Ito et al. 1984a). The contents of soluble sugars and amino acids in the leaves were increased by the O_3 exposure (Dugger et al. 1966, Ting and Mukerji 1967). Only limited information on metabolic changes is available in connection with the NO_2 exposure which may cause the considerable impact on amino acid metabolism as nitrogen from NO_2 can readily be utilized by the plants if nitrite reductase is active (Yoneyama et al. 1979). The present study ws initiated to make clear what changes would take place in the amino acids individually and as a whole upon the exposure to NO_2 and O_3 alone or in combination.

Materials and method

The plant (*Phaseolus vulgaris* L. cv. Shin-edogawa) was grown exactly the same as previously reported (Ito et al. 1984a). In the short-term experiment, 12-day-old plants were

exposed to NO_2 at 4.0 ppm and O_3 at 0.4 ppm singly or in combination for 2, 4 and 8 hrs. The samples of the long-term experiment, where the gas exposures at various concentrations were carried out 10 to 17 days after sowing, were brought over from the previous work (Ito et al. 1984a).

To determine the total amount and the composition of amino acids, ethanol-water-soluble fractions from the trifoliate leaf, primary leaf and root were loaded on an amino acid autoanalyzer (Model 835, Hitachi Co. Ltd.), where lithium buffers were used for better separation of glutamine and asparagine. The total amino acids were the sum of 21 species of amino acids determined by the analyzer. The total nitrogen was measured by a Yanaco CN corder (Model MT 500, Yanagimoto Co. Ltd.). All analyses were performed with duplicate samples, each of which consisted of three plants.

Results

The short-term experiment

Fig. 1 shows the effect of NO_2 and O_3 exposure alone or in combination on the total amount and the composition of amino acids. The total amino acids in the first trifoliate leaf of kidney bean plants exposed to NO_2 at 4.0 ppm increased rapidly, and reached the plateau 4 hours after the start of the gas fumigation. Among 21 species of amino acids tested here, the accumulation of glutamine was the most remarkable and was in parallel with the increase in the amount of total amino acids (Fig. 1a). The amount and composition of amino acids in the first trifoliate leaf of the plants unexposed to the air pollutants stayed almost constant during the course of experiment. The content of glutamine was nearly 10 times of the level at the start of the experiment. The amounts of threonine, serine, γ-aminobutyric acid (GABA) and asparagine also increased, while their extents were only twice as much as the initial and their percents in total amino acids were relatively low as compared with that of glutamine. Other amino acids merely changed the amounts throughout the NO_2 exposure.

The exposure to O_3 also induced the increase in the amino acid pool size (Fig. 1b). In this case, however, the amount of total amino acid content remained at the initial level for the first 2 hr and then increased rapidly. The percent of glutamine became prominently higher with time. The accumulation of glutamine occurred prior to the increase in the total amino acids. Most of amino acids except glycine, alanine and minor amino acids (designated by "others" in this paper) increased their amounts 4 to 8 hrs after the start of the gas exposure.

When NO_2 and O_3 were exposed to the plants in combination, the changes in the amount of total amino acid content followed the same trend as that of the exposure to NO_2 alone. In spite of the co-presence of O_3, nitrogen from NO_2 accumulated quite rapidly in the form of amino acids. It should be noted that the initial level of amino acids was significantly low in this particular experiment as known by the comparison of Fig. 1c with Figs. 1a and b. The difference in amino acid content is thought to be caused by the variation of the plants used in each experiment, though the plants were grown in the artificially-lit growth cabinet from the germination to the harvest under carefully controlled environments. Although the increase in the amount of glutamine was most predominant, the pool size of other amino acids also became bigger.

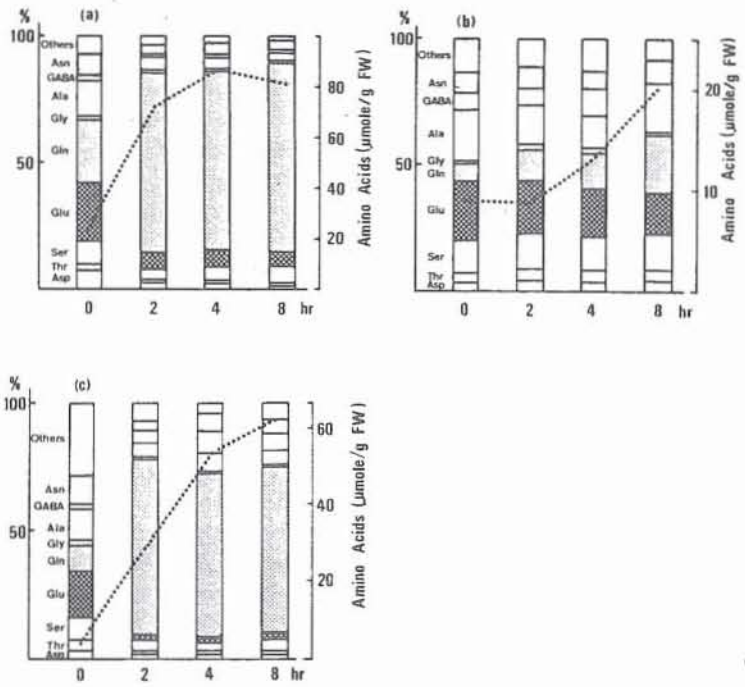

Fig. 1 Changes in the concentration of total amino acids and amino acid composition in the trifoliate leaf of kidney bean plants exposed to NO_2 and O_3 alone or in combination for 2 to 8 hours. (a) NO_2 at 4.0 ppm, (b) O_3 at 0.4 ppm and (c) NO_2 and O_3 at the same concentration as (a) and (b). Asp: Aspartate, Thr: Threonine, Ser: Serine, Glu: Glutamate, Gln: Glutamine, Gly: Glycine, Ala: Alanine, GABA: γ-Aminobutyric aric, Asn: Asparagine and Others include valine, cysteine, isoleucine, leucine, tyrosine, phenylalanine, β-alnine, β-amino-iso-butyric acid, lysine, histidine, arginine and proline.

The long-term experiment

In the next experiment, the effects of air pollutants on the amino acid pool size and composition were overviewed from the longer time scale (2 to 7 days). Fig. 2 shows the changes in the amount of amino acids in the trifoliate leaf, primary leaf and root of kidney beean plants exposed to NO_2 and O_3 alone or in combination. The unexposed plants had a tendency to decrease the amount of amino acids with time in all parts of the plants tested here. The expousre to NO_2 significantly pushed up the size of amino acid pool in the trifoliate and primary leaves, while smaller changes were observed in the root. In particular, the amount of amino acids in the roots of the plants exposed to NO_2 at 2 ppm was nearly equal with that of the control throughout the gas exposure period. The exposure to 0.1 ppm ozone had no effect on all the three parts of plants. The more increase in ozone concentration significantly enlarged the size of amino acid pool. This effect was emphasized especially in the root. The amino acid concentration was also increased by the mixed exposure to NO_2 and O_3 in most of the combinations.

The percent of total amino-nitrogen over total nitrogen in the three parts of kidney bean plants was calculated (data not shown). It has already been reported in the previous paper (Ito et al 1984a) that the percent of total nitrogen is increased by the exposure to NO_2 and O_3 alone or in

combination. The increase in the percent means that the enlargement of amino acid pool size exceeds that of total nitrogen. This trend was observed in most of cases, especially clearly in NO_2 exposure at 4.0 ppm.

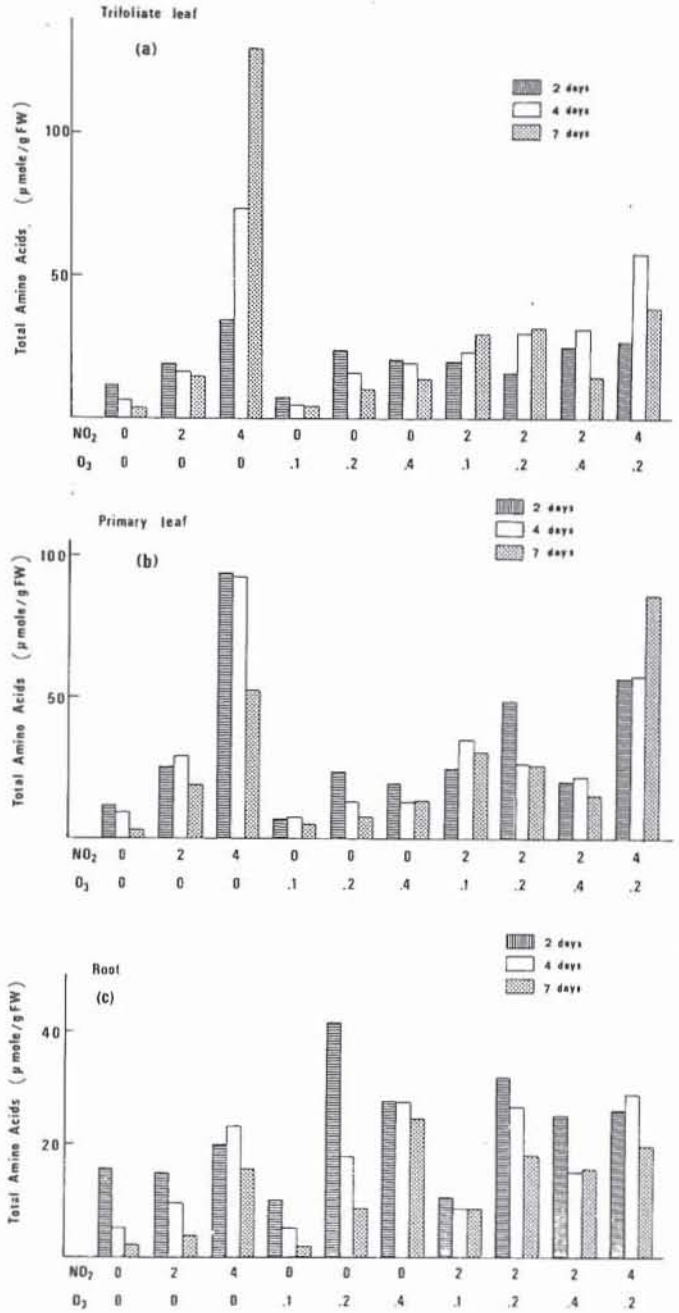

Fig. 2 The concentration of total amino acids in the trifoliate leaf (a), primary leaf (b) and root (c) of kidney bean plants exposed to various concentrations of NO_2 and O_3 alone or in combination.

Ammonium was excluded to calculate the amount of total amino acids, while it is considered to be an important substrate for the synthesis of amino acids. Table 1 shows the ammonium concentration in kidney bean plants. In the control plants, the ammonium concentration decreased with time in all the three parts, of which the primary leaf tended to have the highest concentration in all treatments. The exposure to NO_2 at 4.0 ppm brought about a significant rise in the concentration, particularly 4 to 7 days after the start of the exposure. An obscure pattern of changes in the concentration was observed in the exposure to O_3. In the mixed exposure to NO_2 and O_3, the concentrations in all the four treatments were higher than that in the control 7 days after the start of the exposure. The exposure to NO_2 and O_3 at 4.0 and 0.2 ppm, respectively, gave a extremely high value, which may be caused by the deterioration of cellular metabolism.

Table 1 Concentration of NH_3 in kidney bean plants exposed to NO_2 and or O_3 at various concentrations (umole/g FW)

	NO_2 (ppm)	0	2	4	0	0	0	2	2	2	4
	O_3 (ppm)	0	0	0	.1	.2	.4	.1	.2	.4	.2
Trifoliate Leaf	2 days	0.55	0.80	0.72	0.20	0.78	0.59	0.24	0.53	0.39	0.57
	4 days	0.53	0.64	1.08	0.16	0.40	0.42	0.63	0.39	0.43	0.42
	7 days	0.16	0.21	1.96	0.12	0.33	0.34	0.24	0.44	0.67	1.12
Primary Leaf	2 days	1.34	1.39	2.43	0.34	1.01	0.40	0.40	0.64	0.70	0.46
	4 days	0.98	0.91	3.05	0.27	1.73	0.54	0.79	0.51	0.97	1.14
	7 days	0.37	1.21	3.57	0.40	0.75	0.93	1.20	0.63	0.99	24.3
Root	2 days	0.58	0.24	0.62	0.16	0.65	0.22	0.11	0.27	0.37	0.43
	4 days	0.22	0.40	0.70	0.09	0.45	0.41	0.16	0.30	0.42	1.01
	7 days	0.11	0.11	0.27	0.06	0.20	0.65	0.15	0.20	0.55	1.01

The amides, especially glutamine, are the first acceptors of ammonium and play important roles in the assimilation of inorganic nitrogen and in the storage of the excess nitrogen. The percent of the amides in total amino acids was the highest in the root and decreased with time in all the three parts (Table 2). There was a clear tendency that the gas exposures pushed up the percent of amides in total amino acids, though a few exception existed. The tendency was emphasized by the mixed exposure to NO_2 and O_3. The amides in the trifoliate leaf exposed to 0.1 ppm O_3 could not be separated from the peak of glutamate due to the former's amount lower than the latter's.

Table 2 Percent of glutamine and asparagine over total amino acids in kidney bean plants exposed to NO_2 and/or O_3 at various concentrations

	NO_2 (ppm)	0	2	4	0	0	0	2	2	2	4
	O_3 (ppm)	0	0	0	.1	.2	.4	.1	.2	.4	.2
Trifoliate Leaf	2 days	8.1	12.1	34.2	ND	21.5	19.7	5.3	29.0	31.7	52.4
	4 days	6.4	24.6	33.4	ND	32.5	34.6	25.4	38.3	53.8	52.3
	7 days	3.4	4.4	41.4	ND	7.7	32.9	54.8	50.4	30.8	61.9
Primary Leaf	2 days	25.8	23.2	31.0	22.3	36.4	42.4	61.3	72.7	46.7	78.3
	4 days	22.0	20.9	36.8	24.8	11.8	24.6	62.2	56.3	53.7	75.2
	7 days	4.0	12.5	31.3	17.8	14.2	19.5	55.1	56.7	43.3	61.9
Root	2 days	83.2	81.5	82.4	70.7	82.0	87.3	43.5	90.5	89.4	89.6
	4 days	59.2	74.3	90.5	64.5	80.2	92.9	82.2	90.4	88.9	78.0
	7 days	26.3	37.8	89.8	43.5	82.2	92.0	86.1	86.2	88.9	91.4

ND: Not Determined

Glutamate occupies a key position in amino acid metabolism. In the non-exposed control plants, the percent of glutamate was higher in the leaves than in the root and showed little changes with time (Table 3). The exposure of the plant to air pollutants tended to decrease the percent of glutamate with a few exception as in the case of the trifoliate and primary leaves of the plants exposed to 4.0 pm NO_2. The decrease in the percent of glutamate might mainly be due to the elevated percent of the amides by the gas exposures as mentioned above.

Table 3 Percent of glutamate over total amino acids in kidney bean plants exposed to NO_2 and/or O_3 at various contentrations

	NO_2 (ppm)	0	2	4	0	0	0	2	2	2	4
	O_3 (ppm)	0	0	0	.1	.2	.4	.1	.2	.4	.2
Trifolate Leaf	2 days	37.9	28.4	14.4	3.8	26.2	24.1	2.9	23.2	17.4	0.4
	4 days	27.4	21.6	42.4	22.3	15.8	14.6	13.6	17.0	7.0	9.1
	7 days	27.7	18.0	28.6	21.2	18.6	13.4	11.2	9.1	14.9	5.1
Primary Leaf	2 days	18.8	16.5	42.4	13.2	9.8	5.8	2.8	3.3	5.8	0.8
	4 days	26.7	18.1	34.4	15.3	19.7	10.9	5.7	6.4	4.5	2.2
	7 days	18.8	8.8	4.7	15.0	9.9	3.3	3.8	3.4	4.3	0.2
Root	2 days	0.1	0.1	0.6	1.3	0.3	0	0.1	0	0.1	0
	4 days	3.2	0.2	0.0	2.6	0.1	0	0.3	0	0.1	0
	7 days	5.3	3.7	0.1	5.8	0.3	0	1.6	3.9	0.0	0

Few peaks unidentified with a standard amino acid mixture appeared in the region of acidic amino acids (Fig. 3). The peaks were detected only when the plants were exposed to NO_2. The peak intensities increased with the concentration of NO_2 exposed. The peak #1 was assumed to be ureides such as allantoin, but not yet critically identified. The retention time of the peak #2 was 16.82 ± 0.19 (n=15), which was almost identical with that of glutathione (16.96). The intensities of the peak #2 were not so great as compared with those of neighboring well-known amino acids like aspartate and threonine, which occupied the small portion in total amino acids.

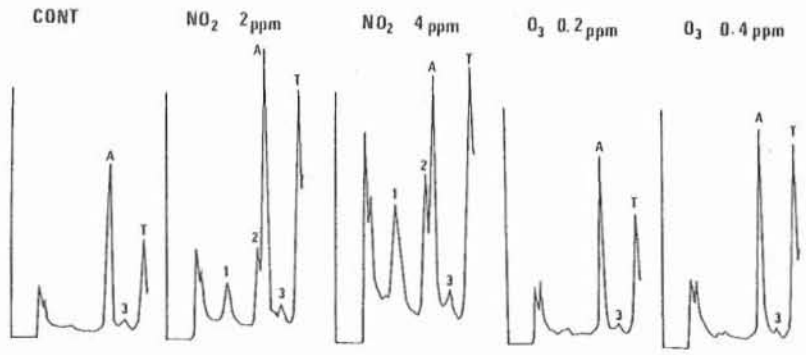

Fig. 3 A part of chromatograms from amino acid autoanalyzer. Extracts from the primary leaf of kidney bean plants exposed to NO_2 at 2.0 and 4.0 ppm or O_3 at 0.2 and 0.4 ppm for 7 days were loaded on the analyzer. A: Aspartate, T: Threonine. The peak #1, 2 and 3 could not be identified with a standard amino acid mixture.

The calculation of correlation coefficient between 16 species of amino acids, ammonium and total amino acid was carried out using the data from the long-term experiment, which consisted of ten treatments and three sampling points (n=30) for each component in the trifoliate and primary leaves of kidney bean plants (Fig. 4). There were 11 and 9 species of amino acids or ammonium which had the correlation coefficient of more than 0.7 with total amino acids in the trifoliate and primary leaves, respectively. Threonine, serine, glycine, alanine, phenylalanine, arginine and asparagine were included in both plant parts. Aspartate, glutamine, tyrosine and GABA were less correlated with total amino acids ($r < 0.7$) in both parts. Among amino acids tested here, threonine and serine had the highest frequency of possessing the correlation coefficient of more than 0.7 with other amino acids. On the other hand, there was no example where glutamine clearly showed any correlations with other amino acids. The pairs of amino acids which were reciprocally correlated with the coefficient of 0.8 to 0.9 in both plant parts were threonine-serine, threonine-phenylalanine, serine-alanine, serine-phenylalanine and asparagine-total amino acids. The amides was well correlated with total amino acids with the coefficients of 0.88, 0.87 and 0.99 in the trifoliate, primary leaves and root, respectively. It is known from the above analysis that the increase in the percent of the amides by gas exposures (Table 2) is mostly explaiend by asparagine.

Trifoliate leaf

	Asp	Thr	Ser	Glu	Gln	Gly	Ala	Val	Cys	Ile	Leu	Tyr	Phe	r-ABA	NH_3	Arg	Asn	T-A
Asp		*	**				*		*				*					
Thr			**				***	***	*	*	*	*	**	*	*			*
Ser		**				*	**	*	**				**				*	*
Glu						**												
Gln																		
Gly		**	***															*
Ala		*	**			**		**	*			*	*	*	**		*	*
Val		*								**	**	*	**	*	*			**
Cys			*			*				***				*			*	
Ile		**	*			*		*			**				*			
Leu		*																
Tyr										**					*			
Phe		**	**			*				**	**							*
r-ABA												*						
NH_3				**														*
Arg																		*
Asn		*	***			**	**		*	*			**					**
T-A		*	**	*		**	*		*	*	*		*			*	**	

Primary leaf

Fig. 4 The correlation between the individual amino acids, ammonium and total amino acids. The number of values used for the calculation of correlation coefficient(r) was 30. ***: $0.9 \leqq r$, **: $0.8 \leqq r < .9$ and *: $0.7 \leqq r < 0.8$.

Discussion

Nitrogen dioxide absorbed by the leaf is dissociated to the liquid phase in the forms of nitrate and nitrite ions, which are then reduced to ammonium ion if the activities of reductases correspondent to nitrate and nitrite exist. Since the plants used in the present experiment were fertilized with nitrate nitrogen as the basal application, it is assumed that both reductases had already been induced before the start of the gas exposure. Trace amounts of nitrate and nitrite were detected by the colorimetric analysis (data not shown), which suggests that the absorbed NO_2 rarely accumulates in the form of nitrate and nitrite. The concentration of ammonium was significantly increased by the exposure to NO_2 alone or in combination with O_3, while the most drastic increase in the amount occurred in glutamine within 2 hour (Fig. 1a and c). The result indicates that the absorbed NO_2-nitrogen is temporally stored in glutamine when the plants are exposed to high concentration of NO_2 that is thought to be above the plants' capabilities to maintain the normal metabolism. The extreme accumulation of glutamine may be caused by the weakness of its further assimilation. The first step of glutamine metabolism is mediated by glutamate synthase which transfers amide base of glutamine to 2-oxoglutarate to form two molecules of glutamate (Lea and Miflin 1974). The activity of the enzyme may not be high enough to convert the enormous amount of glutamine produced from NO_2 to glutamate, from which various species of amino acids are formed by transamination reaction and thus which is located in a key position of amino acid metabolism.

When the plants were exposed to NO_2 alone or in combination with O_3 for more than two days, the concentration of total amino acids and the percent of the amides in total amino acids increased (Fig. 2 and Table 2) as observed in the short term experiment. However,the low correlation coefficient of glutamine with total amino acids (Fig. 4) demonstrates that the increase in the percent is mostly explained by asparagine not by glutamine. Glutamine which accumulates extensively in ealry phase of the gas exposure seems to be gradaully converted to other amino acids, above all asparagine which is highly correlated with total amino acids (Fig. 4) and is thought to be a more stable storage form of amino acids.

The increase in the amino acid pool size was observed also by the exposure to O_3 in both short- and long- term experiments (Figs. 1b and 2). Ting and Mukerji (1971) reported the same phenomenon in cotton plants exposed to O_3 at 0.8 ppm for an hour. The increase in the amino acid pool size by the O_3 treatment can be considered as the result of the inhibition of protein synthesis or the promotion of protein degradation. Tomlinson and Rich (1967) supported the former assumption on the basis that the incorporation of ^{14}C into protein fraction was reduced to one third of the control by the O_3 treatment. Ting and Mukerji (1971) proposed the evidence favorable to the latter according to the fact that the amount of soluble protein was reduced by O_3. The determination of soluble protein was performed in the present experiment, while insignificant changes were obtained during 8 hours' exposure to O_3 at 0.4 ppm (data not shown). It still remains unclear how and from which source amino-nitrogen is supplied in O_3-treated plants.

The increase in amino acids by the O_3 exposure was most striking in the root (Fig. 2c). There might be two possibilities to explain the above phenomenon. One is that the root directly responds to O_3 penetrating from atmosphere to the vermiculite which has a relatively large portion of air space. According to Blum and Tingey (1977), however, the penetration of O_3 is limited only to the surface of soil. The other is a rather indirect effect, that is, the promotion of the translocation of amino acids formed in the leaves to the root, or the production of a substance stimulating the enlargement of amino acid pool size in the root.

The exposure of the plants to O_3 is known to cause stomatal closure (Hill and Littlefield 1969). In fact, nitrogen uptake rate from NO_2 was remarkably reduced upon the mixed exposure to NO_2 and O_3 (Ito et al. 1984a), probably because the penetration of NO_2 into the leaf tissue is prevented by the increased stomatal resistance. From the view-point of amino acid concentration, however, little difference was found between the short term exposure to NO_2 alone and in combination with O_3 (Figs. 1a and c). In spite of the reduction of NO_2 penetration, the amount of amino acids may be considerably enhanced by the supply from the degradation processes of proteins initiated by O_3.

In the long run, the concentration of amino acid and the percent of total amino-N over total-N in the mixed exposure clearly differed from those in the exposure to NO_2 alone (Figs. 2 and 3). Their trends were similar to that of O_3, suggesting that the effect of the mixed exposure on amino acid content is a rather ozone-dominating type. As for the amino acid composition, all the three treatments resulted in some differences, especially in amides (Table 2). It may be possible to distinguish the effects of the three treatments on a basis of amino acid content and composition.

Ureides are considered as important substances for the transport of nitrogen gained by nitrogen fixation from roots to leaves in leguminous plants (McClue and Israel 1979). Nitrogen metabolism may be activated by the NO_2 exposure, thus the excess nitrogen may be converted to ureides, which then are translocated to the leaves and accumulate there. Although the area of the peak #1 in Fig. 4, which is thought to be correspondent to ureides, is relatively small, the significant amount must be present as their sensitivity to ninhydrin is very low.

The appearance of glutathione was characteristic to the NO_2 exposure, though its amount was not so great. Glutathione readily becomes a reduced form catalyzed by glutathione reductase (Wirth and Latzko 1978). It is considered that its reduced form functions in the stabilization of cysteine, homocysteine and protein by maintaining their thiol group, and in the detoxication by degrading the deleterious amount of H_2O_2 (Wolosiuk and Buchanan 1977). The accumulation of glutathione may be a metabolic adaptation of the plants to alleviate the detrimental effect of NO_2 which is thought to be a strong oxident. It is reported that the content of ascorbate is decreased by the exposure to 1.0 ppm NO_2 for 2 hrs every day (Prasad and Rao 1980). This may result from the oxidative decompositon by NO_2.

The majority of amino acids was well correlated with total amino acids in both trifoliate and primary leaves (Fig. 4). This means that the amount of the individual amino acids is increased by the gas exposures and its change proceeds to keep balance as a whole. No example was found for glutamine to have high correlation with other components. This fact suggests that the amount of glutamine changes in a peculiar manner. There were cases where the amides (glutamine and asparagine) became dominant upon the gas expousres especially the mixed exposure of NO_2 and O_3. The increase in the amides must be mostly brought about by asparagine, and not by glutamine, as known from Fig. 4 where the high correlation is obtained between asparagine and total amino acids. Asparagine is reported to accumulate in the plants deficient in minerals such as K, S, P, Mg, Zn and so on (Stwart and Larther 1980), and in water stressed plants (McMichael and Elmore 1977), although the reason for its accumulation under such stressed conditions remains unsolved.

Amino acids can be classified in several categories such as glutamate, aspartate, pyruvate, serine, aromatic families and so on (Manicol 1977), according to the similarity of the synthetic pathway. It is anticipated that amino acids belonging to the same family show the similar behaviour when the environments around the plants are changed. In this experiment, though, it is not the case except with the serine family whose members like serine, glycine and cysteine have relatively high correlation with each other (Fig. 4). The incorporation of ^{13}C from $^{13}CO_2$ into

glycine and serine is enhanced and their amounts are also increased by the exposures to NO_2 and O_3 alone or in combination (Ito et al. 1984b). Good correlation was found between threonine-serine and phenylalanine, and serine-alnine and phenylalanine, even if they belong to different families. Whether the high correlation between them is only accidental or has some meaning is unknown.

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インゲンマメに対する二酸化窒素とオゾンの単独及び複合暴露の影響
Ⅱ　アミノ酸含量と組成

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二酸化窒素とオゾンの単独及び複合暴露がインゲンマメのアミノ酸含量と組成に及ぼす影響を調べることを目的として本実験は行われた。二酸化窒素4.0ppm の短時間暴露により全アミノ酸含量の急激な増大が認められ，その大部分がグルタミンによっていた。全アミノ酸はオゾン処理によっても暴露開始後２時間目からゆっくりではあるが増加した。複合暴露は二酸化窒素単独の場合とほぼ同様な傾向を示した。

暴露期間が播種後10日目から２日から７日に延ばされた場合には，すべての区において全アミノ酸含量の増加が認められ，特に二酸化窒素4.0ppm 区において顕著であった。根においてはオゾン暴露により全アミノ酸含量がより高められた。長期暴露においては，グルタミンに代わってアスパラギンが主要な蓄積形態であった。特に複合暴露の場合にアスパラギンの占める割合が増大した。これに反してグルタミン酸は減少する傾向を示した。これ等の結果は暴露初期に著しく蓄積するグルタミンは暴露時間の経過に伴ないゆっくりと他のアミノ酸，特にアスパラギンに転換されていくことを示唆している。汚染ガス暴露によって引き起こされる個々のアミノ酸含量の変動をみてみると，セリングループに属するグリシン，セリン，システインの間に高い相関が認められた。

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Effects of NO_2 and O_3 Alone or in Combination on Kidney Bean Plants. III. Photosynthetic $^{13}CO_2$ Assimilation Observed by ^{13}C Nuclear Magnetic Resonance

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The effect of exposures to NO_2 and O_3 alone or in combination on the fate of $^{13}CO_2$ assimilated by photosynthesis was examined by means of ^{13}C-NMR with kidney bean primary leaves fed with $^{13}CO_2$ for 10 min. There were more than 70 peaks appearing in the ^{13}C-NMR spectra of ethanol and water soluble fraction from leaf extract, among them 16 relatively well resolved peaks, correspondent to 3 sugars, 2 organic acids and 4 amino acids, were selected for the determinations of the pool size and the ^{13}C incorporation.

Although the gas exposure increased the amount of sucrose and fructose, the increases were not accompanied by the incorporation of the ^{13}C label, which suggests the presence of photosynthetically inactive pools of sucrose and fructose. This phenomenon was especially remarkable in the case of O_3 treatment where no ^{13}C incorporation into sucrose was observed within 10 min. The pool size of glycine and serine and ^{13}C incorporation into them were increased by the gas exposure. This implies that carbon flow through glycolate pathway is stimulated in the leaves exposed to NO_2 and/or O_3.

The incorporation of ^{13}C into alanine was stimulated by NO_2 exposure both alone or in combination with O_3, but not by exposure to O_3 alone. The activation of amino acid metabolism by NO_2 assimilation may accelerate the carbon flow from photosynthetically fixed CO_2 to alanine through three carbon-intermediates in the reductive pentose cycle.

It is proven in the present study that, with only simple procedure of sample preparations, ^{13}C-NMR provides satisfactory informations on the metabolic changes in the photosynthetic pathway under the conditions stressed by the air pollutants.

Key Words: NO_2 – O_3 – Kidney bean – Photosynthesis – CO_2 assimilation – ^{13}C-NMR.

Recent studies have proven that Fourie transform nuclear magnetic resonance (FT-NMR) is a useful tool for the study on metabolism in the living materials as it offers an enormous amount of informations on each carbon atom of molecules present in inhomogenous samples. Due to this great advantage objective compounds can be observed by FT-NMR with only a little single separation and purification procedures. The improvements of sensitivity and versatility

achieved by the development of high magnetic field NMR made it possible even to delineate metabolism in whole cells and intact organs.The FT-NMRis thought to be the only method that has the potential to follow biosynthetic pathway rapidly and non-destructively. Researches performed with meticulous attention to the intactness of samples have so far been confined to bacteria and animals (Shylman 1979). In most of those studies phosphorus is selected as a target nucleus to get information about the quantity, and kinetics of phosphorus metabolites or to determine physiological environment and intracellular pH (Gadian and Radda 1981). The oxygenated *Saccharomyces cerevisiae* revealed a higher pH by 0.5-0.7 pH unit and a higher ATP level than those of resting and anaerobic cells, suggesting the operation of active proton transport (Navon et al. 1979). A similar phenomenon was observed with phototrophic bacterium *Rhodopseudomonas shaeroides* when they were illuminated by argon ion laser beam in the NMR tube located at superonducting fields (Nicolay et al. 1981).

The ^{13}C NMR has been used for isotopic tracer experiment as its natural abundance is sufficiently low for that purpose. Hollander et al (1979) calculated backward/forward ratios of aldolase-triosephosphate isomerase reactions in yeast cells supplied with 1-and 6-^{13}C glucose from the accumulated spectra in 1 min blocks. Attempts have already been made to apply to this field the ^{15}N, which has much lower sensitivity to NMR measurement than those isotopes (^{31}P and ^{13}C) mentioned above. The incorporation of ^{15}N into nitrogenous compounds which stand at intermediary points of nitrogen metabolism was studied with intact mycelia of *Neurospora crassa*, and the turnover time of glutamine was estimated from the results (Legerton et al. 1981). NMR studies with intact tissues and organs have recently made further great progress in medical science with increasing needs to observe metabolic activities for the diagnosis of patients by non-invasive manners.

In contrast, the application of NMR to plants is far behind. The intracellular pH changes caused by fusicoccin treatment (Roberts et al. 1981) and the difference in the cytoplasmic and vacuolar pHs (Roberts et al. 1980) were estimated with detached maize root tips. Protein incorporation of asparagine,which is thought to be a major translocating nitrogenous substance from roots, was followed by magic-angle single and double cross-polarization ^{13}C and ^{15}N NMR with lyophilized soybean cotyledons (Schafer et al. 1981). These are pioneer works where NMR technique was applied to study the physiology of plant tissues, although those plant tissues are not intact.

In other attempts, crude extracts or partially purified compounds from plant materials were subjected to NMR measurements. Photorespiration was characterized by the appearance of ^{13}C-^{13}C doublet in anomeric carbon of glucose component of sucrose separated from soybean and corn, which had been fed with $^{13}CO_2$ under various CO_2 and O_2 concentrations (Schafer et al. 1980). The incorporation of ^{13}C into photosynthetic metabolites was detectable by NMR within 30 sec after the feeding of $^{13}CO_2$, and the differences in CO_2 assimilation pathways between C_3 and C_4 plants were clearly manifested in ^{13}C-NMR spectra of ion-exchanged fractions (Mitsumori 1982).

Photosynthetic CO_2 assimilation has been investigated by means of ^{14}C as the only tracer with an enormous number of plant species and under different environmental conditions, though it requires rather complicated, skilful and laborious techniques. The ^{13}C NMR, however, has a great advantage over the conventional method on account of its simplicity of sample preparations and the extensive amount of obtainable information in one analysis.

In the previous papers (Ito et al. 1984a and b) we reported that the exposures of kidney beans to O_3 and/or NO_2 resulted in considerable changes in the level of active metabolites such as sugars and amino acids. This implies that CO_2 assimilation pattern might be modified by these gas exposures. The present study was undertaken to elucidate this point with the analytical

assistance of ^{13}C-NMR.

Materials and Methods

Plant Culture

Kidney bean plants (*Phaseolus vulgaris* L. cv. Shin-edogawa) were grown in the growth cabinet under exactly the same conditions as mentioned in the previous paper (Ito at al. 1984a). The plants were transferred to the gas-exposure chamber 10 days after the germination and exposed to either O_3 at 0.2 ppm, NO_2 at 2 ppm alone or in combination. The gas concentration was maintained at those levels throughout the gas exposure treatments.

$^{13}CO_2$ Feeding

Twelve plants were selected from more than 70 plants in each treatment on the basis of visible uniformity of the height and leaf size. One of the primary leaves in each plant was placed inside a gas-tight chamber where cooling units, a heater and a humidifier were installed to make an automatic regulation of the temperature and humidity at the same level as outside the chamber, and were sealed at their petioles with adhesive vinyl tapes. The air inside was replaced by CO_2-free air to deplete non-labeled CO_2. When CO_2 concentration went down to 30 to 50 ppm, for which it usually took 10 to 12 min, 1 N HCl was injected through a tygon tubing to $Ba^{13}CO_3$ (30 atom %) suspension in a beaker placed inside the chamber with continuous stirring. The CO_2 concentration was monitored with an infrared CO_2 gas analyzer (Fuji Electric CO., ZFD) and maintained at around 350 ppm throughout the feeding experiment. The primary leaves were taken out from the sealed chamber 10 min after the injection of HCl and put into liquid nitrogen as quickly as possible. Opposite leaves of the fed leaves were also harvested as zero time controls at the end of the experiment. It has been proven that there is a negligible intermovement of photosynthates between the primary leaves within a short term period.

Sample Preparation for NMR Measurement

The frozen leaves were homogenized with 80% ethanol by a Polytron (type PT 10/35 from Kinematica in Switzerland). The homogenates were kept in a refrigerator overnight for the further extraction. The residues were removed with Toyo filter paper No.6. The filtrates were then evaporated completely dry and dissolved with a small volume of distilled water. Chlorophyll insoluble to water was spun down by the centrifugation at 15,000 rpm for 10 min. The supernatants were again dried up and supplied for the NMR mesurement.

NMR Measurement

The dried ethanol and water soluble compounds were dissolved with a small volume of water and pH was adjusted at 7.0 with 1N NaOH and HCl. In the case of the formation of the suspended matter as a result of pH changes, another centrifugation was performed. The final volume was adjusted to 2.0 ml which contained D_2O at 10% to achieve the field frequency locking. The size of sample tubes was 10 mm in outside diameter. The ^{13}C-NMR spectra were obtained by Bruker SXP-4-100 spectrometer in the pulsed Fourier transform mode operated at 22.63 MHz with wide band proton noise decoupling (10 watts). The temperature of samples was maintained at 5° C throughout the experiment. For all samples in the present experiment 50,000 free induction decays were accumulated after 45° pulses with a recycle time of 1.0 sec. The quadratune phase detection was employed. Data points were 4K × 2 words for the spectral witdh of 6,000 Hz. Fourier transformation was done in 8K × 2 words with zero supplements. To improve the signal to noise ratio the digital exponential window was employed which caused the 1 Hz line broadening.

Chemical shifts calculated by $(\gamma s - \gamma ref)/\gamma ref \times 10^6$ in which γs and γref are the absolute resonance frequencies of a particular sample and the reference peaks, respectively, were reported with respect to TMS (tetramethylsilane). Neat methanol sealed in the coaxial capilary tube was used here as an actual reference whose chemical shift value was determined at 48.8 ppm downfield from that of TMS.

Results and Discussion

^{13}C-NMR Spectra

Primary leaves used in this experiment for $^{13}CO_2$ feeding had nearly reached the fully expanding stage at the start of gas exposures. The leaves were kept in atmosphere containing air pollutants for four full days, which resulted in the most severe influences from the gases compared with leaves at other leaf positions. The leaves turned darker green by NO_2 treatment than the ones in control, probably due to the enhancement of chlorophyll synthesis from the assimilated NO_2. Minute silvery flecks were associated with O_3 treatment. When NO_2 and O_3 were exposed in combination, the visible symptom seemed to be an O_3-dominant type. The flecks grew into the coalescent lesions to form necrotic large spots. The leaf fresh weight was also affected by gas exposures. The NO_2 exposure caused only a slight decrease in fresh weight, 1.02g per leaf over 1.10g control, while the significant changes were obtained in O_3 and mixture treatments, 0.95g and 0.78g per leaf.

Fig. 1 shows ^{13}C-NMR spectra of the ethanol-water soluble fraction of the leaf extract from control and from O_3 treatment. Three sugars, four amino acids, two organic acids, and an unknown compound were identified in the spectra. The presence of a considerable amount of sugars such as sucrose, glucose, fructose rather complicates the spectra, because they give totally 36 peaks in theory, most of which stand at the region of 60–80 ppm and are undistinguishable from each other. The peaks correspondent to sucrose 2′ and 5′, glucose-β1 and fructose-2 were relatively well-resolved and satisfactory enough to be used for the quantitative determination. The peaks of sucrose-1 and glucose-α1 are unseparated but looked quite sharp. The peak intensities of carboxyl carbons in amino acids and organic acids were suppressed due to the too short recycling time and the lack of nuclear overhauser effect as compared with those expected from other carbons in these compounds. There were two weak peaks which can be slightly distinguished over the noise level. They are unseparated glycine and serine-1 and the unknown compound. The hitherto unknown compounds has not yet been indentified clearly, while it is assumed to be 2-methyltetronic acid or its isomer on the basis of the signal location of its methyl carbon at 22.6 ppm and carboxyl carbon at 181.9 ppm (Mitsumori 1982).

Peak intensities measured from the spectra shown in Fig. 1 were calibrated against those for methanol used as an external standard at evey measurement, and then were expressed per gram fresh weight. The results are shown in Fig. 2. The white bar represents the peak intensity of the zero time sample which contains ^{13}C at natural abundance, and is thought to be quantitatively correlated with the pool size of each compound. The dotted bar represents the peak intensity of labeled samples fed with $^{13}CO_2$ for 10 min. The differences in the peak intensity between zero time and labeled samples must correspond to the incorporation of ^{13}C into the compound.

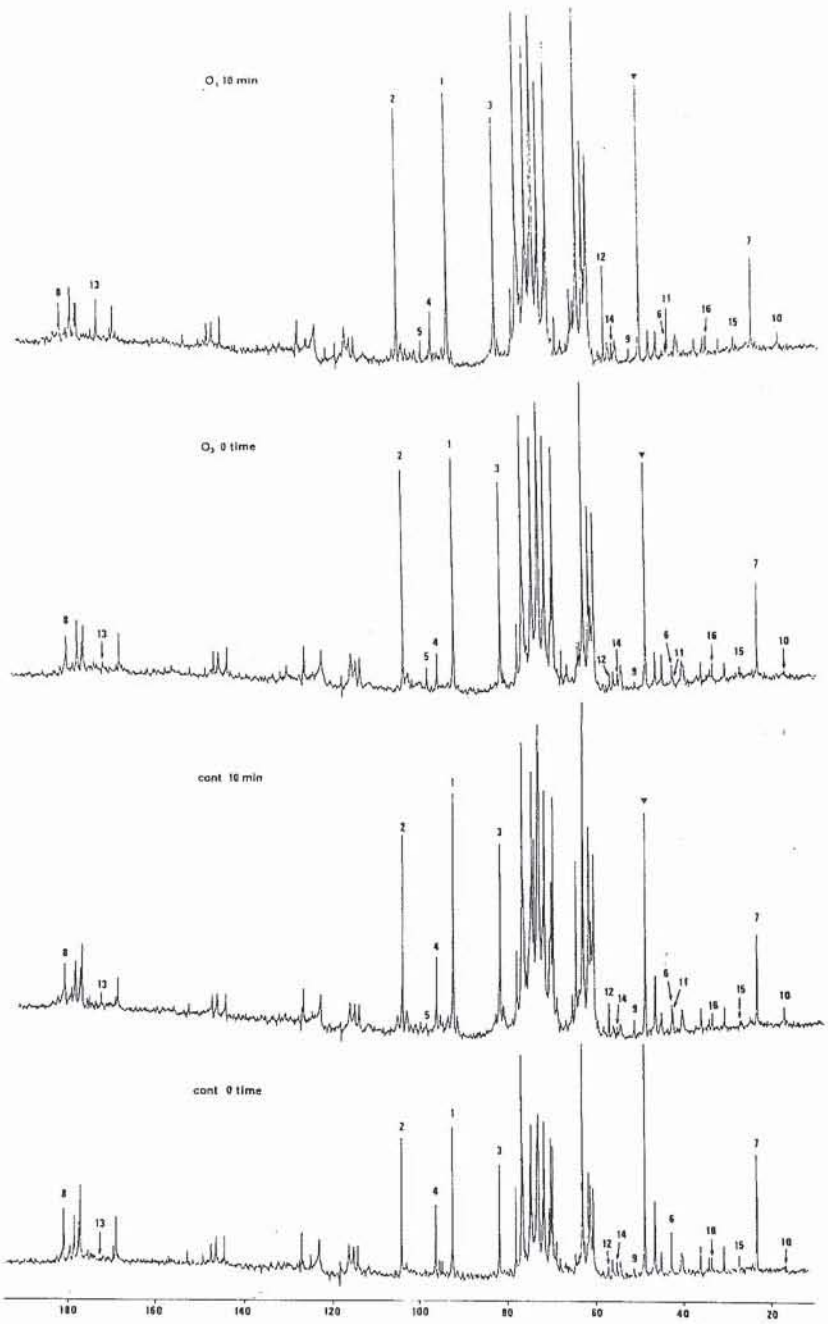

Fig. 1 ^{13}C-NMR spectra of ethanol-water soluble fraction from kidney bean primary leaves before and after $^{13}CO_2$ feeding for 10 min. The lower two spectra are from non-gas exposed control plants and the upper two from O_3 exposed plants. Peak numbers are as follows: (1) sucrose-1 and glucose-α-1, (2) sucrose-2′, (3) sucrose-5′, (4) glucose-β-1, (5) fructose-1, (6) malate-3, (7) unknown acid-CH_3, (8) unknown acid-COOH (9) alanine-2, (10) alanine-3, (11) glycine-2, (12) serine-2, (13) glycine-1 and serine-1, (14) glutamate-2, (15) glutamate-3 and (16) glutamate-4.

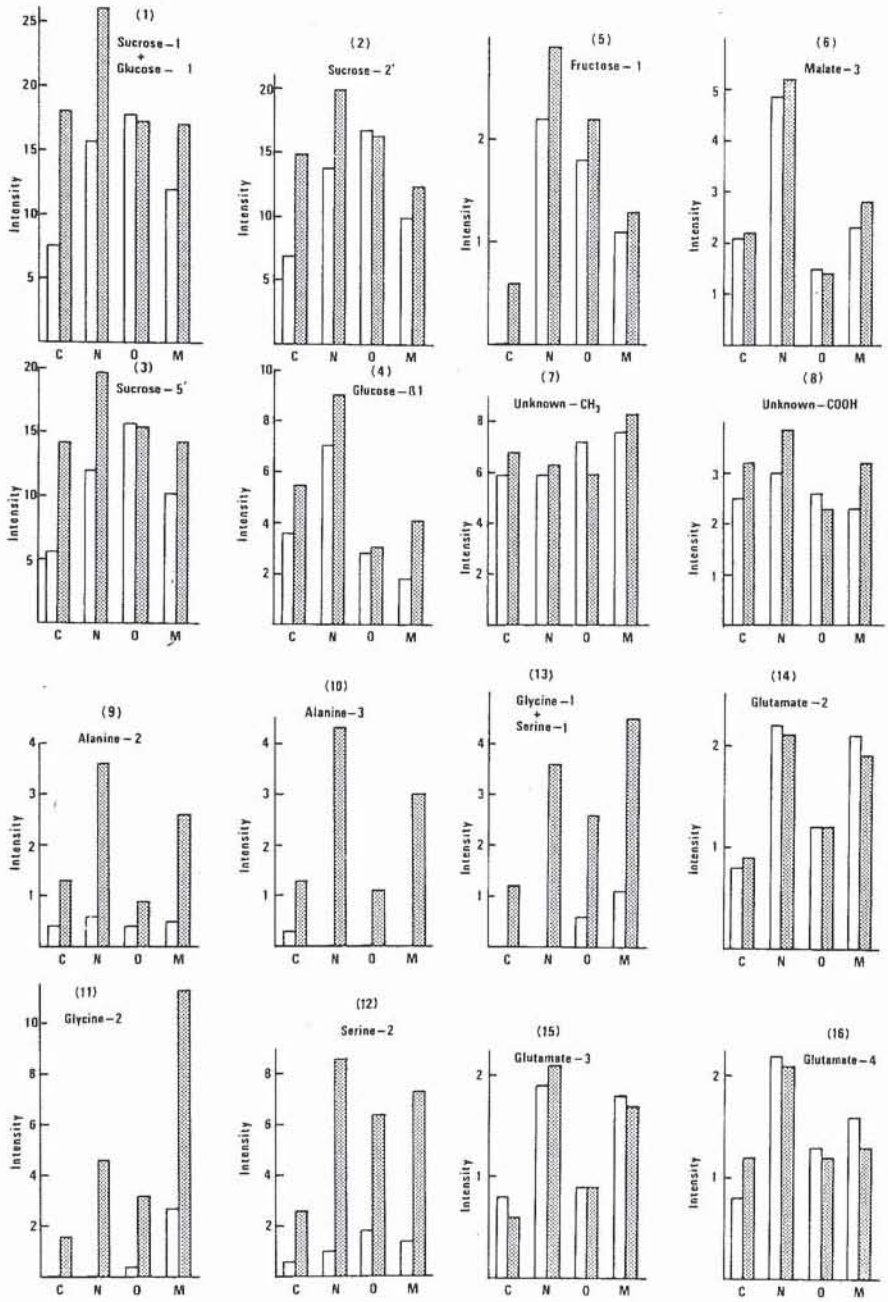

Fig. 2 Peak intensities of individual atom of ethanol and water soluble compounds observed in ^{13}C-NMR spectra. The white bar represents peak intensity at zero time and dotted bar at 10 min after $^{13}CO_2$ feeding. C: control, N: NO_2 exposure at 2 ppm. 0: O_3 exposure at 0.2 ppm and M: Mixed exposure to NO_2 and O_3 at the same concentration as the single exposure. The number above the name of each compound corresponds to peak number shown in Fig. 1.

Sugars

It is shown from the peak intensity at zero time of sucrose-2′ and 5′ that the amount of sucrose is significantly increased by all gas treatments. The highest amount was observed in the leaves exposed to O_3 at 0.2 ppm. Barnes (1972) reported that the chronic exposure of pine seedlings to 0.05 ppm O_3 for 11 weeks resulted in significantly higher amounts of total soluble sugars mainly consisting of sucrose. Little change in sucrose level, however, is obtained with lemon seedlings exposed to 0.25 ppm O_3, 8 hours a day, 5 days a week for 9 weeks (Dugger et al. 1966). The discrepancy between those two reports may come from the difference in plant species used, analytical method or some other factors.

In our experiment,no incorporation of ^{13}C into sucrose was observed in O_3-treated leaves, which meant a negligible direct synthesis of sucrose from photosynthetic assimilates. Other sources of sucrose should be considered to account for its increased level, which will probably be supplied from the degradation of starch. It has been reported that the amount of starch decreased in lemon seedlings exposed to O_3 (Dugger et al. 1966). The degraded starch might be converted into sucrose and temporarily stored in its form in O_3 exposed kidney bean leaves. In the experiment which has been performed exactly by the same manner as the present studies, O_3 exposure was reported to decrease the export of ^{13}C-labeled photosynthetates from the primary leaves to other plant organs (Okano et al. 1984). The inhibition of ^{13}C incorporation into sucrose may be one of the causes to decrease the translocation of photosynthetically assimilated CO_2, as sucrose is a major translocating substance among photosynthates (Ziegler 1975).

From the experiment with $^{14}CO_2$ as a tracer, it is substantiated that the distribution of ^{14}C among soluble compounds in pine seedlings exposed to O_3 was decreased in soluble sugar fraction where sucrose was predominant, and was increased in sugar phosphates (Wilkinson and Barnes 1973). The result suggests that enzymes converting sugar phosphates derived from Calvin cycle to sucrose are impaired by O_3 exposure. Unfortunately this could not be proven in our experiment because the level of sugar phosphates was below the sensitivity of ^{13}C-NMR.

In the treatments other than O_3 exposure, considerable incorporation of ^{13}C into sucrose was observed within 10 min. Since the present experiments have not been performed quantitatively, the real specific activity of each carbon in a compound cannot be calculated. The ratio of the difference in peak intensities of zero time and 10 min over one at zero time, however, must have a direct relationship with specific activity. When we look back on the data from this point, specific activities of sucrose-2′ and 5′ in the control seem to be the highest, which suggests the most active turnover.The incorporation of ^{13}C in NO_2 and mixed treatments was lower than that in the control in spite of larger pool sizes, resulting in lower specific activities. This suggests the presence of photosynthetically inactive pool in those two treatments. NO_2 seems to alleviate the complete inhibition of ^{13}C incorporation into sucrose by O_3 exposure when it is mixed with O_3. The mixture of NO_2 and O_3 had produced the synergistic effect on the appearance of visible symptoms and the growth depression as shown in the previous paper (Ito et al. 1984a). The alleviative effect of NO_2 is contrary to the generally known observations reported so far. Its mechanism has not yet been clarified.

Glucose showed somewhat different behaviors from those of sucrose.The pool size was the highest in the NO_2 exposure, but the pool size in O_3 and mixed treatments was lower than that in the control unlike in the case of sucrose.The significant incorporation of ^{13}C into glucose was observed except in O_3 treatment where the incorporation was negligible as the same as sucrose. The peak intensity of fructose was too weak to be detected in the control at zero time, while a considerable amount of fructose was present in the gas-exposed leaves. Prasad and Rao (1980) reported the appearance of fructose, which was absent in the control plants, by the exposure of wheat plants to 1 ppm NO_2 for 2 hrs daily over a total period of 80 days. In spite of the small pool

size, the control plant showed relatively high incorporation of ^{13}C into fructose which will certainly resulted in the highest specific activity, suggesting faster turnover than any other treatments.

Organic Acids

Malate seems to be photosynthetically inactive in all treatments due to little incorporation of ^{13}C into its C-3 position carbon.Since the kidney bean belongs to C_3-plant, malate will receive ^{13}C-label from CO_2 through sugars, which are then metabolized via Embden-Meyerhof pathway and enter TCA cycle. Because of that fact and the supression of the glycolysis and TCA cycle operation under photosynthetic condition, it will need a much longer feeding time to observe the ^{13}C label detectable by NMR in malate. The amount of malate in the NO_2-treated leaves was remarkably larger than in other treatments. This may be the result of the increasing demand for organic acids, substrates of amino acid synthesis which was greatly enhanced by NO_2 exposure.

The acid tentatively called "the unknown acid" (UA) in this paper will probably be the isomer of 2-methyl tetronic acid or its closely related compounds. The ^{13}C-NMR spectra of partially purified acid were similar to the spectra of the acid isolated from sunflower leaves (Mitsumori 1982). Since the slight differences was observed in chemical shift value of its methene carbon, we still cannot definitely identify the compound. The presence of 2-methyl tetronic acid in kidney beans had been confirmed by Schramn (1979) with GC-MS. Although UA present in both kidney beans and sunflowers in relatively high concentration as compared with other substances, there had been so far no report relating to its physiological function. The gas exposures caused insignificant changes both in the amount of UA and in the incorporation of ^{13}C into UA.

Amino Acids

Comparing peak intensities of alanine-2 and 3 at zero time, we found a slight disagreement there. No significant difference in pool size of alanine was demonstrated from the result of alanine-2, while as for alanine-3 the apparent peak above the noise level was observed only in the control. Fundamentally both should show a similar trend. This may be due to the error in measuring the peak height which is extremely small especially in unlabeled zero time samples. Then it can be conclusively mentioned that the gas exposures hardly affect the pool size of alanine in the leaves. Ting and Mukerji (1971) reported a slight reduction of alanine in cotton leaves exposed to 0.8 ppm O_3 for 1 hr.

The ^{13}C label may be introduced into alanine from $^{13}CO_2$ through three-carbon intermediates in Calvin cycle, which is finally converted into pyruvate before the transamination from glutamate. The appearance of the label in alanine will indicate the rapid progress of the above mentioned pathway. Actually the considerable ^{13}C incorporation into alanine was observed in all treatments within the $^{13}CO_2$ feeding period chosen in this experiment (10 min). The ^{13}C incorporation was especially enhanced by NO_2 exposures alone or in combination with O_3. The stimulation of amino acid metabolism by NO_2 exposure may accerelate the flow of carbon from photosynthetically fixed CO_2 to alanine. It is concluded that in NO_2 treated leaves alanine shows more rapid turnover than in the other two treatments. On the other hand, the O_3 exposure resulted in the similar incorporation of ^{13}C into alanine with the control.In contrast to our result,the distributionof ^{14}Cin alanine among soluble compounds was remarkably increased in the white pine (Wilkinson and Barnes 1973) and the bean (Tomlinson and Rich 1967) exposed to O_3.

Glycine and serine are thought to be related with photorespiration, and hold an important position in glycolate pathway through which a part of the fixed CO_2 flows fast especially in

C_3-plants like kidney beans used in this experiment (Zelitch 1971). The signal of carboxyl carbon for glycine and serine overlaped at the similar chemical shift and cannot be separated. The pattern of its peak intensity, however, was nearly identical to that of glycine-2. Therefore, this carboxyl signal may correspond mainly to glycine. The amount of glycine was increased by O_3 alone and mixed exposures of O_3 and NO_2. This trend stood out particularly in the latter treatment. The incorporation of ^{13}C into glycine was made much faster by all treatments. A similar tendency was observed in serine as regards ^{13}C incorporation, but in conjunction with the pool size the increase was not as intensive as in glycine. Those results suggest that the gas exposure should accelerate the carbon flow through glycolate pathway. It had been shown that the enrichment of nitrogen nutrient in the medium increased enzyme activities relating with photorespiration such as RuBP oxygenase, glycolate oxidase and catalase (Cresswell et al. 1979, Fair et al. 1974). In pine leaves exposed to O_3, the distribution of ^{14}C in serine was greatly increased as compared with control plants (Wilkinson and Barnes 1973). Such reports support the assumption derived from our result that the photorespiration should be enhanced by the exposure to NO_2 and O_3.

As for glutamate, the increase of peak intensities during 10 min of $^{13}CO_2$ feeding was insignificant in all treatments, which indicated that the compound located considerably far from the photosynthetic site in metabolic pathway. Three carbons in glutamate (2,3 and 4) gave a consistent trend in terms of its pool size. The exposures of NO_2 alone and in combination with O_3 increase the amount of glutamate. From the view-point that glutamate plays an important role in amino acid metabolism, in other words that various amino acids are synthesized from glutamate as a fundamental substance, our result is quite reasonable because NO_2 receives the active assimilation under the condition used in this experiment which will result in the activation of amino acid synthesis. The amount of glutamate was also increased by O_3 exposure, but not as greatly as by NO_2 exposure.

In the present experiment the ^{13}C-NMR was successfully applied to the studies on photosynthetic CO_2 assimilation pathway which would be affected by many environmental and nutritional factors like O_3 and NO_2 exposure examined here as one of those factors. Although the compounds observable by ^{13}C-NMR are limited to those present at a relatively higher concentration in plant tissues, major changes in metabolic activities can be deduced from the information provided by the ^{13}C-NMR.

In conclusion it is demonstrated by the present experiment that increase in the pool size of sucrose by the exposure to NO_2 and O_3 can be distinguished from the incorporation of ^{13}C label, and that glycolate pathway may be accerelated by the exposure to NO_2, O_3 and their mixture.

Acknowledgement: Authors wish to thank Mrs. I. Takakura for the preparation of figures and typing the manuscript.

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インゲンマメに対する二酸化窒素とオゾンの単独及び複合暴露の影響
Ⅲ　光合成によって同化された $^{13}CO_2$ の ^{13}C-NMR による観測

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光合成による炭酸ガスの同化に対する二酸化窒素とオゾンの単独及び複合影響がインゲンマメの初生葉に $^{13}CO_2$ を10分間供与し ^{13}C-NMR の手法を用いることにより調べられた。葉からの抽出液のエタノール—水画分の ^{13}C-NMR スペクトル上には70以上のピークが確認された。それ等のうち3種の糖，2種の有機酸，4種のアミノ酸に相当する比較的分離の良好な16ピークが，標識炭素の取り込みと含量の測定のために選ばれた。

汚染ガス暴露によりシュークロースとフラクトースの含量は増加するが，標識炭素の取り込みは影響を受けなかった。このことはシュークロースとフラクトースの合成分解には光合成から離れたプールが存在することを示唆している。この現象はオゾン暴露処理の場合に特に顕著で，10分間ではシュークロースへの標識炭素の取り込みは全く認められなかった。グリシンとセリンの含量とそれ等への標識炭素の取り込みは汚染ガス処理により増大した。このことはグリコレート経路を通る炭素の流れが二酸化窒素又はオゾンを暴露された葉においては促進されることを示している。

アラニンへの標識炭素の取り込みは二酸化窒素の単独及びオゾンとの複合暴露により促進されるが，オゾン単独によっては影響されなかった。二酸化窒素の同化によりアミノ酸代謝が活性化されると光合成により固定された炭酸ガスからカルビン回路の三単糖中間体を経てアラニンへの炭素の流れが速められるものと推察される。

本実験により ^{13}C-NMR が，非常に簡単な試料調製だけで，大気汚染ガス暴露などによって引き起こされるストレス条件下での光合成経路における代謝変動に関する有用な情報を与えることが示された。

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Effects of NO_2 and O_3 alone or in combination on kidney bean plants. IV. Alteration of ^{13}C-assimilate partitioning induced by O_3*

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A quantitative tracer experiment was conducted in order to elucidate the effects of continuous exposure of ozone (O_3) at 0.2 ppm on the translocation and the distribution of ^{13}C-labelled assimilates from the individual source leaves in 14-day-old kidney bean plants.

The amount of labelled assimilates exported from the primary leaf, which acted as a main source of photosynthates for the root growth, decreased remarkably by O_3 due to both a considerable reduction of $^{13}CO_2$ fixation (by 62%) and the inhibition of the translocation. Whereas, that from the 1st trifoliate leaf, which mainly nourished the immature growing leaves, was not so decreased by O_3 because a smaller reduction of $^{13}CO_2$ fixation (by 24%) was almost compensated by the acceleration of the translocation. The pattern of the assimilate distribution was altered by O_3, so that a greater proportion of assimilates was partitioned to the growing leaves at the expense of the root and the stem. Consequently, the amount of labelled assimilates translocated to the non-photosynthetic organs (stem and root) decreased by 53%, while that to the photosynthetic organs (leaves) was reduced by only 28%. Those results suggest that the plants might have adapted themselves to O_3 environment so that the reduction of growth efficiency caused by O_3 could be minimized.

Key words: O_3 — Assimilate — ^{13}C — Translocation — Partitioning, — Kidney bean

Ozone, which is a major component of photochemical oxidants, is the most important and widespread of the air pollutants phytotoxic to plants in Japan. Growth responses of plants to O_3 have been extensively documented. Tingey et al. (1971) first reported that O_3 affected differently the growth of various plant parts; the root growth of radish was depressed more severely than the foliage growth. Other studies on soybean (Tingey et al. 1973), carrot (Bennett and Oshima 1976) and crimson clover and annual ryegrass (Bennett and Runneckles 1977) have also shown the root system to be highly sensitive to O_3. Blum and Tingey (1977) demonstrated that O_3 did not penetrate into the soil to cause a direct effect on root growth, rather the reductions in root growth might result indirectly from O_3-induced alterations in foliar metabolism and transloca-

*This study will be published in; *New Phytol.*.97(2) (1984)

tion.

Oshima et al. (1978, 1979) showed the dynamic response of plants to O_3 stress by employing the growth analysis techniques. The relative growth rates (RGR) of both parsley and cotton plants were initially lowered by the exposure of low concentration of O_3 due to the reduction of net assimilation rate (NAR), thereafter RGR in the O_3-treated plants recovered above that of the contorl plants. The O_3-fumigated plants were characterized by larger leaf area ratio (LAR), which could account for the elevated RGR. Consequently, O_3 depression of leaf dry weight was less than that of the other parts such as root and stem. A similar growth response to O_3 was observed in several species of grasses (Horsman et al. 1980) and sunflower (Shimizu et al. 1981). Those results suggest that O_3 should initially depress the photosynthetic rate and then cause an alteration in the pattern of the assimilate partitioning in favour of the leaves. There have been, however, no report which verifies directly that this sequence of events indeed occurs in the O_3-exposed plants.

In the present study, we attempted to demonstrate the effect of continuous exposure of 0.2 ppm O_3 on the fixation of $^{13}CO_2$ and on the translocation and distribution of ^{13}C-labelled assimilates from the individual source leaves in 14-day-old kidney bean plants. Although the O_3 concentration of 0.2 ppm used in this study was fairly higher than the ambient level, the concentration was selected in order to obtain the reasonably definitive effects within a relatively short-term exposure to O_3.

Materials and methods

Plant materials

Two kidney bean (*Phaseolus vulgaris* L. cv. Shin-edogawa) seeds were sown in a pot containing 300 ml of artificial soils (mixture of vermiculite, peat moss, perlite and fine gravel, 2:2:1:1, v/v), 1 g of Magamp K (N: P_2O_5: K_2O = 6:40:5, W.R. Grace Co., Tennessee, U.S.A.) and 3 g of magnesia lime. The germination and the consecutive growth were achieved in an artificially-lit growth room (30 klx, light period 14h, 25° C, R.H. 70%). One of the two seedlings in a pot was eliminated at seven days after the sowing to increase the uniformity among the plants in a set of the culture which usually consisted of nearly 90 pots.

O_3 exposure and $^{13}CO_2$ feeding

The exposure of the plants to O_3 at 0.2 ppm was started 10 days after the sowing. The exposure treatment was conducted continuously throughout day and night in an artificially-lit fumigation room as described by Shimizu et al. (1981). After four days-exposure (14 days after the sowing), 24 uniform plants were selected for the $^{13}CO_2$ feeding experiment. In this growth stage, the plants had a pair of fully-expanded primary leaves, the 1st trifoliate leaf with 70 to 80% of its maximum leaf area and unexpanded immature leaves (Fig. 1). Since the immature leaves scarcely exported the asimilates to the other parts (Okano and Ito, unpublished data), all of the carbon translocating in the plant seemed to be derived from either a pair of the primary leaves or the 1st trifoliate leaf. Therefore, ^{13}C-labelled carbon dioxide was administered to either one side of the primary leaves or the 1st trifoliate leaf.

Feeding of $^{13}CO_2$ to single leaves was conducted in a rectangular feeding box (130 × 50 × 30 cm, made of plexiglass) placed in another artificially-lit fumigation room. The primary leaves and the 1st trifoliate leaves of 12 plants were inserted into the box and sealed in with vinyl tapes at their petioles. The air in the box was purged out by CO_2-free air down to 20 ppm CO_2 within 10 min, and then $^{13}CO_2$ was introduced into the box from a cylinder. The procedure to produce $^{13}CO_2$ gas was as follows; $^{13}CO_2$ gas was evolved by the addition of 1 N hydrochloric acid to

^{13}C-labelled barium carbonate (90.7 atom % ^{13}C) *in vacuo*, and trapped into the cylinder refrigerated in the liquid nitrogen. The concentration of $^{13}CO_2$ in the box was monitored continuously and regulated at 350 ppm by a feedback system consisted of an infrared gas analyzer (Type ZFD, Fuji Electric Co., Ltd., Tokyo), a PID controller (Chino manufactory Co., Ltd., Tokyo) and a mass flow controller (Ohkura Electric Co., Ltd.. Tokyo) as shown in Fig. 2

In parallel with the feeding of $^{13}CO_2$, O_3 was simultaneously introduced to both inside and outside of the feeding box. The concentration of O_3 inside the box was monitored every 30 sec and maintained at 0.20 ± 0.02 ppm by a controlling system with a UV absorptive O_3 analyzer (TUV-1100, Tokyo Industries Inc. Tokyo) (Fig. 2). Each primary leaf and trifoliate leaf inserted into the box were fed with $^{13}CO_2$ for eight hours under the fumigation of 0.2 ppm O_3 in the

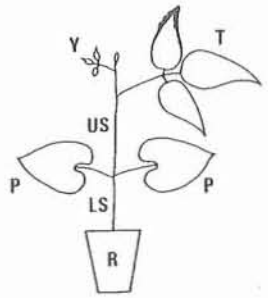

Fig. 1 Schematic diagram of a 14-day-old kidney bean plant used in the present experiment. For details, see text.

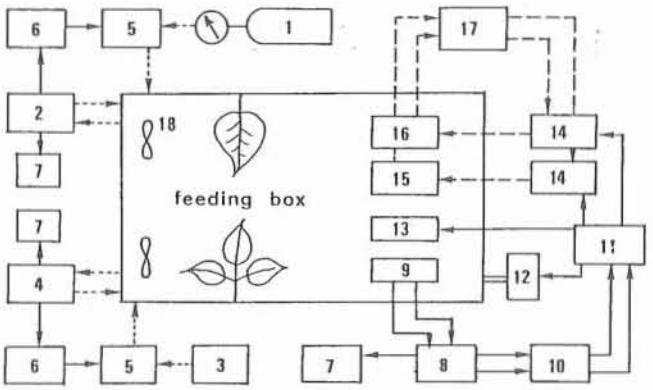

Fig. 2 Diagram of $^{13}CO_2$ feeding system under constant environmental conditions.
1. compressed $^{13}CO_2$ cylinder, 2. IR gas analyzer, 3.O_3 generator, 4. UV absorptive O_3 analyzer, 5. mass flow controller, 6. PID controller, 7. recorder, 8. R/V converter, 9. temperature and humidity sensor, 10. temperature and humidity controller, 11. on-off relay, 12. humidifier, 13. heater, 14. water pump, 15. dehumidifier, 16. cooler, 17. water chiller, 18. fan.
—— electric signal, ·--· gas flow, —·— water flow.

controlled environment. The air in the box was mixed with the fans set in the box. The temperature and relative humidity in the box were monitored continuously and regulated at 25.0 ± 1.5°C and R.H. 70 ± 5% by a controlling system consisted of a cooler, a heater, a humidifier and a dehumidifier described in Fig. 2. Oxygen concentration in the box rose gradually during the 8h-feeding period (from 21% at the start to 30% at the end of feeding period) probably due to the introduciton of O_3.

Sampling

The plants wer sampled at 2, 4, 6 and 8 hours after the start of $^{13}CO_2$ feeding. Two groups of three plants were taken at every sampling time. One group of the plants which were fed $^{13}CO_2$ through the primary leaf were separated into the $^{13}CO_2$-fed leaf (P1), the leaf opposite to the fed leaf (P2), the 1st trifoliate leaf (T), the immature growing leaves (Y), the upper stem (US, between the primary leaves and the 1st trifoliate leaf), the lower stem (LS, between the primary leaves and the root; hypocotyl and epicotyl) and the root (R) (Fig. 1). Another group of the plants which were fed $^{13}CO_2$ through the 1st trifoliate leaf were separated into the $^{13}CO_2$-fed leaf (T), primary leaves (P), Y, US, LS and R.

Determination of ^{13}C

Separated plant parts were dried in an oven at 80°C for three days, and ground to a fine powder with a vibrating sample mill (TI-100, Heiko manufactory Ltd., Tokyo). Total carbon content of the samples was determined with a Yanaco CN-corder (MT-500, Yanagimoto Co., Ltd., Kyoto) and ^{13}C content was measured by the infrared absorption spectrometry which had been recently developed (Okano et al. 1983) using a JASCO EX-130 $^{13}CO_2$ analyzer (Japan Spectroscopic Co., Ltd., Tokyo).

The experiments were repeated three times on consecutive three days. The results are expressed as a mean value of nine plants from three replications. Another series of experiments without the exposure to O_3 were designated as the control.

Results

Foliar injuries called "white fleck" or "bronzing" were observed on the primary leaves by the continuous exposure to 0.2 ppm O_3 for four days. In contrast, the 1st trifoliate leaf which was younger than the primary leaves was less visibly injured; only a slight shrinkage and dark greening were observed.

Table 1 shows the effects of O_3 exposure on the $^{13}CO_2$ fixation of each source leaf. The amount of ^{13}C fixed by the primary leaf of O_3-treated plants decreased by 62% as compared with the control plant, whereas that by the 1st trifoliate leaf was reduced by only 24%. Thus, photosynthesis of the primary leaf was inhibited more severely than the 1st trifoliate leaf by the exposure to O_3. Menser et al. (1963) observed that recently matured tobacco leaves were more O_3-sensitive than overmature or rapidly expanding leaves. Ozone sensitivity of the leaves seems to be inversely related to the leaf maturity. No respiratory loss of ^{13}C from the whole plant was detected at least within 8h-experimental period.

The effects of O_3 exposure on the export of labelled carbon from the individual source leaves are shown in Table 2. Twenty to thirty percent of the ^{13}C assimilated in each source leaf was exported to the other parts throughout the feeding period, and this value increased with the feeding time. The export ratio of ^{13}C from the primary leaf was slightly depressed by the exposure to O_3. On the contrary, that from the 1st trifoliate leaf was significantly elevated by the same treatment. Ozone affected the export of labelled carbon from the two kinds of source

leaves in the opposite way.

Table 3 summarizes the relative inhibition of photosynthesis and translocation in the individual source leaves of O_3-fumigated plants. Ozone-inhibition of translocation in the primary leaf was greater than that of photosynthesis, while any inhibition of translocation was not detected in the 1st trifoliate leaf despite the depression of photosynthesis by 25 to 30%.

Table 1 Effects of 0.2 ppm O_3 on the $^{13}CO_2$ fixation[a] of individual source leaves in 14-day-old kidney bean plants.

Source leaf	Cont	O_3
Primary leaf	9.26 ± 1.29[b] (100)	3.50 ± 0.46 (38[c])
1st trifoliate leaf	11.81 ± 1.02 (100)	9.02 ± 1.15 (76)

a. The amount of ^{13}C (mg) in whole plant at the end of 8h-feeding period.
b. Mean of three replications ± standard deviation.
c. Relative values to control (100).

Table 2 Effects of 0.2 ppm O_3 on the export ratio[a] of fixed ^{13}C from the individual source leaves.

Time (h)	Primary leaf		1st trifoliate leaf	
	Cont	O_3	Cont	O_3
2	18.4 ± 1.7[b]	14.8 ± 2.8	16.2 ± 2.1	19.9 ± 3.2
4	21.4 ± 2.4	18.6 ± 1.6	19.7 ± 1.6	27.1 ± 1.0
6	24.5 ± 3.2	22.6 ± 6.8	22.6 ± 1.9	29.0 ± 1.2
8	27.8 ± 4.2	25.2 ± 7.1	22.4 ± 2.6	30.1 ± 2.3

a. Export ratio $= \frac{\text{amount of } ^{13}C \text{ exported from the source leaf}}{\text{amount of } ^{13}C \text{ in whole plant}} \times 100$

b. Mean of three replications ± standard deviation.

Table 3 Relative inhibitions of photosynthesis and translocation in the individual source leaves of O_3-treated plant.

Time	Primary leaf		1st trifoliate leaf	
(h)	Fixed ^{13}C	Exported ^{13}C	Fixed ^{13}C	Exported ^{13}C
2	41[a]	32	68	84
4	36	31	71	98
6	36	33	77	99
8	38	34	76	103

a. Numerals are relative values to control plant (100).

The effects of O_3 exposure on the distribution percentage of labelled carbon in the various plant parts are shown in Table 4. In the control plant, 67% of the ^{13}C exported from the primary leaf was distributed to the lower parts including the lower stem and the root, and 22% was partitioned to the younger leaves. In contrast, 39% of the ^{13}C exported from the 1st trifoliate leaf was distributed to the lower parts, and 36% was partitioned to the younger leaves. By the exposure to O_3, percentage distribution of ^{13}C exported from the primary leaf to the lower parts decreased from 67% to 53%, in contrast, that to the younger leaves increased from 22% to 36%. Similarly, the distribution of ^{13}C from the 1st trifoliate leaf to the younger leaves also increased from 36% to 42% due to the decrease of distribution to the stems. Thus, the pattern of assimilate distribution was altered by the exposure to O_3; a greater proportion of the assimilates was partitioned to the growing leaves at the expense of the stem and the root.

Table 4 Effect of 0.2 ppm O_3 on the distribution (%)[a] of exported ^{13}C in the various parts of 14-day-old kidney bean plant.

Plant part	Primary leaf		1st trifoliate leaf	
	Cont	O_3	Cont	O_3
Younger leaves	22 ± 1[b]	36 ± 4	36 ± 3	42 ± 2
1st trifoliate L.	4 ± 1	2 ± 1	—	—
Upper stem	6 ± 0	8 ± 1	24 ± 2	19 ± 3
Primary L.-1	—	—	1 ± 1	1 ± 0
Primary L.-2	1 ± 1	1 ± 1		
Lower stem	40 ± 1	31 ± 2	20 ± 1	17 ± 2
Root	27 ± 2	22 ± 1	19 ± 4	21 ± 4

a. Values are averages for all samples from 2 h to 8 h.

b. Mean ± standard deviation.

Figure 3 shows the effect of O_3 on the amount of ^{13}C translocated from the individual source leaves to the other leaves and the stem and root during the 8h-feeding period. Figure 3 also indicates the contributions of the two kinds of source leaves to the various sink organs. Since all of the carbon translocating at this growth stage of the plant would be derived from those fixed by either a pair of the primary leaves or the 1st trifoliate leaf as mentioned in Materials and Methods, total amount of carbon imported into the sink organs could be determined quantitatively. In the control plant, two-thirds of the assimilates required for the stem and root growth was originated from the primary leaves. On the other hand, the leaves were supplied with the assimilates from both source leaves by nearly equal proportion. By the exposure to O_3, the amount of ^{13}C exported from the primary leaves decreased remarkably due to both the large reduction of $^{13}CO_2$ fixation and the inhibition of translocation (Table 3). Whereas, the amount of ^{13}C exported from the 1st trifoliate leaf hardly or barely decreased by O_3 because the reduction of $^{13}CO_2$ fixation was almost compensated by the acceleration of the translocation (Table 3). Furthermore, since the pattern of assimilate distribution was altered by O_3 in favour of the growing elaves (Table 4), the amount of ^{13}C translocated to the stem and root decreased by 53%, while that to the leaves was reduced by only 28%.

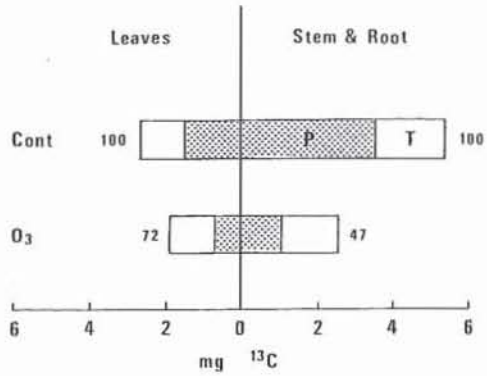

Fig. 3 Effect of 0.2 ppm O_3 on the amount of ^{13}C translocated from the individual source leaves to the other leaves and the stem and root during 8h-feeding period.
P; ^{13}C translocated from a pair of the primary leaves.
T; ^{13}C translcoated from the 1st trifoliate leaf.
Numerals in the figure show the relative values to control (100)

Discussion

Direct inhibition of translocation by O_3

Translocation of the assimilates from the fed leaf may be altered by the rate of photosynthesis. Ryle and Powell (1976) reported that the barley leaves kept under low light showed enhanced export of ^{14}C-assimilates as compared with those kept under high light. Generally, the ratio of the amount of carbon translocated to that fixed increases as the rate of carbon fixation decreases (Teh and Swanson 1982). This was also true in the 1st trifoliate leaf of the present experiment (Table 2) where the export ratio of the labelled carbon was significantly accelerated by O_3 since the carbon fixation of the leaf was considerably lowered. However, the export ratio of the labelled carbon in the primary leaf was not enhanced, rather slightly inhibited by O_3, although photosynthesis of the primary leaf was severely depressed by the treatment (Table 2). Those results suggest that the translocation processes in the primary leaf might be impaired directly by the exposure to O_3. Noyes (1980) reported with kidney bean plants that the translocation process of ^{14}C-assimilates from the fed leaf was directly inhibited by the low concentration of sulfur dioxide (SO_2) whose concentration was below the level to affect the net photosynthesis. His autoradiographic observations suggested that one mechanism of SO_2 damage on translocation might be the inhibition of sieve-tube loading. Teh and Swanson (1982) showed that the translocation rates determined experimentally from SO_2-stressed bean leaves were lower than the rates expected from the observed reduction in photosynthesis, and also inferred that under SO_2 stress the phloem loading system became a major limiting step in controlling the translocation rate. Recently, McCool and Menge (1983) reported that tomato leaf tissue exposed to O_3 retained more of the labelled photosynthetic products than did unexposed controls. Ozone may act on the translocation process in the same way as SO_2.

Alteration in the pattern of assimilate distribution

Percentage distribution of the labelled carbon exported from both source leaves to the root and the stem decreased by the exposure to O_3, while that to the younger leaves increased (Table 4). Thus, a greater proportion of the assimilates was partitioned to the growing leaves at the

expense of the root and the stem in the O_3-stressed plants. This kind of alteration in the patern of assimilate partitioning seems to be brought about not only by the exposure to O_3 but also by other stresses which cause the reduction of photosynthesis. Ryle and Powell (1976) reported that grasses grown in low light exported a greater proportion of their assimilates to immature growing leaves and a smaller proportion to their roots and tillers than the corresponding plants in high light. This genereal pattern of the response to the limitation of assimilates has some similarity with the pattern obtained when a stress was imposed by the physical removal of a part of the photosynthetic organs (Ryle and Powell 1975). Therefore, no matter what the causes may be, a reduction in the amount of available assimilate seems to cause such a change in the assimilate distribution (Wardlaw 1968).

From the results indicated in Fig. 3, the specific inhibition of the root growth caused by O_3 could be explained by the following two factors. First, the physiological functions of the lower mature leaves which acted as main sources of assimilates for the root growth were impaired more severely by O_3 than those of the upper younger leaves which mainly nourished the newly growing leaves. Secondly, resultant lower production of photosynthates caused an alteration in the pattern of the assimilate distribution, and a greater proportion of the assimilates was partitioned to the growing leaves at the expense of the root and stem. The reduction in the amount of assimilates translocated to the root should have led to the eventual decline of the root growth (Ito et al. 1984). Consequently, the root growth would be inhibited drastically by the exopsure to O_3, while the growth of the new leaves would not so reduced by the same treatment.

Adaptive response of plant to O_3 exposure

Although the amount of ^{13}C exported from the source leaves was reduced in the ozonated plant, a larger proportion of ^{13}C was distributed to the photosynthetic organs (leaves) and a smaller to the non-photosynthetic organs (stem and root) (Fig. 3). This alteration in the assimilate partitioning would result in a greater LAR which could maintain RGR at a higher level by compensating the reduced NAR, as observed in many species of the plants (Oshima et al. 1978, 1979, Horsman et al. 1980, Shimizu et al. 1981). The plants seem to show the adaptive response to O_3 exposure in order to minimize the reduction in growth efficiency caused by O_3. Walmsley et al. (1980) demonstrated that the adaptation process of a radish to continuous O_3 fumigation consisted of two components. The first component was an alteration in the pattern of the assimilate distribuiton, so that new leaves were produced more rapidly in the ozonated plants at the expense of hypocotyl development. The second component was the progressive increase in tolerance to O_3 shown by the new leaves emerging successively. Their measurement of stomatal resistance suggested that this was an acquired, rather than an inherent, characteristic of the new leaves. As for a similar phenomenon to the latter component, Tanaka and Sugahara (1980) reported that the younger poplar leaves exposed to SO_2 at 0.1 ppm increased more superoxide dismutase (a scavenger for active oxygen) activity than the older ones, and thereby became more tolerant to SO_2 toxity.

Ozone-induced increase of assimilate partitioning to the newly growing leaves which will become the sites of carbon assimilation may be advantageous to the plant growth with respect to the dry matter production. On the other hand, it may be worthy to consider the changes of root functions in ozonated plants. Ozone causes a reduction of the assimilate supply to the root, which may subsequently bring about the lowered carbohydrate level in the root (Tingey et al. 1976, Ito et al. 1984), the reduced root respiration (Hofstra et al. 1981) and changes in mycorrhizal association (Tingey and Blum 1973, McCool and Menge 1983). The decline in root functions due to the lack of energy substrates will result in the reduction in the supply of water

and mineral nutrients to the shoot. These changes in root metabolism will ultimately be expressed as a reduciton of whole plant growth.

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インゲンマメに及ぼす NO_2 と O_3 の混合ガスの影響
Ⅳ　O_3 暴露による ^{13}C 標識同化産物の分配の変化

岡野邦夫[1]・伊藤　治[1]・竹葉　剛[2]・清水　明[3]・戸塚　績[1]

オゾン（O_3）や亜硫酸ガス等の大気汚染ガスに暴露された植物では，地上部に比べ地下部の生長が特異的に阻害されることが多い。この理由を明らかにする目的で，安定同位体の ^{13}C を使ったトレーサー実験を行い，インゲン幼植物の個々の葉からの光合成産物の転流・分配に及ぼす0.2ppm O_3 の影響を調べた。

根への光合成産物の主要な供給源である初生葉の $^{13}CO_2$ 固定能は O_3 暴露により著しく低下し，葉からの同化産物の転流も阻害された。一方，新葉へ主として光合成産物を送っている第１本葉では，O_3 暴露による $^{13}CO_2$ 固定能の低下は相対的に小さく，葉からの同化産物の転流は O_3 により逆に促進された。

また O_3 暴露により光合成能が低下し同化産物が不足した結果，各部位への同化産物の分配パターンが変化し，根や茎に比べ新葉に相対的に多くの同化産物が転流した。

以上より，新葉の生長を促進する方向へと同化産物の分配パターンが変化することに加えて，根への同化産物の主な供給源である下位葉の生理活性が O_3 により強く影響を受けるために，根の特異的な生長阻害が生ずることが明らかとなった。非同化器官である根や茎への分配を減らし，将来同化器官となる新葉へ少ない同化産物を優先的に分配する体制は，生長効率の低下を防ぐのに有利であり，O_3 暴露下の植物の示すひとつの適応反応と考えられる。

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Res. Rep. Natl. Inst. Environ. Stud., Jpn., No.66, 1984.

Effects of NO_2 and O_3 alone or in combination on kidney bean plants. V. ^{13}C-assimilate partitioning as affected by NO_2 and/or O_3

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The effects of ozone (O_3) at 0.2 ppm and nitrogen dioxide (NO_2) at 2.0 ppm alone or in combination on the translocation and distribution of ^{13}C-labelled assimilates from the individual source leaves in 14-day-old kidney bean plants were investigated.

The plants exposed to NO_2 showed no visible foliar injury, while the mixture of O_3 and NO_2 caused severe ozone-type injuries on the primary leaves.

Exposure to 2.0 ppm NO_2 scarcely affected the translocation processes except for the export from the primary leaves. Since the photosynthetic activities of the both source leaves were significantly enhanced by the exposure to NO_2, the amount of labelled assimilates translocated to the various sink organs was considerably increased.

The mixture of O_3 and NO_2 reduced the $^{13}CO_2$ fixation of both source leaves more severely, and altered the pattern of assimilate distribution more drastically than would be expected from the effects induced by the exposure to each pollutant alone. Consequently, the amount of labelled assimilates translocated to the root and the lower stem was remarkably reduced by the mixture of O_3 and NO_2 (by 85% in the root and 80% in the lower stem), whereas that to the younger leaves was less decreased (by 33%).

Those results suggest that the plants might respond to the mixture of O_3 and NO_2 by diverting relatively more assimilates into the growing leaves and that the subthreshold concentration of NO_2 could modify the susceptibility of the plants to O_3. Possible mechanisms fo the greater than additive effect induced by O_3 and NO_2 are discussed.

Key words: Pollutant combination – O_3 – NO_2 – ^{13}C – Assimilate – Translocation – partitioning – Kidney bean

In the urban and industrial areas, the atmosphere contains a complex mixture of phytotoxic gases and plants growing in the fields will often be exposed to several pollutants

simultaneously. Menser and Heggestad (1966) first reported that mixture of sulfur dioxide (SO_2) and O_3 caused foliar injury to tobacco at concentrations of the single gases which would not normaly cause the visible foliar injury. They suggested that this combination of pollutants lowered the threshold concentration for the injury and that the effect was synergistic. Thereafter, a number of investigations have been conducted to define the effects of pollutant combinations, mainly SO_2 and O_3 or SO_2 and NO_2, on the foliar injury and plant growth (see Reinert et al. 1975, Arai and Totsuka 1979, Ormrod 1982). The information relating to the other combination of pollutants, however, have been limited hitherto. Ozone and NO_2 occur together in photochemical oxidants which are the most serious air pollutants in Japan, but little is known of how such a combination might affect the plants.

In the previous study (Okano et al. 1984a), we demonstrated that the pattern of assimilate distribution in kidney bean plants was altered by 0.2 ppm O_3 so that a greater proportion of assimilate was partitioned to the growing leaves and a smaller proportion to the root and the stem. The present paper describes the tracer experiments designed to assess the effects of 0.2 ppm O_3 and 2.0 ppm NO_2, applied both singly or in combination, on the translocation and the distribution of ^{13}C-labelled assimilates exported from the two kinds of source leaves in 14-day-old kidney bean plants. The objective of this study is to determine whether NO_2 modify the susceptibility of the plants to O_3, as measured by the changes in the translocation and distribution of the assimilates.

Materials and Methods

Plants

Kidney bean (*Phaseolus vulgaris* L. cv. Shin-edogawa) plants were grown in a controlled-environment growth room (30klx, 25° C, R.H. 70%, photoperiod 14 h) as described previously (Okano et al. 1984a).

Exposure to pollutants

Exposure of plants to O_3 and/or NO_2 were started 10 days after the sowing. All fumigations were carried out in a controlled-environment fumigation room with the same environmental conditions as the growth room. Ozone was generated by a silent electrical discharge in dry oxygen. Nitrogen dioxide was supplied from a pressurized cylinder of predetermined concentration. Controlled levels of pollutants were released through a thermal mass-flow controller into the air stream entering the fumigation room. The treatments were as follows; (a) control: charcoal filtered-air, (b) 0.2 ppm O_3, (c) 2.0 ppm NO_2, (d) 0.2 ppm O_3 + 2.0 ppm NO_2. Pollutant concentrations within the fumigation room were monitored continuously using a chemiluminescent O_3 analyzer (Model 806, Kimoto Electric) for O_3 and a chemiluminescent NO-NO_2-NO_x analyzer (Model 14, Thermo Electron) for NO_2 to ensure that they remained at the required levels.

Feeding of $^{13}CO_2$

After four days-exposure to O_3 and/or NO_2 (14 days after the sowing), 24 uniform plants were selected for the $^{13}CO_2$ feeding experiment. Feeding of $^{13}CO_2$ to single leaves was conducted in a rectangular feeding box placed in an another controlled-environment fumigation room. The apparatus and techniques used to provide a constant concentration (350 ppm) of $^{13}CO_2$ to single leaves have been described elsewhere (Okano et al. 1984a). ^{13}C-labelled carbon dioxide (made from 90.7 atom % $Ba^{13}CO_3$) was administered continuously for eight hours to either one side of the primary leaves or the 1st trifoliate leaf.

In parallel with the feeding of $^{13}CO_2$, O_3 and/or NO_2 were simultaneously introduced to both inside and outside of the feeding box. The concentrations of O_3 and NO_2 inside the box were regulated by a system similar to that described for controlling $^{13}CO_2$ concentration (Okano et al. 1984a) using a UV absorptive O_3 analyzer (TUV-1100, Tokyo Industries) and a chemiluminescent NO_x analyzer (Model 258, Kimoto Electric), and were maintained at 0.20 ± 0.02 ppm for O_3 and at 2.0 ± 0.1 ppm for NO_2. The fans set in the box created sufficient air movement to ensure the complete mixing of gases and to lower the boundary layer resistance around the leaves. The temperature and relative humidity in the box were monitored continuously and regulated at 25.0 ± 1.5°C and 70 ± 5% respectively by a controlling system as described previously (Okano et al. 1984a).

Sampling and ^{13}C determination

The plants were sampled at 2, 4, 6 and 8 hours after the start of $^{13}CO_2$ feeding, and separated into each part in the same way as previously reported (Okano et al. 1984a). Total carbon and ^{13}C content of the samples were determined as described previously (Okano et al. 1984a). Each experiment was repeated three times on consecutive three days, so the results are expressed as a mean value of nine plants from three replications.

Result

Although the effects of 0.2 ppm O_3 on the translocation of the assimilates in the kidney bean plants have been fully described in the previous paper (Okano et al. 1984a), the same data are included in the following tables and figures for the comparison with the additional data.

The plants exposed to 2.0 ppm NO_2 for four days did not show any foliar visible injury, rather appeared to be greener and healthier than those grown in charcoal filtered-air. The total nitrogen contents of all leaves in the NO_2-fumigated plants were significantly higher than those in the control plants (data not shown). The mixture of 0.2 ppm O_3 and 2.0 ppm NO_2 caused severe ozone-type injuries on the primary leaves, while produced no foliar injury on the 1st trifoliate leaf except for a downward curvature of the leaf.

The effect of O_3 and/or NO_2 on the $^{13}CO_2$ fixation of each source leaf were shown in Fig. 1. Exposure to 2.0 ppm NO_2 did not depress the $^{13}CO_2$ fixation, rather enhanced the photosynthetic activity by 18% in the primary leaf and by 39% in the 1st trifoliate leaf. However, the mixture of O_3 and NO_2 inhibited the photosynthesis of the both source leaves more severely than expected from summing up the effects of the individual gases. The mixture effect was greater than additive with respect to photosynthetic CO_2 fixation. It means that the subthreshold concentration of NO_2 promoted the inhibitory effect of O_3 on the photosynthesis of the kidney bean leaves. The mixed gases reduced the photosynthetic activity of the primary leaf more severely (by 70%) than that of the 1st trifoliate leaf (by 48%). The mature leaves appeared to be more sensitive to the mixture of O_3 and NO_2 than the younger leaves. A similar result was also observed in 0.2 ppm O_3 treatment.

Table 1 shows the effects of O_3 and/or NO_2 on the export of the labelled carbon from the individual source leaves. As well as O_3 treatment, NO_2 slightly lowered the export ratio of ^{13}C from the primary leaf, whereas elevated that from the 1st trifoliate leaf. The mixture of O_3 and NO_2 inhibited the export of ^{13}C from the primary leaf more furiously than O_3 or NO_2 alone, and the effect of pollutant combination was equal to additive effects of single pollutants. The mixture also accelerated the export of the labelled carbon from the 1st trifoliate leaf, but the degree of acceleration was less than that caused by O_3 alone.

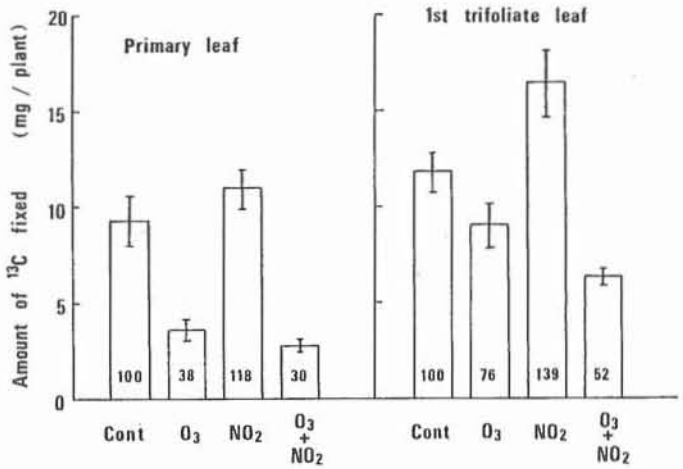

Fig. 1 Effects of O_3 and NO_2 alone or in combination on the $^{13}CO_2$ fixation of individual source leaves in 14-day-old kidney bean plants during 8h-feeding period. Vertical bar indicates the standard deviation. Numerals in the figure show the relative value to control (100).

Table 1 *Effects of O_3 and NO_2 alone or in combination on the export ratio[a] of fixed ^{13}C from the individual source leaves at the end of 8h-feeding period.*

Treatment	Source leaf	
	Primary leaf	1st trifoliate leaf
Cont[c]	27.8 ± 4.2[b]	22.4 ± 2.6
O_3[c]	25.2 ± 7.1	30.1 ± 2.3
NO_2	25.2 ± 2.7	26.2 ± 1.1
$O_3 + NO_2$	22.2 ± 0.7	27.6 ± 1.3

a. Export ratio $= \frac{\text{amount of } ^{13}C \text{ exported from the source leaf}}{\text{amount of } ^{13}C \text{ in whole plant}} \times 100$

b. Mean of three replications ± standard deviation.

c. Data from Okano et al. (1984a).

Figure 2 and 3 show the changes in the distribution patterns of the labelled carbon in the plants by the exposure to O_3 and/or NO_2. Exposure to NO_2 alone did not significantly affect the distribution pattern of assimilates exported from both source leaves. In the NO_2-treated plants as well as the control ones, two-thirds of the assimilate exported from the primary leaf were distributed to the lower parts, such as the lower stem and the root, and a quarter to the younger leaves (Fig. 2). Whereas, the 1st trifoliate leaf contributed to both the lower parts and the younger leaves by similar proportion (Fig. 3). The mixture of O_3 and NO_2 increased the distribution percentages of ^{13}C exported from both source leaves to the younger leaves or the upper stem, while decreased those to the root or the lower stem. Especially, the 1st trifoliate leaf of the plant exposed to the mixture distributed a large proportion (60%) of its assimilates to the

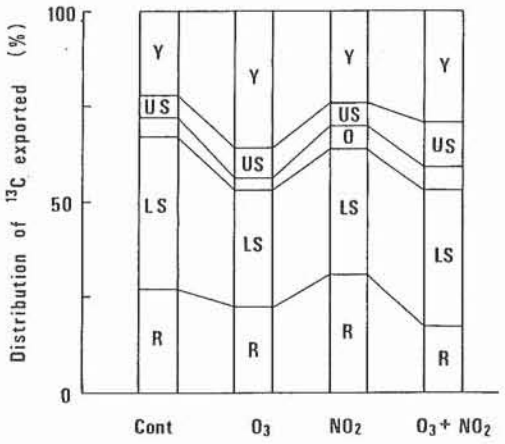

Fig. 2 Effects of O_3 and NO_2 alone or in combination on the distribution of ^{13}C exported from the primary leaf of kidney bean plants. Y: younger leaves, US: upper stem, LS: lower stem, R: root, and O: others (P or T).

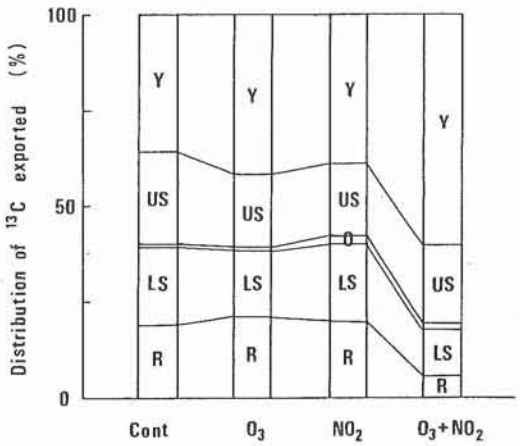

Fig. 3 Effects of O_3 and NO_2 alone or in combination on the distribution of ^{13}C exported from the 1st trifoliate leaf of kidney bean plants. Symbols are the same as in Fig. 2.

growing leaves, and only a small proportion (6%) to the root (Fig. 3). The combined effect of O_3 and NO_2 on the distribution of the assimilate was greater than additive for the 1st trifoliate leaf, while seemed to be equal to additive for the primary leaves.

From the data described above, the actual amount of the labelled carbon translocated to the various parts from both a pair of the primary leaves and the 1st trifoliate leaf could be calculated, and summarized in Fig. 4. Exposure to NO_2 alone increased the amount of labelled assimilates translocated to both the younger leaves and the root about 1.5 times as compared with the control. This was mainly due to the increase of $^{13}CO_2$ fixation in the source leaves of NO_2-treated plants (Fig. 1). The mixture of O_3 and NO_2 caused a greater reduction of the

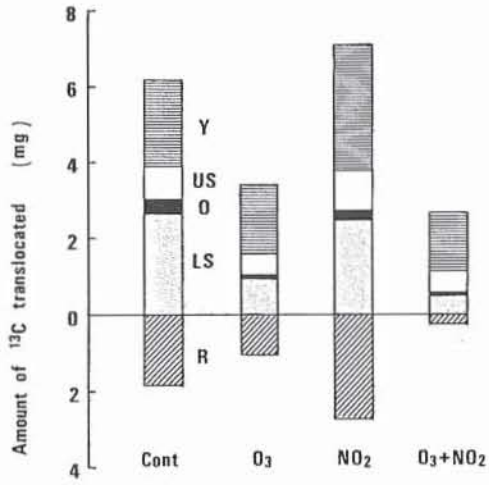

Fig. 4 Effects of O_3 and NO_2 alone or in combination on the amount of ^{13}C translocated from a pair of primary leaves and the 1st trifoliate leaf to the various sink organs. Symbols are the same as in Fig. 2.

assimilate translocated to all sinks than expected from the effects induced by the exposures to each pollutant alone. In particular, the amount of assimilate translocated to the root and the lower stem was remarkably reduced by the mixture of O_3 and NO_2 (by 85% in th root and by 80% in the lower stem), while that to the younger leaves was less decreased (by 33%). A larger reduction of $^{13}CO_2$ fixation of the primary leaves (Fig. 1) and the alteration of the pattern of assimilate distribution (Fig. 2 and 3) could account for the observed greater reduction of the assimilates translocated to the root and the lower stem in the mixed gas-treated plants.

Discussion

Effects of exposure to NO_2 alone

The exposure of the kidney bean plants to 0.2 ppm O_3 for four days inhibited the $^{13}CO_2$ fixation and export of the assimilate from the mature leaves, and altered the pattern of assimilate distribution in the plants (Okano et al. 1984a). In contrast to O_3, 2.0 ppm NO_2 scarcely prevented the translocation processes except for the export from the primary leaves. The photosynthetic activities of both source leaves were rather significantly enhanced by the exposure to NO_2 (Fig. 1). Consequently, the amount of assimilates translocated to the various sink organs were considerably increased (Fig. 4). Those results imply that 2.0 ppm NO_2 may not affect the plants as a toxic air pollutant, but act as a nitrogen fertilizer from the atmosphere (Okano et al. 1984b). The fact that the nitrogen content of the leaves was significantly increased by the exposure to NO_2 may also support the above assumption. Although high concentrations (above 3 to 4 ppm) of NO_2 could cause the acute injurious effects on the physiological functions of the plant leaves probably through the accumulation of nitrite (Kato et al. 1974, Yoneyama et al. 1979), low concentrations of NO_2 absorbed in the leaf could be easily converted into nitrite and nitrate, and further assimilated into amino acids (Yoneyama and Sasakawa 1979). A beneficial effect of low concentration of NO_2 on the plant growth might be therefore be

anticipated, and has been actually confirmed with rice (Fujiwara 1973), tomato (Troiano and Leone 1977) and cucumber (Yoneyama et al. 1980). Exposure to 2.0 ppm NO_2 exceptionally inhibited the export of the assimilate from the mature primary leaves (Table 1). Even low concentrations of NO_2 which unaffects the net photosynthesis may inhibit the phloem loading of the assimilate in a similar way with low concentrations of SO_2 as proposed by Noyes (1980).

Combined effects of O_3 and NO_2

Although singly applied 2.0 ppm NO_2 might act beneficially on the plant growth, the inhibitory effect of 0.2 ppm O_3 on the plants was amplified by the presence of 2.0 ppm NO_2. The mixture of O_3 and NO_2 reduced the $^{13}CO_2$ fixation of both source leaves more severely, and altered the pattern of assimilate distribution more drastically than O_3 alone did. Furukawa and Totsuka (1980) reported that the simultaneous fumigation of O_3 at 0.2 ppm and NO_2 at 0.2 or 1.0 ppm inhibited the net photosynthesis of sunflower to a greater extent than expected from the sum of the inhibition caused by individual gases. Omasa et al. (1979) also showed with sunflower that the mixture of O_3 and NO_2 at the concentrations of the each gas below the threshold caused the stomatal closure and the foliar injury.

The mechanisms causing the greater than additive effect induced by O_3 and NO_2 are not known at present, but several possible mechanisms can be raised. First, O_3 and NO_2 may react chemically with each other in the fumigation room to produce unknown chemicals which are more toxic to the plants than the original pollutants. In the present experiment, however, the air in the fumigation room was frequently ventilated (50 to 60 times/h) with the charcoal-filtered ambient air, and the concentrations of the reaction products in the fumigation room could be controlled at a lower level which would not affect the plants (Matsumoto et al. 1979). However, the possibility that the unknown chemicals might generate in the feeding box during the 8h-$^{13}CO_2$-feeding period coult not be ruled out. Secondly, O_3 might destroy the ability of the plant to reduce the nitrite originated from NO_2 fumigation into the amino acids. Wellburn et al. (1981) found that although NO_2 induced a significant increase in nitrite reductase (NiR) activity of the several grasses, the mixture of SO_2 and NO_2 completely prevented the induction of NiR activity. They assumed that this inhibition of a potential detoxification mechanism of nitrite might be one of the main reasons why the $SO_2 + NO_2$ combination exhibited more than additive effects upon the growth of grasses. Since NiR activity of the plants has been found to be quite sensitive to ozonation (Leffler and Cherry 1974), the mixture of O_3 and NO_2 might also inhibit the reduction of nitrite in a similar way as $SO_2 + NO_2$ combination. Thirdly, NO_2 might amplify the inhibitory effects of O_3 on the plants. The fact that the mixture of O_3 and NO_2 caused severe ozone-type injuries on the primary leaves suggests that this will be most possible. Although many workers have contributed to a better understanding of the injuries brought about by O_3, the precise biochemical mechanisms of O_3 demage to the organism have not yet been satisfactorily characterized. Therefore, there was little information about the mechanism of the third possibility that NO_2 might modify the sensitivity of the plants to O_3.

Alteration of assimilate partitioning

No matter what the mechanisms may be, the mixture of O_3 and NO_2 inhibited the leaf photosynthesis more severely than the sum of their individual effects. A larger reduction of available assimilates would result in the more pronounced alteration of assimilate partitioning; a greater proportion to the growing leaves and a smaller proportion to the root and the stem. This was clearly indicated in the 1st trifoliate leaf of the plants exposed to the mixture of O_3 and NO_2, where 60% of the labelled carbon was distributed to the growing leaves and only 6% to the root (Fig. 3). Besides the alteration of assimilate partitioning, the pollutant mixture injured the

primary leaves, which mainly nourished the root and the lower stem, more severely than the 1st trifoliate leaf. Consequently, the amount of assimilates translocated to the root and the lower stem remarkably reduced by the mixture of O_3 and NO_2, while that to the younger leaves was less decreased (Fig. 4). When environmental factors such as light, water and air pollutants become limiting to the plant growth, the alteration in photosynthate partitioning may be one of strategies for the plants to minimize the detrimental effects of the unfavourable conditions. For example, Silvius et al. (1977) and Chatterton and Silvius (1980) found that soybean root growth was favoured over shoot growth when vegetative plants were exposed to low soil moisture, while more priority was given to the shoot growth than root growth when the plants were grown under a short photosynthetic period. The kidney bean plants used in the present experiment might respond to the mixture of O_3 and NO_2 by diverting relatively more assimilate into the growing leaves which will become the sites of carbon assimilation.

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インゲンマメに及ぼす NO_2 と O_3 の混合ガスの影響
V ^{13}C-同化産物の分配に及ぼす混合ガスの影響

岡野邦夫[1]・伊藤　治[1]・竹葉　剛[2]・清水　明[3]・戸塚　績[1]

光化学オキシダントの主成分である O_3（0.2ppm）と NO_2（2.0ppm）の混合ガスが，インゲン幼植物の個々の葉からの同化産物の転流・分配に及ぼす影響を，^{13}C をトレーサーとして用いて調べた。

NO_2 単独による葉面可視害は認められなかったが，NO_2+O_3 の混合ガスは初生葉に著しい O_3 型の可視害を引き起こした。

NO_2 は同化産物の転流・分配にほとんど影響を与えず，葉の ^{13}C の固定能をかえって促進したため，各部位への同化産物の転流量を増大させた。NO_2+O_3 の混合ガスは O_3 単独以上に葉の ^{13}C 固定を抑制し，その程度は初生葉でより大であった。また初生葉からの同化産物の転流を阻害し，逆に第1本葉からの転流を促進した。さらに新葉や上位茎への同化産物の分配率を高め，逆に根や下位茎への分配率を低下させた。その結果，根や下位茎への同化産物の転流量は O_3 単独以上に減少した。

本実験の結果から，NO_2+O_3 の混合ガスは同化産物の転流・分配に対して O_3 単独と類似した影響を与えること，共存する NO_2 はそれ自身では植物にほとんど悪影響を与えなくとも，O_3 の毒性を増幅する作用があること，等が明らかとなった。O_3 と NO_2 による相乗効果のメカニズムについて，いくつかの可能性を指摘した。

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Comparison of the fates of $^{15}NO_2$ and $^{13}CO_2$ absorbed through a leaf of rice plants*

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To investigate the fate of NO_2-nitrogen in plants after it was absorbed, ^{13}C-labelled carbon dioxide and ^{15}N-labelled nitrogen dioxide were simultaneously administered to a mature leaf of rice plants at reproductive stage, and the behaviours of the two isotopes in the plants were followed and compared over eight days.

Most of the transfer of ^{13}C from the fed leaf to the other parts took place within one day, and during this time 21% of the photoassimilated ^{13}C was lost through respiration. Transfer of NO_2-nitrogen from the fed leaf occurred gradually throughout the experimental period with a rapid efflux in the first several hours, and no significant loss of ^{15}N from the plant was detected during this period.

Higher abundances of both ^{13}C and ^{15}N were observed in the panicle, the tillers and the upper roots which were the organs actively developing. The close similarity of labelling patterns of ^{13}C and ^{15}N in the various plant parts suggested that the ^{13}C-labelled sugars and the ^{15}N-labelled nitrogenous compounds derived from NO_2 might flow together in phloem as a bulk stream at least during the phase of primary distribution. The $^{13}C/^{15}N$ ratios in the various plant parts indicated that the relative distribution of carbon and nitrogen might be partly regulated by the characteristics of the sink organs.

We concluded that NO_2-nitrogen once incorporated into proteins in plant leaves was then hydrolyzed to amino acids and transferred to the rapidly growing organs probably through phloem, and that this kind of nitrogen would be utilized for the growth of new tissues in the same manner as the nitrogen taken up by roots.

Key words: NO_2 — Nitrogen assimilation — Transfer — Absorption of NO_2 — ^{15}N — ^{13}C —Rice plant

The plants are considered to have the capacity to remove air pollutants, such as nitrogen dioxide (NO_2) and sulfur dioxide (SO_2), from the atmosphere (Hill 1971, Bennett and Hill 1973). A large proportion of the air pollutants is absorbed through stomata of the plant leaves.

* A part of this study was presented in; *Japan. Jour. Crop Sci.* 52: 331–341 (1983).

Yoneyama and Sasakawa (1979) demonstrated, using ^{15}N, that NO_2 absorbed in spinach leaves was converted into nitrate and nitrite, which were then rapidly assimilated into amino acids and proteins. Most of NO_2-nitrogen taken up by plant leaves was once incorporated into protein fraction, thereafter hydrolyzed and transferred to the other organs, although some of NO_2-nitrogen was directly exported form the fed leaf (Kaji et al. 1980, Okano et al. 1981)

However, the fate of NO_2-nitrogen in plants after it is absorbed has not been sufficiently determined hitherto. It is necessary to clarify the behaviour of NO_2 and its derivatives in plants for an adequate understanding of the NO_2 effects on plant growth and the removing capacity of NO_2 from the atmosphere by plants. We expected that a part of this problem could be ascertained by comparing the movement of NO_2-nitrogen with that of CO_2-assimilates. Previous work on sunflower (Yoneyama et al. 1980) indicated that some nitrogenous compounds originated from NO_2 were transferred from the fed leaf as fast as CO_2 assimilates and that NO_2-nitrogen was mainly translocated to growing organs.

In the present study, ^{15}N-labelled nitrogen dioxide and ^{13}C-labelled carbon dioxide were simultaneously administered to a mature leaf of rice plants at reproductive stage, and the fate of NO_2-nitrogen and CO_2-assimilates in the plants was followewd over eight days.

Materials and methods

Plants

Pre-germinated rice seeds (*Oryza sativa* L. cv. Nihonbare) were grown on a salan net floating in a vat with a 10-fold dilution of Kasugai's rice culture solution (Baba and Takahashi 1956) in a naturally-lit growth room with a constant temperature at 25°C and a relative humidity at 70%. The plants were grown under a short-day condition during October to November. At the 5th leaf emergence (Oct. 27, 1980), 120 seedlings were transplanted spacing 4 cm × 4 cm into a water culture bed filled with a 4-fold dilution of Kimura's B culture soluton (Baba and Takahashi 1956) which contained both NH_4-N and NO_3-N as nitrogen source. The culture solution was renewed twice a week and adjusted to pH 5.5 with 1 N HCl and 1 N KOH. The roots of main stem were classified into three sets of different nodal roots by the method of Tatsumi and Kono (1980). 1) Lower roots (LR): the roots emerged from lower than the 2nd node. 2) Middle roots (MR): the roots from the 2nd and 3rd nodes. 3) Upper roots (UR): the roots from the 4th node.

Feeding of $^{13}CO_2$ and $^{15}NO_2$ to single leaves.

On November 20, 1980, when the plants were at boot stage and the 7th leaf (terminal leaf) had fully expanded, 50 plants were selected and transplanted to an another water culture bed in an artificially-lit growth room (30 klx, light/dark 14h/10h, 25°C, R.H. 70%). Composition of the nutrient solution was the same as before. The feeding experiment was carried out in the same room on the next day. Feeding of $^{13}CO_2$ and $^{15}NO_2$ to single leaves of each plant was conducted in an assimilation chamber (130 × 50 × 30 cm, made of plexiglass) into which the 7th leaf blades of 50 plants were inserted and sealed in with urethane resin at their basal parts. The air in the assimilation chamber was purged by CO_2-free air for 10 min and CO_2 concentration was reduced to around 20 ppm. Then, the evolution of ^{13}C-labelled CO_2 was initiated by addition of an appropriate amount of 1 N HCl to $Ba^{13}CO_3$ (90.7 atom % ^{13}C). The concentration of $^{13}CO_2$ in the chamber was monitored with an infrared gas analyzer (Tye ZFD, Fuji Electric Co., Ltd., Tokyo), and was maintained between 350 to 400 ppm by occasional addition of 1 N HCl to $Ba^{13}CO_3$.

^{15}N-labelled NO_2 was introduced simultaneously with the generation of $^{13}CO_2$ by a syringe. $^{15}NO_2$ gas was made from $K^{15}NO_3$ (99.7 atom % ^{15}N) as described elsewhere (Yoneyama et al.

1978). The concentration of $^{15}NO_2$ in the chamber was monitored continuously every minute with a NO_x analyzer (Model 258, Kimoto Electric Co., Ltd., Osaka) and was maintained between 2.5 to 3.5 ppm by additional injection of $^{15}NO_2$.

The 7th leaf blades of 50 plants were fed with $^{13}CO_2$ and $^{15}NO_2$ for two hours in light. Any visible NO_2 injury was not observed on the leaf surface. At the end of the feeding period, the concentrations of $^{13}CO_2$ and $^{15}NO_2$ remaining in the chamber were reduced to around 20 ppm and 0.2 ppm respectively through the introduction of CO_2-free air for 10 min, then the assimilation chamber was opened. Ten plants were sampled immediately after the termination of isotopes feeding (Day 0). The rest of the plants were kept in the same growth room, then sampled at 1/4 (6 h, Day 1/4), 1 (Day 1), 3 (Day 3) and 8 (Day 8) days after. Roots of the harvested plants were washed with running tap water, then the plants were separated into the fed leaf (FL), the lower leaves (LL, 3rd to 6th leaf), the young panicle (P), the culm (C), the upper roots (UR), the middle roots (MR), the lower roots (LR) and the tillers (T). The leaf sheath was included in each leaf smaple. Separated parts of the plant were dried in an oven at 80° C for three days, and ground to a fine powder with a vibrating smaple mill (TI-200, Heiko Seisakusho Ltd., Tokyo).

Determination of ^{13}C and ^{15}N

Total carbon content of the smaple was determined with a Yanaco CN-corder (MT-500. Yanagimoto Co., Ltd., Kyoto), and ^{13}C content was measured by infrared absorption spectrometry (Okano et al. 1983) using a JASCO EX-130 $^{13}CO_2$ analyzer (Japan Spectroscopic Co., Ltd., Tokyo). Total nitrogen content was determined by the distillation method after Kjeldahl digestion where salicylic acid was used so that the nitrate nitrogen would be measured. ^{15}N content was measrued by emission spectrography (Muhammad and Kumazawa 1974) with a JASCO NIA-1 ^{15}N analyzer.

Results

Plant growth

Since rice plants used in the present experiment were grown under short-day condition during October to November, the young panicle had already differentiated on the main stem at the 6th leaf stage. The tillers had also formed at the axils of the 4th and 5th leaf at the time of feeding experiment. Changes in dry weight of various parts of the plant during the isotope-chase period are shown in Fig. 1. The tillers, the roots, the culm and the young panicle increased their dry weight with their growth, especially in the tillers dry weight increased vigorously. On the contrary, gradual decrease of dry weight was observed in the fed leaf and the lower leaves. Dry weight of the upper roots increased linearly. In contrast, dry weight did not show any significant changes in the middle roots, while some decrease occurred in the lower roots. Changes in total carbon content and total nitrogen content in the various plant parts during the isotope-chase period were similar to those in dry weight, therefore detailed data are not listed here.

Export and respiratory loss of isotopes

Table 1 shows the changes in the amount of ^{13}C and ^{15}N recovered in the whole plant during the isotope-chase period. Rapid respiratory loss of ^{13}C from the whole plant occurred during the first one day, followed by a gradual loss throughout the isotope-chase period. By Day 1, 21% of the originally assimilated ^{13}C was respired and a further 11% was lost in the following seven days. In contrast, ^{15}N recovered in the whole plant did not show any significant loss over eight days.

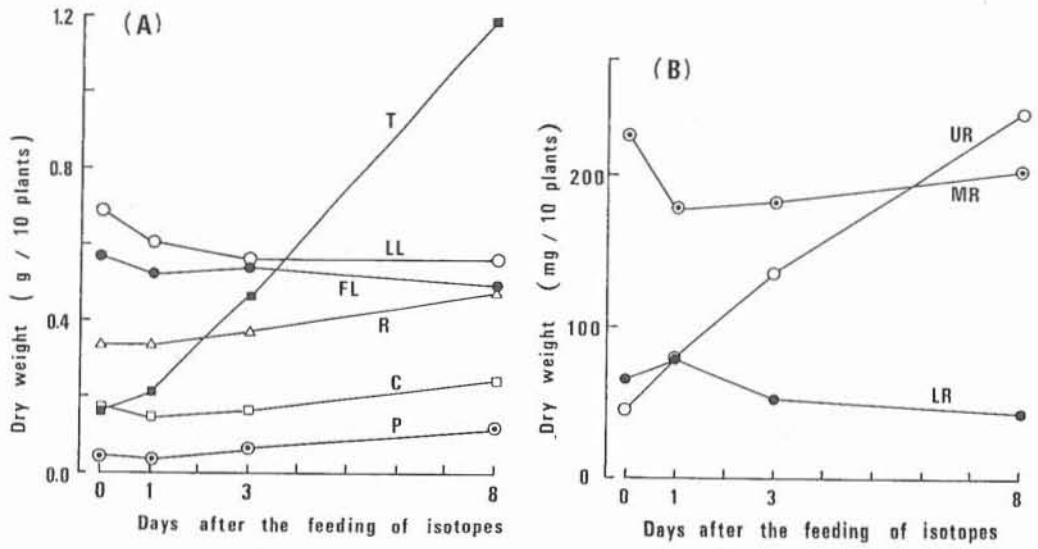

Fig. 1 Changes in dry weight of the various plant parts (A) and the different nodal roots (B).
A: Isotope-fed leaf (●), young panicle (⊙), lower leaves (○), culm (□), tillers (■), and roots (△).
B: Upper roots (○), middle roots (⊙), and lower roots (●).

Table 1 Recoveries of ^{13}C and ^{15}N in whole plants

Isotopes	Days after the feeding of isotopes				
	0	1/4	1	3	8
^{13}C	5134*	4476	4028	3812	3481
	(100)**	(87)	(79)	(74)	(68)
^{15}N	236*	229	247	259	225
	(100)**	(97)	(105)	(110)	(95)

* μg/10 plants
** Relative value to that in Day 0.

Most of the efflux of ^{13}C from the fed leaf also took place within one day, and subsequent slow efflux continued thereafter (Fig. 2). In contrast, ^{15}N was exported more slowly than ^{13}C from the fed leaf. Gradual decrease of ^{15}N from the fed leaf was observed throughout the experimental period with a rapid decrease in the first several hours. By Day 1, 75% of the fixed ^{13}C had been exported to the other parts or lost in respiration, whereas only 21% of the assimilated ^{15}N was exported to the other parts from the fed leaf. This general pattern of export of ^{13}C and ^{15}N, and of the loss of ^{13}C in respiration, were very similar to those previously reported on sunflower plant (Yoneyama et al. 1980).

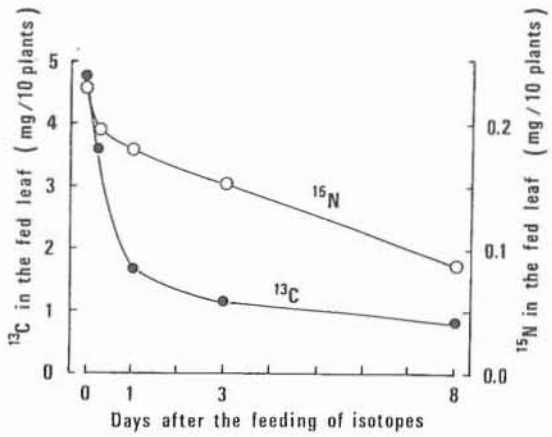

Fig. 2 Efflux of ^{13}C and ^{15}N from the fed leaf.

Distribution of ^{13}C and ^{15}N in the plant

Table 2 shows the changes in ^{13}C and ^{15}N abundances (atom % excess) in the various parts of the rice plant. ^{13}C and ^{15}N abundances in the fed leaf decreased continuously with the outflow from the leaf or respiratory loss. ^{13}C abundance in all parts except for the fed leaf increased rapidly up to Day 1 and then decreased mainly due to the dilution by non-labelled carbon newly assimilated. ^{15}N abundance in vegetative parts also increased up to Day 1, followed by a gradual decrease thereafter. In the young panicle, ^{15}N abundance increased up to Day 3, then very small changes were observed. The young panicle showed the highest ^{13}C and ^{15}N abundances among the plant parts at Day 1, followed by the tillers, the culm and the root. Within the whole root, the highest abundances of both ^{13}C and ^{15}N were detected in the upper roots followed by the middle and the lower roots. Very low isotopes abundances were detected in the lower mature leaves.

Table 2 Changes in ^{13}C and ^{15}N abundances in various parts of rice plant fed from a terminal leaf on the main stem with $^{13}CO_2$ and $^{15}NO_2$.

Plant Part	^{13}C abundance (atom % excess)					^{15}N abundance (atom % excess)				
	0*	1/4	1	3	8	0	1/4	1	3	8
Young panicle	0.58	1.14	2.16	1.36	0.84	0.13	0.33	0.54	0.65	0.61
Leaves 7**	2.04	1.50	0.78	0.53	0.43	1.18	1.06	0.93	0.79	0.59
6	0.00	0.08	0.16	0.02	0.05	0.01	0.05	0.07	0.03	0.03
5	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.02	0.02	0.01
3-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Culm	0.17	0.38	0.62	0.54	0.44	0.05	0.19	0.25	0.24	0.15
Tillers	0.15	0.47	1.18	0.75	0.28	0.04	0.21	0.35	0.34	0.24
Roots upper	0.03	0.24	0.88	0.58	0.22	0.05	0.09	0.20	0.15	0.11
middle	0.00	0.03	0.21	0.12	0.07	0.00	0.05	0.08	0.06	0.02
lower	0.00	0.00	0.05	0.01	0.00	0.01	0.04	0.03	0.01	0.02
whole	0.01	0.06	0.33	0.27	0.14	0.01	0.06	0.11	0.09	0.07

* Days after the feeding of isotopes.

** $^{13}CO_2$ and $^{15}NO_2$ were fed to 7th leaf for two hours.

The distributions of the two isotopes in the plant are summarized in Fig. 3. Extremely large amount of ^{13}C was transported to the tillers. Transfer of ^{13}C to the tillers continued up to Day 3 when the tillers received more than 50% of the exported ^{13}C. The amount of ^{13}C in the young panicle, the culm and the roots increased rapidly up to Day 1. Labelled carbon in the young panicle and the culm tended to increase slightly from Day 1 to Day 8, while significant decrease was observed in the roots during this period.

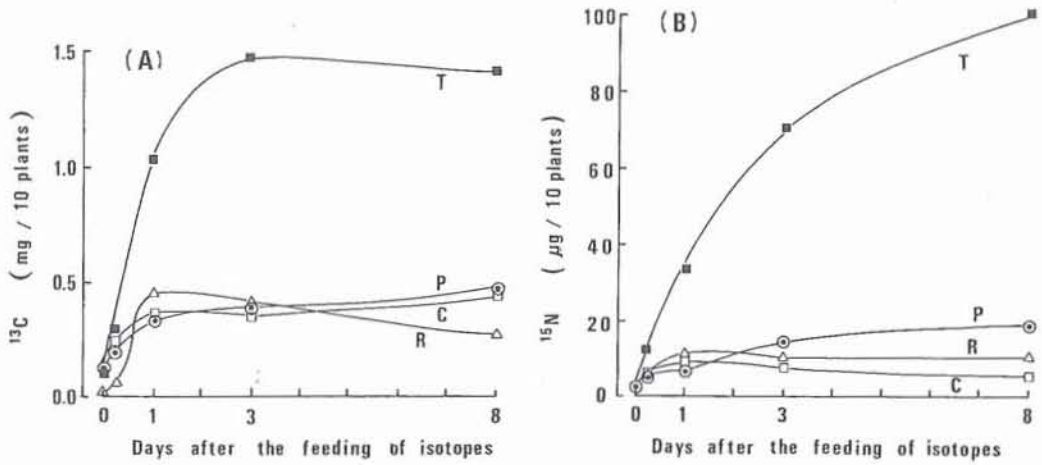

Fig. 3 Distribution of ^{13}C (A) and ^{15}N (B) in the various parts of rice plants fed from a terminal leaf on the main stem with $^{13}CO_2$ and $^{15}NO_2$.
Young panicle (◎), culm (□), tillers (■) and roots (△).

The amount of ^{15}N in the tillers and the young panicle continued to increase up to Day 8 with a gradual efflux of ^{15}N from the fed leaf. The tillers received a large proportion (73%) of exported ^{15}N at Day 8. Labelled nitrogen in the roots and the culm increased up to Day 1, thereater ^{15}N in the roots was maintained at a steady level. Only a small amount of both ^{13}C and ^{15}N was translocated to the lower leaves.

Discussion

Carbon dioxide taken up in rice leaves could be converted into sugars and translocated to the other parts as mainly sucrose (Asada et al. 1960). Most of the transfer of ^{13}C from the fed leaf to the other parts took place within one day, and during this time 21% of photoassimilated ^{13}C was lost from the whole plant through respiration (Table 1, Fig. 2). The end of the rapid phase of respiration was associated with the cessation of export of labelled carbon from the fed leaf. The synchronism between the intense respiratory loss of $^{13}CO_2$ and the translocation of ^{13}C to the growing tissues observed during one day is indicative of a causal relationship. Although we did not determine the respiratory loss of ^{13}C by each plant part, the bulk of the respiratory loss of ^{13}C might occur from the plant parts importing ^{13}C from the fed leaf (Gordon et al. 1979). Retransfer of the carbon from once distributed part seemed to be very small, and most of ^{13}C in the plant appeared to be immobilized at the sites to which it had been translocated during the

first one day. Continuous transfer of ^{13}C to the actively growing parts such as the tillers, the young panicle and the upper roots observed after Day 1 (Table 2, Fig. 3) might be due to the transfer of the temporary metabolites accumulated in the fed leaf. These characteristics of carbon transfer in rice plant were very similar to those in uniculm barley (Gordon et al. 1977) and sunflower (Yoneyama et al. 1980). It seems that the utilization system of carbon in rice plant may be also organized on a diurnal basis with little carry-over of one day's assimilates to another as Gordon et al. (1977) indicated on uniculm barley.

Transfer of NO_2-nitrogen from the fed leaf occurred gradually throughout the experimental period with a rapid efflux in the first several hours (Fig. 2). Nitrogen dioxide absorbed in the leaf through stomata could be converted into nitrite and nitrate and further assimilated into amino acids via glutamine synthetase and glutamate synthase system (Yoneyama and Sasakawa 1979). Some of amino acids synthesized may be directly exported from the fed leaf to the other parts through phloem in the first several hours, while others may be incorporated into proteins and other nitrogenous compounds in the fed leaf. The latter could be hydrolyzed later and amino acids produced were exported from the leaf gradually (Kaji et al. 1980, Okano et al. 1981). Yoneyama and Sano (1978) reported that in mature rice leaves the proteins involved were turned over and that the nitrogen once incorporated into the constituents of the mature leaves was then retranslocated to the young growing parts. Thus, in contrast to carbon, NO_2-nitrogen once incorporated into the leaf constituents may be easily remobilized and utilized repeatedly according to the demand of meristematic tissues.

Sink activity for NO_2-nitrogen expressed as the abundance of ^{15}N was highest in the yong panicle, followed by the tillers, the culm, the whole roots and the lower leaves (Table 2). This labelling pattern of ^{15}N in the various plant parts was very similar to that of ^{13}C. Only small amounts of both ^{13}C and ^{15}N were translocated to the mature lower leaves where the phloem might be active in export but not in import (Kaneko et al. 1980). These results suggest that the ^{13}C-labelled sugars and the ^{15}N-labelled nitrogenous compounds derived from NO_2 might flow together in the phloem as a bulk stream at least during the phase of primary distribution (probably within the first one day). This assumption may be supported by the finding (Hanson and Tully 1979) that the translocation velocities of ^{14}C-labelled glutamate and proline in phloem were similar to those of $^{14}CO_2$ assimilates.

The $^{13}C/^{15}N$ ratios at Day 1 or Day 3 may indicate the relative requirement of carbon and nitrogen in the various plant parts (Table 3). In the young panicle and the tillers, the ratio decreased from Day 1 to Day 3; this was attributed mainly to a gradual influx of ^{15}N. A high $^{13}C/^{15}N$ ratio observed in the young panicle, the culm and the upper roots may reflect the high synthetic activity of cell wall constituents in these parts. Active protein synthesis probably occurred in the tillers might result in a relatively low $^{13}C/^{15}N$ ratio in this part. Those results suggest that the relative distribution of carbon and nitrogen might be partly regulated by the characteristics of the sink organs.

The present result on rice and the previous ones on sunflower and corn (Yoneyama et al. 1980, Okano et al. 1981) show that NO_2-nitrogen incorporated into amino acids and proteins in plant leaves was then retransferred to the rapidly growing organs probably through phloem along with the photosynthates. The distribution pattern of nitrogen derived from NO_2 in rice plant was very similar to that of nitrogen retransferred from mature rice leaves whose nitrogen was supplied through root as ammonium sulfate (Yoneyama and Sano 1978). Therefore, NO_2-nitrogen absorbed through plant leaves would be utilized for the synthesis of the new tissues in the same manner as the nitrogen taken up by roots as nitrate or ammonium. This implys that in some cases, low concentrations of NO_2 which would not cause acute injuries may affect beneficially on plant growth as a nitrogen fertilizer from the atmosphere.

Table 3. The C/N and $^{13}C/^{15}N$ ratios in the various parts of rice plant fed $^{13}CO_2$ and $^{15}NO_2$ from a terminal leaf on the main stem.

Plant part	Days after the feeding of isotopes			
	1		3	
	C/N	$^{13}C/^{15}N$	C/N	$^{13}C/^{15}N$
Young panicle	12.4	49.8	12.8	26.8
7th leaf*	11.2	9.4	11.4	7.7
Lower leaves	12.4	—	12.7	—
Culm	17.1	42.3	20.3	45.6
Tillers	9.3	31.2	9.5	21.0
Roots upper	10.8	47.8	11.7	45.4
middle	12.2	31.9	14.2	28.4
lower	21.1	—	28.4	—

* $^{13}CO_2$ and $^{15}NO_2$ were fed to 7th leaf for two hours.

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水稲葉に吸収された $^{15}NO_2$ と $^{13}CO_2$ の体内移動

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葉から吸収された二酸化窒素（NO_2）の植物体内での挙動を明らかにする目的で，^{15}N 標識の NO_2 と ^{13}C 標識の二酸化炭素を幼穂形成期の水稲の最上位葉に同時に供与し，2種類の安定同位体の行方を8日間追跡・比較した。

同化葉からの ^{13}C の転流は1日で大部分が終了し，その間に固定された ^{13}C の21％が呼吸により失なわれた。^{15}N の同化葉からの流出は ^{13}C に比べて遅く，かつ実験期間中継続して起り，その間における植物体からの ^{15}N の損失は認められなかった。

いずれの同位体の存在比も活発に生長している器官，すなわち幼穂，分げつ，新根において高かった。^{15}N と ^{13}C の体内分布パターンの類似性から，少なくとも一次分配過程では ^{15}N と ^{13}C は一緒に師管中を動くと考えられた。一方，各部位の $^{13}C/^{15}N$ 比の違いは，炭素と窒素の相対的分布がシンク器官の性格によっても影響されることを示していた。

これらの結果から，葉面の気孔を通じて吸収され葉のタンパク質に取り込まれた NO_2 由来の窒素は，その後徐々にアミノ酸に加水分解され，光合成産物とともに師管を通って生長中の部位へ転流し，新しい組織の形成に利用されると考えられた。

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Image instrumentation of plants exposed to air pollutants (1) Quantification of physiological information included in thermal infrared image

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In order to evaluate spacial patterns of physiological informations such as transpiration rate, stomatal resistance, and air pollutant sorption from leaf temperature distribution measured by an instrumentation system of thermal infrared image, heat and mass transfer on a leaf was examined, and then models for the evaluating were derived. Furthermore, parameters of the models, accuracies of environmental controls and measurements and so on were examined by the experiments. As a result, transpiration rate and stomatal resistance to water vapor diffusion on the leaf were evaluated within accuracies of 0.02×10^{-5} g·cm^{-2}·s^{-1} and 0.3 s·cm^{-1}, respectively. Accuracy of transpiration rate may be applied correspondingly to that of the sorption rate of air pollutants such as SO_2, NO_2, O_3 and PAN from an analogy between the models for the evaluating transpiration rate and sorption rates of these air pollutants. Finally, changes in spacial distributions of physiological informations mentioned above were evaluated from the change in distribution of leaf temperature during SO_2 exposure.

Key words: Thermal infrared image — Air pollutant — Leaf temperature — Sorption —Stomatal resistance — Transpiration

In the research fields concerned with the production of plants, the effects of environmental pollutants on plants, and their purification functions, it is necessary to measure the physiological reaction and the heat and mass transfer of growing plants without destroying or touching them (Takatsuji et al. 1979, Hashimoto et al. 1982, Omasa and Aiga 1984). Recently, methods for measuring and evaluating these physiological informations as the image have been developed (Hashimoto et al. 1982, Omasa and Aiga 1984).

Leaf temperature of the plants is not only an important factor determining physiological reactions such as photosynthesis and respiration (Berry and Björkman 1980) but also a useful factor as a physiological information because it relates to water balance and heat and mass transfer (Raschke 1960, Takiuchi and Hashimoto 1977, Omasa et al. 1980). The thermography has been used for measuring surface temperature of the object. Cetas (1978) examined problems in measuring the surface temperature with it. Schurer (1975) applied it to the observation of leaf

temperature distribution. Recently, Hashimoto et al. (1979, 1980) and Omasa et al. (1980) introduced image instrumentation systems with a thermal camera of scanning type and a computer in order to process very numerous image data. They also reported that the leaf temperature distribution gave qualitatively spacial patterns of physiological informations such as stomatal movement, transpiration, and air pollutant sorption. However, the mathematical model and the experimental apparatus must be closely examined in order to quantify these physiological informations. Therefore, we made the present study to develop a method for obtaining quantitative distributions of physiological informations such as stomatal resistance, transpiration rate, and air pollutant sorption rate from the leaf temperature distributions.

Principle for quantification of physiological informations of plants

Heat and mass transfer model at the local site on a leaf

Water vapor evaporates from cell wall of stomatal bottom and diffuses into the free air through substomatal cavity, stomatal throat, and leaf boundary layer (Fig. 1). Flux of the

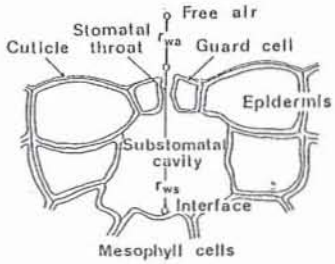

Fig. 1 Schematic cross-section of a stoma.

transpiration at the local site on a leaf is fundamentally determined by the difference of the water vapor density and the diffusion resistance between the free air and the gas-liquid interface of stoma. If the leaf temperature, stomatal conditions and leaf boundary layer are assumed to be equal at both sides on a leaf, transpiration rate, W_x (g·cm^{-2}·s^{-1}) at the local site is

$$W_x=2\{X_{sx}(T_{lx}) - \Phi X_s(T_a)\}/(r_{wax} + r_{wsx}) \quad (1)$$

where T_l is leaf temperature (°C), T_a is air temperature (°C), $X_s(T)$ is saturated water vapor density at T °C (g·cm^{-3}), Φ is relative humidity, r_{wa} is boundary layer resistance to water vapor diffusion (s·cm^{-1}) and r_{ws} is stomatal resistance to water vapor diffusion (s·cm^{-1}). The subscript x denotes the values at the local site x on the leaf.

On the other hand, air pollutants intrude into plant's tissues through the opposite process to that of water vapor. As for the model for the transfer of air pollutants, it is necessary to consider the reaction and the transfer in the tissues. These are represented by the gas concentration of the gas-liquid interface in the stomata. Therefore, air pollutant sorption rate, Q_x (g·cm^{-2}·s^{-1}) at the local site is given by

$$Q_x = 2(P_a - P_{lx})/(r_{gax} + r_{gsx}) \quad (2)$$

$$r_{gwx} = (D_w / D_g)^{2/3} r_{wwx} \tag{3}$$

and

$$r_{gsx} = (D_w / D_g) r_{wsx} \tag{4}$$

where P_1 is gas concentration of the gas-liquid interface in the stomata ($g \cdot cm^{-3}$), P_a is gas concentration of air ($g \cdot cm^{-3}$), r_{ga} is boundary layer resistance to gas diffusion ($s \cdot cm^{-1}$), r_{gs} is stomatal resistance to gas diffusion ($s \cdot cm^{-1}$), D_w is air-water vapor diffusivity (= 0.249 $cm^2 \cdot s^{-1}$; 25° C) and D_g is air-gas diffusivity (= 0.129 $cm^2 \cdot s^{-1}$; SO_2, 25° C). The gas concentration of the gas-liquid interface of major air pollutants such as SO_2, NO_2, O_3 and PAN, P_{1x} can be assumed to be nearly equal to zero, because the metabolism and transfer in the plant's tissues are very large (Omasa and Abo 1978, Omasa et al. 1979, Omasa 1979, Nouchi 1980).

Heat balance at the local leaf site

Heat balance at the local site on the leaf is

$$c_1 w_1 \mathrm{d}T_{1x} / \mathrm{d}t = E_x + S_x - LW_x + M_x + G_x \tag{5}$$

where E is net radiation ($cal \cdot cm^{-2} \cdot s^{-1}$), S is sensible heat transfer by convection ($cal \cdot cm^{-2} \cdot s^{-1}$), M is reaction heat ($cal \cdot cm^{-2} \cdot s^{-1}$), G is heat conduction from other sites ($cal \cdot cm^{-2} \cdot s^{-1}$), c_1 is specific heat ($cal \cdot g^{-1} \cdot °C^{-1}$), w_1 is weight ($g \cdot cm^{-2}$), t is time (s), and L is latent heat by evapolation (= 583.3 $cal \cdot g^{-1}$; 25 °C).

Net radiation, E_x in Eq. (5) is

$$E_x = \alpha_p E_{sx} + \epsilon \{E_{wx} - 2\sigma(273.15 + T_{1x})^4\} \tag{6}$$

and sensible heat transfer, S_x is

$$S_x = 2\rho c_p (T_a - T_{1x}) / r_{kax} \tag{7}$$

where E_s is shortwave radiation from the environment ($cal \cdot cm^{-2} \cdot s^{-1}$; wavelength $\lesssim 3\ \mu m$), E_w is longwave radiation from the environment ($cal \cdot cm^{-2} \cdot s^{-1}$; wavelength $\gtrsim 3\mu m$), r_{ka} is boundary layer resistance to heat transfer ($s \cdot cm^{-1}$), α_p is absorption coefficient of shortwave radiation of the leaf (=0.67, sunflower), ϵ is emissivity of longwave radiation of the leaf, σ is Stefan-Boltzmann constant (= 1.354×10^{-12} $cal \cdot cm^{-2} \cdot s^{-1} \cdot K^{-4}$) and ρc_p is volumetric heat capacity of air (= 0.285×10^{-3} $cal \cdot cm^{-3} \cdot °C^{-1}$; 25° C).

From an analogy of heat transfer and mass transfer, ratio of boundary layer resistance to water vapor diffusion to that to heat transfer, r_{wax} / r_{kax} is given by

$$r_{wax} / r_{kax} = (\kappa / D_w)^{2/3} \tag{8}$$

where κ is thermal diffusivity of air (=0.222 $cm^2 \cdot s^{-1}$; 25 °C).

Reaction heat, M_x by photosynthesis, respiration and so on is usually within 2 to 3% of shortwave radiation. In the case of 0.2° C·min^{-1} change of leaf temperature of a thin leaf such as sunflower plant, the accumlated heat, $c_1 w_1 \mathrm{d}T_{1x} / \mathrm{d}t$ is ca. 2 to 3% of latent heat by transpiration or net rediation. Heat conduction from other sites, G_x is ca. 5%, when heat conduction by 1° C·cm^{-1} heat gradient arises in 0.5 cmϕ site. Usually, $c_1 w_1 T_{1x} / \mathrm{d}t$ and G_x at the local site of the leaf exposed to air pollutants are within above-mentioned values. Therefore, Eq. (5) can be simplified as follows.

$$E_x + S_x - LW_x = 0 \tag{9}$$

Substituting Eqs. (6) and (7) in Eq. (9) gives the following equation for transpiration rate at the local site.

$$W_x = [\alpha_p E_{s_x} + \epsilon\{E_{w_x} - 2\sigma(273.15 + T_{1_x})^4\} + 2\rho c_p(T_a - T_{1_x})/r_{ka_x}]/L \tag{10}$$

When environmental conditions such as air temperature, humidity, radiation and air current are kept constant, only leaf temperature, T_{1_x} is left as a variable in the right side of Eq. (10). Therefore, transpiration rate can be evaluated by measuring the leaf temperature. Furthermore, stomatal resistance to water vapor diffusion, r_{ws_x}, which is an indicator of the degree of stomatal opening, is evaluated from Eqs. (1) and (8).

$$r_{ws_x} = 2\{X_{s_x}(T_{1_x}) - \Phi X_s(T_a)\}/W_x - (\kappa/D_w)^{2/3} r_{ka_x} \tag{11}$$

In addition, since the stomatal resistance to air pollutant diffusion, r_{gs_x} and the boundary layer resistance to air pollutant diffusion, r_{ga_x} can be determined from the relationship between Eqs. (3), (4) and (8), the sorption rate, Q_x of air pollutants, such as SO_2, NO_2, O_3 and PAN, at the local site is evaluated by substituting these resistances in Eq. (2).

Epitome and performance of apparatus

Thermal infrared image instrumentation system

The block diagram of a thermal infrared image instrumentation system is shown in Fig. 2. The thermal camera is an optical-mechanical scanning type and its detector is an HgCdTe (8 to 13 μm, cooled by liquid nitrogen). The detected signals from the thermal camera are converted into 12-bit digital signals (256^Hx240^V, quantization error 0.0125° C) by a video processor and analyzed by a computer. The system also has a vidicon camera for evaluating the visible injury on a leaf surface.

The detected image was enhanced by integrating using the video processor. The temperature resoving power, the uniformity of image, and the drift were within 0.05° C, ± 0.1° C and ± 0.05° C/4hr, respectively. The image resolving power was ca. 50^Hx40^V in the measuring at error within 4% (0.2° C).

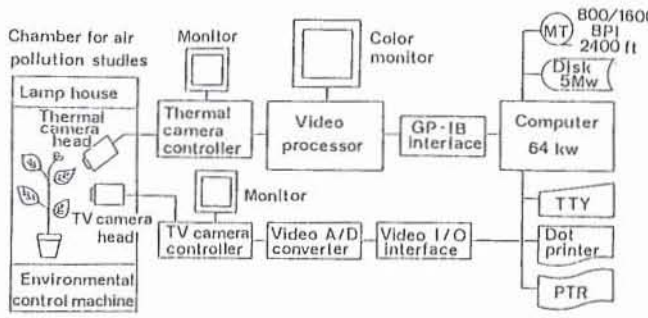

Fig. 2 Block diagram of a system for image instrumentation of plants.

Environment control system

Experiments were carried out in an environmental control chamber which was designed and constructed for studies of air pollution effects on plants (Aiga et al. 1982). Control accuracies of air temperature, humidity and air pollutant concentration in the chamber were within ±0.1°C, ±1% and ±0.04 volppm, respectively. An apparatus for fixing an intact leaf or a model leaf horizontally and fans for maintaining uniform air current on the leaf surface were set in the chamber (Fig. 3). The intact leaf or the model leaf was attached to a thin plastic sheet (20 × 20 cm) which was cut out in a shape similar to that of the leaf, and was placed on the fixing plate with stretched fishing-line (Fig. 3). The Yoko lamp (Toshiba) with heat absorbing filter was used as a light source. The scattering filter was placed between the light source and the fixing apparatus in order to prevent the direct exposure of shortwave radiation from the light source to the leaf. The wall in the chamber was covered with a black wool paper and a lawn. Consequently, the spatial distribution of shortwave radiation on a leaf surface set on the fixing apparatus was within ±2% of the mean value. Furthermore, the temperature distribution of the model leaf (black wool paper) was kept within ± 0.2°C except at the leaf edges by controlling air current. This result means that local boundary layer resistance is constant at every sites on the leaf. Boundary layer resistances of both sides on the leaf were kept the same by adjusting the air current.

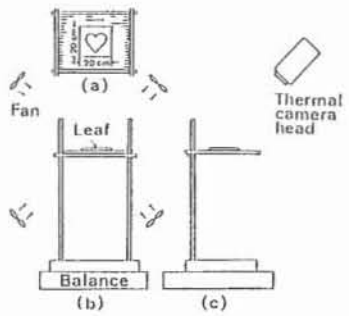

Fig. 3 Schematic diagram of an apparatus for fixing a leaf. Environmental conditions on the leaf were maintained constant.

Other instruments

Temperature was calibrated with a standard glass thermometer (accuracy ±0.03°C) and thermocouples of 0.1 mm diameter. Humidity was measured with a dew-point instrument (EG and G, Model 660, accuracy ±0.2°C), shortwave radiation with radiometers (Eko, Model MS-42 and LI-COR Model LI-185), SO_2 concentration with a pulsed fluorescent SO_2 analyzer (Thermo Electron, Model 43), and weight with electric balances (Mettler, Model PK16 and PL3000, limit of weighing 0.1g).

Problem in quantification and its experimental accuracy

Absorption coefficient of shortwave radiation and emissivity of longwave radiation of intact plant leaf or model leaf

The absorption coefficient of shortwave radiation of sunflower leaves grown for 3 to 6

weeks after sowing, α_p was 0.68 ± 0.03 and that of model leaves (black wool paper) was 0.98 ± 0.02, these were measured with the Model MS-42 radiometer (effective wavelength; ca.0.4 to 3 μm). Their longwave emissivities, ϵ measured with the thermal camera were 0.98 ± 0.02. Although the effective wavelength of the thermal camera is 8 to 13 μm, the obtained emissivity is regarded as the mean value in the range of the longwave because the spectral emissivity of the leaf is approximately constant in the range above 3 μm. The emissivity of healthy leaf was compared with that of leaf exposed to SO_2 for about 2 hours, but there was no difference.

Influence of environment in measuring the leaf temperature

Although the thermal camera has a function for correcting the influence of radiation from the environment, it may cause the error when the radiation from the environment is not uniform. Therefore, we examined the influence of the environment in measuring leaf temperature in the chamber. Experiment was carried out in such a way that the infrared camera was horizontally fixed, and then the angle of the leaf surface to the camera face was altered from 60° to −60°. Although the leaf surface received different radiation from various parts of inside of the chamber according to altering the angle between the leaf surface and camera face, the difference between temperature measured with the infrared camera and that with the thermocouple was within ±0.1°C regardless of difference of the angle.

Longwave radiation from environment and boundary layer resistance

Substituting Eqs.(6) and (7) in Eq.(9) gives the following heat balance equation.

$$\alpha_p E_{s_x} + \epsilon E_{w_x} - 2\epsilon\sigma(273.15 + T_{l_x})^4 + 2\rho c_p(T_a - T_{l_x})/r_{ka_x} - LW_x = 0 \qquad (12)$$

Since E_{s_x}, E_{w_x}, W_x, T_{l_x} and r_{ka_x} are constant all over the model leaf surface on the above-mentioned apparatus, Eq.(12) may be regarded as a heat balance equation of one leaf. Therefore, longwave radiation from the environment, E_{w_x} and boundary layer resistance to heat transfer, r_{ka_x} can be evaluated by substituting leaf temperature, transpiration rate and shortwave radiation obtained from experiments of a dry or wet model leaf in Eq.(12) and by solving the obtained simultaneous equations. In order to examine the accuracy of this method, r_{ka_x} evaluated from Eq.(12) was compared with that from the following equation obtained by substituting Eq.(8) in Eq.(1) where r_{ws_x} was assumed to be zero s·cm^{-1}.

$$r_{ka_x} = 2\{X_{s_x}(T_{l_x}) - \Phi X_s(T_a)\}/\{W_x(\kappa/D_w)^{2/3}\} \qquad (13)$$

where T_{l_x} and W_x of a wet model leaf were substituted. This result is shown in Fig. 4. Although r_{ka_x} obtained by Eq.(12) tended to be smaller than that by Eq.(13), the difference between both values was within 0.1 s·cm^{-1}. Furthermore, the difference in E_{w_x} evaluated by substituting r_{ka_x} in Eq.(12) was within 1×10^{-4} cal·cm^{-2}·s^{-1}.

Accuracy of quantification of plant physiological information

In order to examine the accuracy of the evaluataion using the above-mentioned method, transpiration rate and stomatal resistance to water vapor diffusion of sunflower leaves obtained by this method were compared with those obtained by the weighing method. This result is shown in Fig. 5. Fig. 5 (a) shows the relationship between transpiration rate, W_w, obtained by the weighing method and W_x calculated from Eq.(10). Symbol I denotes distribution of W_x and symbol ∘ denotes the mean value of all over the leaf surface in this figure. Being the mean value of one leaf, W_w are compared with the mean value of W_x. When transpiration rate was below 0.1×10^{-5} g·cm^{-2}·s^{-1}, the leaf wilted and the leaf color slightly altered. The difference between W_w and the mean value of W_x was within 0.02×10^{-5} g·cm^{-2}·s^{-1} except above case. Fig. 5 (b) shows

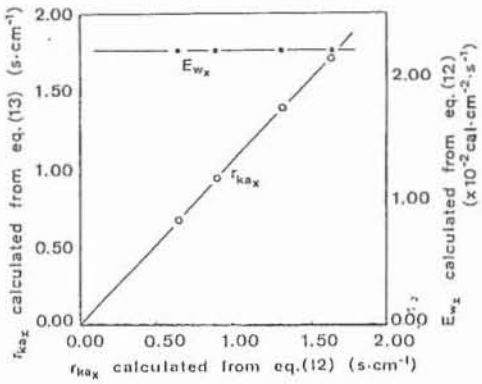

Fig. 4 Accuracies in evaluating boundary layer resistance on the leaf to heat transfer (r_{ka_x}) and longwave radiation from environment (E_{w_x}).
Conditions: T_a=25.0°C, Φ=59 − 62%RH, E_{s_x}=2.91 − 3.01 × 10^{-3} cal·cm^{-2}·s^{-1}.

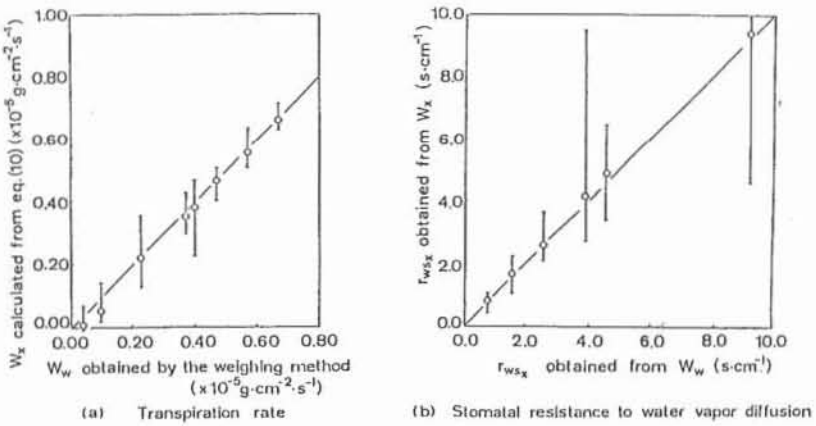

Fig. 5 Accuracies in evaluating transpirtion rate (W_x) and stomatal resistance to water vapor diffusion (r_{ws_x}). Conditions and parameters: T_a=24.9 − 25.4°C, Φ=58 − 60%RH, r_{ka_x}=1.64s·cm^{-1}, E_{s_x}=3.01 × 10^{-3} cal·cm^{-2}·s^{-1} (ca. 30 klx), E_{w_x}=22.21 × 10^{-3} cal·cm^{-2}·s^{-1}.

the relationship between stomatal resistance to water vapor diffusion, r_{ws_x} obtained from W_w and that obtained from W_x. When r_{ws_x} was below 10 s·cm^{-1}, the difference between r_{ws_x} from W_w and that from the mean value of W_x was within 0.3 s·cm^{-1}. Although the accuracy of evaluation of transpiration rate and stomatal resistance depend on the environmental conditions, we can expect sufficient accuracy under usual environmental conditions from this result. Furthermore, the accuracy of transpiration rate may be applied correspondingly to that of the sorption rate of air pollutants such as SO_2, NO_2, O_3 and PAN from an analogy between the models for the evaluating the transpiration rate and the sorption rate of these air pollutants.

Image instrumentation of leaf temperature of intact plant exposed to air pollutant and quantification of its physiological information

The above-mentioned method for the image instrumentation of leaf temperature and the quantification of physiological information was applied to the evaluation of physiological reaction of a sunflower plant under SO_2 exposure. An intact leaf of the test plant was placed on the fixing apparatus, and then environmental factors such as air temperature, humidity, radiation from environment and boundary layer resistance were maintained constant. After the plant was sufficiently acclimatized to the new conditions, the exposure to ca. 1.5 volppm SO_2 was carried out for 60 minutes. Figure 6 shows changes in the leaf temperature distribution. The leaf temperature was 23.4 to 24.5° C before the SO_2 exposure, began to rise after the exposure and reached 24.7 to 26.8° C after 60 minute exposure. Figure 7 shows changes in distributions of transpiration rate, stomatal resistance to water vapor diffusion and SO_2 sorption rate evaluated from the leaf temperature shown in Fig. 6. Transpiration rate, stomatal resistance and SO_2 sorption rate before the exposure were 0.56 to 0.72×10^{-5} g·cm^{-2}·s^{-1}, 0.6 to 1.5 s·cm^{-1} and 0.0 g·cm^{-2}·s^{-1}, and after 60 minute exposure, they became 0.19 to 0.52×10^{-5} g·cm^{-2}·s^{-1}, 1.8 to 9.1 s·cm^{-1} and 0.04 to 0.17×10^{-8} g·cm^{-2}·s^{-1}, respectively. The stomatal resistance, which is an indicator of the stomatal aperture, increased and the transpiration rate and SO_2 sorption rate decreased with lapse of exposure time. However, their behaviors varied randomly at different sites on a leaf. This result means that stomatal sensitivity to SO_2 varies at the local site of a leaf. SO_2 caused physiological effects such as decrease of photosynthesis, fading of vegetal pigments and destruction of cells. Although these phenomena also vary at different sites of the leaf, the relationship between the sorption at local site and their phenomena will be elucidated by introducing this method.

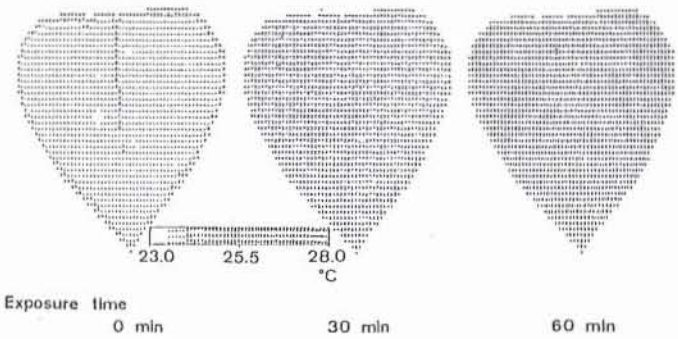

Fig. 6 An example of changes in temperature distribution of a sunflower leaf during SO_2 exposure.
Conditions: T_a=25.1° C, Φ=69%RH, r_{ka_x}=0.875s·cm^{-1}, E_{s_x}=2.91 × 10^{-3} cal·cm^{-2}·s^{-1}, E_{w_x}=22.13 × 10^{-3} cal·cm^{-2}·s^{-1}, P_a=1.5 volppm.

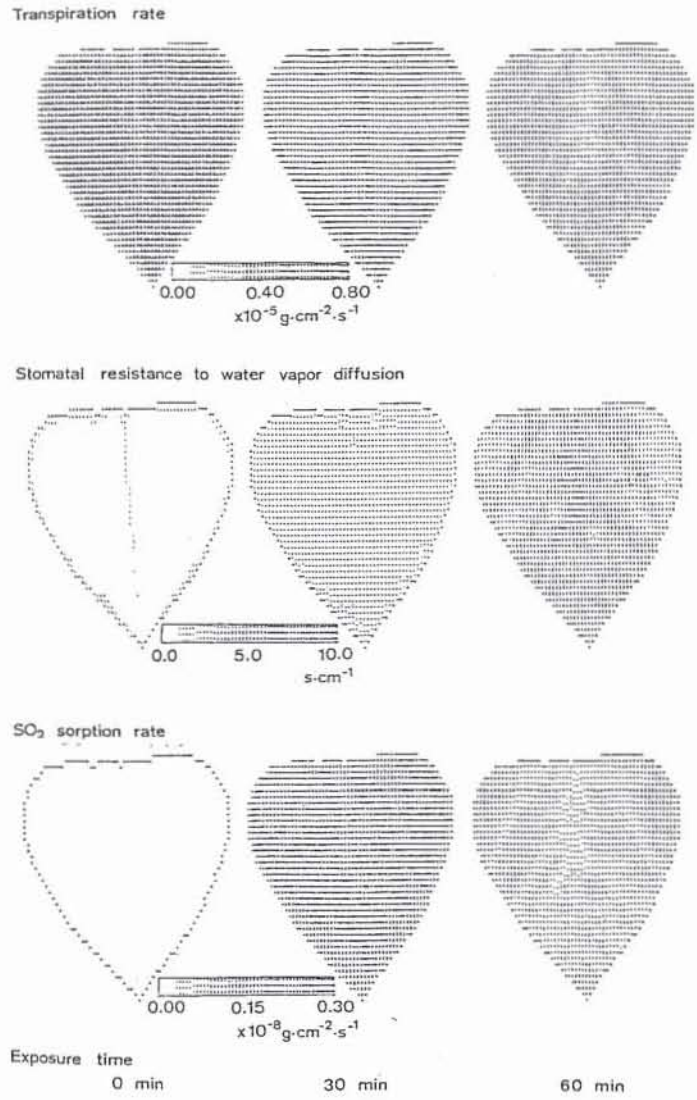

Fig. 7 Changes in distributions of transpiration rate, stomatal resistance to water vapor diffusion and SO_2 sorption rate obtained from the leaf temperature distributions in Fig. 6.

Conclusion

In this paper, we have examined heat and mass transfer at the local site on a leaf and derived models for evaluating the spacial distribution of physiological information such as transpiration rate, stomatal resistance and sorption rate of air pollutants from the leaf temperature distribution. We have also examined problems in quantification of these physiological information experimentally. Consequently, the transpiration rate and the stomatal resistance to water vapor diffusion on the leaf were evaluated within accuracies of 0.02×10^{-5} g·cm^{-2}·s^{-1} and

0.3 $s \cdot cm^{-1}$, respectively. The accuracy of transpiration rate may be applied correspondingly to that of sorption rate of air pollutants such as SO_2, NO_2, O_3 and PAN from an analogy between the models for the evaluating the transpiration rate and the sorption rate of these air pollutants. Finally, the change in distribution of the physiological information during SO_2 exposure was evaluated as an application.

This method may be effective for elucidation of the mechanism of stomatal movement, the relationship between sorption of air pollutants and their effects, and the physiological resistance to air pollutants. Furthermore, this method may be applied to not only analysis of the plant reaction to air pollutant, but also that of the phenomena related to mass transfer of plants such as plant production.

This study was published in Transactions of the Society of Instrument and Control Engineers (Omasa et al. 1981).

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植物の画像計測

(1) 熱赤外画像に含まれる生体情報の定量化について

大政謙次[1]・橋本　康[2]・相賀一郎[1]

本論文では，葉の局所部位における熱および物質の輸送について検討し，葉温分布から蒸散速度，気孔抵抗，汚染ガス吸収速度などの生体情報の葉面分布を推定するモデルを導いた。そして，実際に，これらの生体情報を定量化する際の諸問題について実験的検討を行い，蒸散速度および水蒸気拡散に対する気孔抵抗を0.02×10^{-5}g・cm^{-2}・s^{-1}，0.3s・cm^{-1}の精度で推定することができた。また，主要な汚染ガスであるSO_2，NO_2，O_3，PANなどの吸収速度の推定精度は，蒸散速度と汚染ガス吸収速度の推定モデルの相似性から，蒸散速度のそれに準ずるものと考えられた。最後に，SO_2に被暴した植物葉の反応について検討したが，ここで述べた面領域での生体情報の定量化の手法が，気孔開閉のメカニズムや植物葉の被害と汚染ガス吸収との関係，さらには，汚染ガスに対する生理的な抵抗性の解明に有効であることが示唆された。

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Res. Rep. Natl. Inst. Environ. Stud., Jpn., No.66, 1984.

Image instrumentation of plants exposed to air pollutants (2) Relationships between SO_2 or NO_2 sorption and their acute effects on plant leaves

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We have reported on a method for evaluating the distributions of stomatal resistance to water vapor diffusion and SO_2 or NO_2 sorption on a leaf, using a thermal infrared image instrumentation system. In the present paper, we examined quantitatively the relationships between the acute effects, such as stomatal response and visible injury, of SO_2 or NO_2 on a leaf and gas sorption, using the image instrumentation method. The results obtained were as follows.

1) There was a tendency for stomata to close during SO_2 or NO_2 exposure. However, the behavior varied randomly at different sites on a leaf. The differences in stomatal response at local sites were not dependent on those in integrated SO_2 or NO_2 sorption for 60 minutes exposure. These results suggest that there are differences in the stomatal sensitivity to SO_2 or NO_2 at local sites on a leaf.

2) There was a tendency for visible injury to occur at sites where the integrated SO_2 or NO_2 sorption was over a threshold value. Injured leaves were generally separated into two areas, a healthy area and an injured one. It was seen that the characteristic visible injuries were caused by differences in boundary layer and stomatal resistances at local sites governing the gas sorption.

Key words: Image – Leaf temperature – SO_2 or NO_2 sorption – Acute effects – Stomatal response – Leaf injury

Sulfur dioxide and nitrogen dioxide are major air pollutants that cause various effects on plants (Mudd 1975, Taylor *et al.* 1975, Horsman and Wellburn 1976). The degree of the effects of SO_2 or NO_2 in relation to the concentration, dosage size and the amount of sorption on one leaf or a whole plant has been frequently discussed (Thomas 1961, Mudd 1975, Taylor et al. 1975, Bressan *et al.* 1978). However, visible injury and stomatal response, which are conspicuous symptoms of the acute effects, vary strikingly at different sites on the leaf (Barrett and Benedict 1970, Taylor and Maclean 1970, Omasa *et al.* 1980, 1981a). Under the usual exposure conditions in the field and growth chamber, the leaf boundary layer and stomatal resistances governing SO_2 and NO_2 sorption also vary (Monteith 1973, Omasa *et al.* 1980, 1981a). Therefore, to better understand the effects of SO_2 and NO_2 sorption, it is necessary to clarify relationships between local sorption, factors governing the sorption, and the degree of

the effects at local sites.

We have recently developed an instrumentation method for studying the distribution of the sorption of air pollutants, transpiration, and stomatal diffusion resistance of leaves using a thermal infrared image instrumentation system, and have measured changes in the distributions of leaf temperature, transpiration rate, sorption rate and stomatal resistance to water vapor diffusion during SO_2 exposure(Omasa *et al.* 1981a). However, the relationships between sorption and its effects all over the leaf surface have not been determined.

We therefore made the present study to elucidate the relationships between the distribution patterns of SO_2 or NO_2 sorption and its acute effects such as visible injury and stomatal response on leaves.

Materials and methods

Plant materials

Sunflower plants (*Helianthus annuus* L. cv. Russian Mammoth) were grown in a phytotron at 25/20°C day/night temperature and 70%RH under natural light for 6 to 7 weeks (1,800 to 2,500 cm^2 leaf area/plant and 20 to 25 leaves/plant) after sowing in pots (10 cm in diameter and 20 cm in height). The pot was filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss and fine gravel which was moistened with nutrient solution. The plants were irrigated daily. Intact mature leaves (130 to 140 cm^2 leaf area) were used in the experiments.

Environment control system(Omasa et al. 1981a)

SO_2 and NO_2 were introduced into the environment control chamber designed and constructed for studies of air pollution effects on plants. Air temperature and humidity in the chamber were maintained at 25.0±0.1°C and 62±1%RH. SO_2 and NO_2 concentrations were kept at the desired values of ca. 2 volppm and 7 volppm, respectively. An apparatus for fixing an intact leaf horizontally and fans for maintaining a uniform air current on the leaf surfaces were set in the chamber. The intact leaf was attached to a thin plastic sheet (20 × 20 cm^2) cut out geometrically in a shape similar to that of the leaf (cut area; ca. 100 cm^2), and was placed on the fixing apparatus. The distributions of shortwave radiation, longwave radiation, illumination and boundary layer resistance to heat transfer on the leaf surface were maintained at $2.37\pm0.05 \times 10^{-3}$ cal·cm^{-2}·s^{-1}, $2.23\pm0.01 \times 10^{-2}$ cal·cm^{-2}·s^{-1}, ca. 25klx, and 1.5±0.1 s·cm^{-1} except at the leaf edges.

Instrumentation system(Omasa et al. 1981a)

Distributions of integrated SO_2 or NO_2 sorption and stomatal resistance to water vapor diffusion were evaluated from the leaf temperature distribution, measured by using a thermal infrared image instrumentation system. Equations for evaluating their distributions were as follows:

(1) Stomatal resistance to water vapor diffusion at local site x, r_{wsx} (s·cm^{-1}), is expressed by

$$r_{wsx} = \frac{2\{X_{sx}(T_{lx}) - \Phi X_s(T_a)\}}{W_x} - \left(\frac{\kappa}{D_w}\right)^{2/3} r_{kax} \qquad (1)$$

and

$$W_x = \frac{\alpha_p E_{sx} + \epsilon\{E_{wx} - 2\sigma(273.15 + T_{lx})^4\} + 2\rho c_p(T_a - T_{lx}) / r_{kax}}{L}, \qquad (2)$$

where T_l is leaf temperature (°C), T_a is air temperature (°C), X_s (T) is saturated water vapor density at T°C (g·cm^{-3}), Φ is relative humidity, W is transpiration rate (g·cm^{-2}·s^{-1}), r_{ka} is boundary layer resistance to heat transfer (s·cm^{-1}), κ is thermal diffusivity of air (=0.222cm^2·s^{-1}), D_w is air-water vapor diffusivity (=0.249cm^2·s^{-1}), E_s is shortwave radiation from the environment (cal·cm^{-2}·s^{-1}), E_w is longwave radiation from the environment (cal·cm^{-2}·s^{-1}), α_p is absorption coefficient of shortwave radiation of the leaf (=0.68, sunflower), ϵ is emissivity of longwave radiation of the leaf (=0.98, sunflower), σ is Stefan-Boltzmann constant (=1.354 × 10^{-12} cal·cm^{-2}·s^{-1}·K^{-4}), ρc_p is volumetric heat capacity of air (=0.285 × 10^{-3} cal·cm^{-3}·°C^{-1}) and L is latent heat by evaporation (=583.3 cal·g^{-1}). The suffix x denotes the values at local site x on the leaf surface.

(2) Integrated SO_2 or NO_2 sorption at local site x, Q_{intx} (g·cm^{-2}), is expressed by

$$Q_{intx} = \int_0^T Q_x dt \tag{3}$$

$$Q_x = \frac{2P_a}{r_{gax} + r_{gsx}} \tag{4}$$

$$r_{gax} = \left(\frac{\kappa}{D_g}\right)^{2/3} r_{kax} \tag{5}$$

and

$$r_{gsx} = \left(\frac{D_w}{D_g}\right) r_{wsx}, \tag{6}$$

where Q is SO_2 or NO_2 sorption rate (g·cm^{-2}·s^{-1}), T is exposure time (s), t is time (s), P_a is SO_2 or NO_2 concentration of air (g·cm^{-3}), r_{ga} is boundary layer resistance to SO_2 or NO_2 diffusion (s·cm^{-1}), r_{gs} is stomatal resistance to SO_2 or NO_2 diffusion (s·cm^{-1}) and D_g is air-SO_2 or -NO_2 diffusivity (=0.129m^2·s^{-1} (SO_2), =0.155cm^2·s^{-1} (NO_2)).

This image instrumentation system was calibrated by a blackbody source (Electro Optical Industries, Models PD1401X and D254) with chromel-constantan thermocouples which were traceable to the National Bureau of Standards in the U.S.A. The error in measuring leaf temperature using this system was within ±0.1°C, and errors in evaluating sorption and stomatal resistance to water vapor diffusion were within ca. 10% and 0.3s·cm^{-1}, respectively, until beginning of fading of plant pigments. The resolution of this system was $256^H \times 240^V$ pixels. Air temperature was measured with a calibrated copper-constantan thermocouple of 0.1 mm diameter, humidity with a dew-point instrument (EG and G, Model 660), SO_2 concentration with a pulsed fluorescent SO_2 analyzer (Thermo Electron, Model 43), NO_2 concentration with a chemiluminescent NO-NO_2-NO_x analyzer (Thermo Electron, Model 14), and shortwave radiation or illumination with radiometers or a photometer (Eko, Model MS-42 and LI-COR, Model LI-185). Longwave radiation and boundary layer resistance to heat transfer were evaluated by substituting values obtained from experiments, where dry and wet model leaves were used instead of a real leaf, in the following Eq. 7 and by solving the simultaneous equations thus obtained.

$$\alpha_p E_{sx} + \epsilon E_{wx} - 2\epsilon\sigma\,(273.15 + T_{lx})^4 + \frac{2\rho c_p\,(T_a - T_{lx})}{r_{kax}} - LW_x = 0 \tag{7}$$

where E_{sx}, E_{wx}, W_x, T_{lx} and r_{kax} were maintained constant all over the leaf surface. Leaf temperature and transpiration rate were measured with this image instrumentation system and electric balances (Mettler, Models PK 16 and PL 3000). The errors in evaluating longwave radiation and boundary layer resistance to heat transfer were within 1×10^{-4} cal·cm^{-2}·s^{-1} and 0.1 s·cm^{-1}, respectively. The signals detected with these instruments were converted into digital signals and were transmitted to a computer for the image instrumentation system.

Experimental methods

An intact leaf of a test plant grown in a phytotron was attached to a thin plastic sheet and was placed horizontally on the fixing apparatus in the chamber. After the plant was sufficiently acclimatized to the new conditions, exposure to SO_2 or NO_2 was carried out for 60 minutes. The changes in leaf temperature distribution were measured at intervals of 2 minutes during the gas exposure by using the image instrumentation system, and the measured data were filed on magnetic tapes. The changes in distributions of stomatal resistance to water vapor diffusion and integrated SO_2 or NO_2 sorption were evaluated from the filed image data of the leaf temperatures, according to Eqs. 1 to 6. The visible injury images on the leaf surfaces were photographed one day later.

Results and discussion

Figure 1 shows changes in the distribution of stomatal resistance to water vapor diffusion on a leaf surface during exposure to ca. 2 volppm SO_2. The stomatal resistances distributed in the range of 0.4 to 1.4s·cm^{-1} before the start of SO_2 exposure, began to increase within 5 minutes after the start and reached 0.7 to 3.2s·cm^{-1} after 60 minutes exposure. From a series of image data, there was an observable tendency for the stomatal resistance to be great and to increase especially in the vicinity of veins and leaf edges. Stomatal resistance is generally an indicator of the degree of stomatal opening and increases at sites of stomatal closure under usual growing conditions, except in cases of water-soaking and wilting (Monteith 1973, Omasa *et al.* 1981a). Figure 2 shows changes in the distribution of integrated SO_2 sorption during the exposure and a photograph (f) taken one day later under lighting. The integrated SO_2 sorptions were 0.05 to 0.10×10^{-5} g·cm^{-2} after 8 minutes of SO_2 exposure, 0.31 to 0.50×10^{-5} g·cm^{-2} after 30 minutes exposure and 0.62 to 1.05×10^{-5} g·cm^{-2} after 60 minutes. Visible injuries progressed successively with water-soaking, wilting, fading of pigments and necrosis or chlorosis. Water-soaking began to appear slightly at sites of the smallest stomatal resistance after 60 minutes exposure. However, wilting and fading of vegetal pigments did not appear during SO_2 exposure. The injuries in Fig. 2, (f) which occurred one day later, reached the stage of necrosis and chlorosis. There was a tendency for the visible injury to occur at sites where the integrated SO_2 sorption at the end of 60 minutes exposure was over a threshold value of ca. 0.85×10^{-5} g·cm^{-2}. The differences in stomatal response at local sites were not dependent on those in the integrated SO_2 sorption.

Figure 3 shows changes in the distribution of stomatal resistance to water vapor diffusion on a leaf surface during exposure to ca. 7 volppm NO_2. The stomatal resistances were 0.4 to 1.4s·cm^{-1} before the start of NO_2 exposure, began to increase within a few minutes after the start and reached 4.8 to 17s·cm^{-1} after 60 minutes exposure. Unlike the phenomena observed during SO_2 exposure, the sites where increase of the stomatal resistance was striking could not be specified. Figure 4 shows changes in the distribution of integrated NO_2 sorption during the exposure, and a photograph (f) taken one day later under lighting. The integrated NO_2 sorptions were 0.08 to 0.14×10^{-5} g·cm^{-2} at 8 minutes exposure, 0.55 to 1.0×10^{-5} g·cm^{-2} after 30 minutes

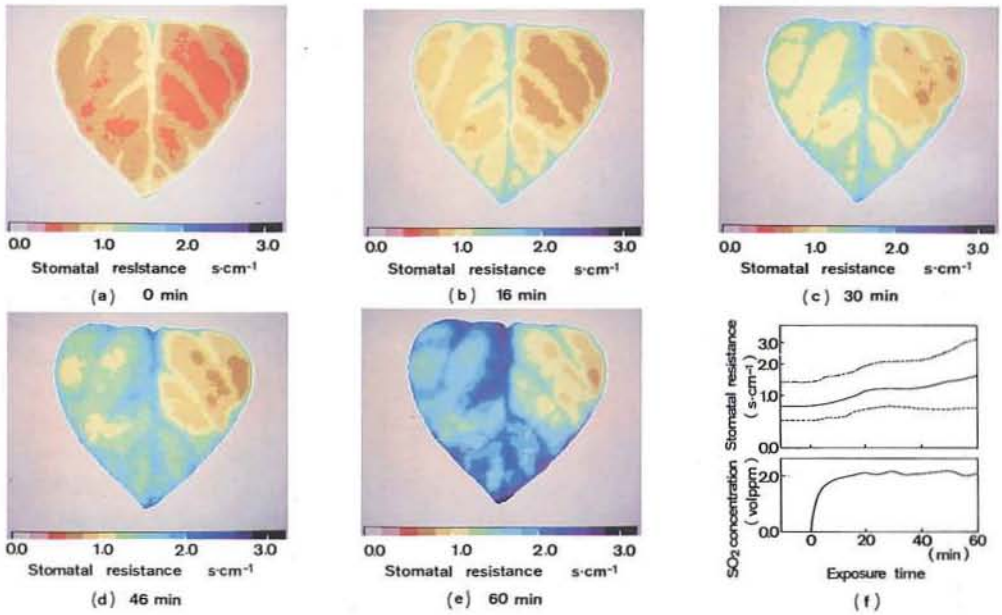

Fig. 1 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 2 volppm SO_2. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (——), minimum (-----) and mean (———) stomatal resistances and SO_2 concentration during the exposure. Environmental conditions: air temperature, 25.0°C; humidity, 62%RH; shortwave radiation, 2.37×10^{-3} cal·cm^{-2}·s^{-1}; longwave radiation, 2.23×10^{-2} cal·cm^{-2}·s^{-1}; illumination, 25klx; boudary layer resistance to heat transfer, 1.5s·cm^{-1}.

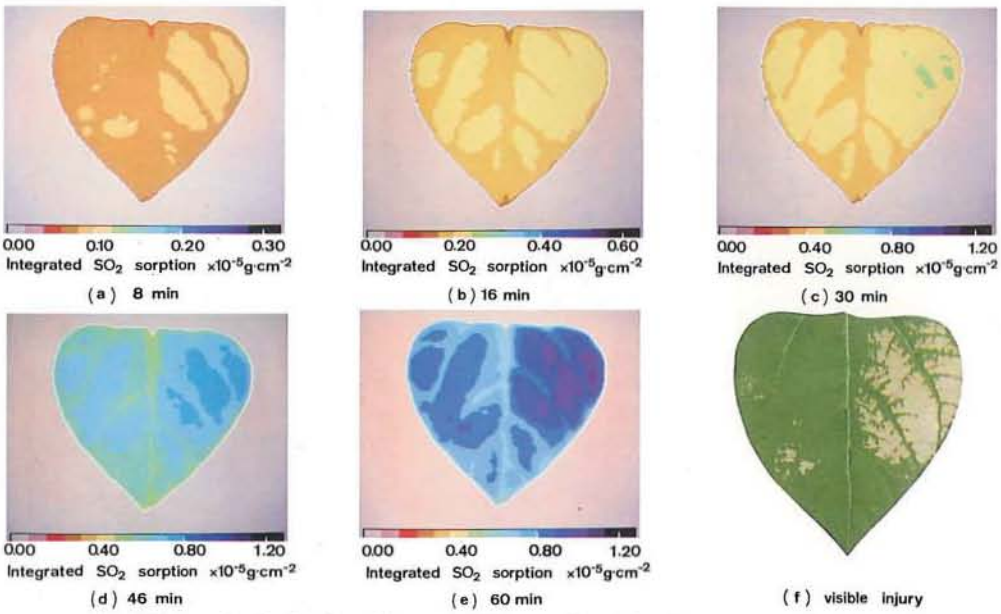

Fig. 2 Changes in distribution of integrated SO_2 sorption during the exposure in Fig. 1 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated SO_2 sorption at given periods of exposure. (f) shows the distribution pattern of the visible injury.

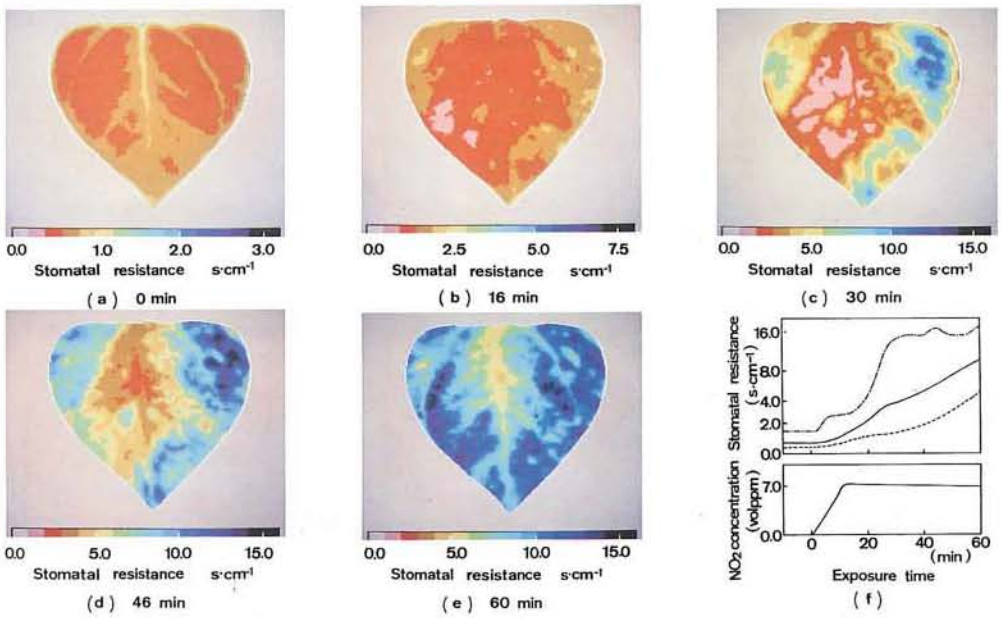

Fig. 3 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 7 volppm NO_2. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (—·—), minimum (-----) and mean (———) stomatal resistances and NO_2 concentration during the exposure. Environmental conditions were the same as those in Fig. 1.

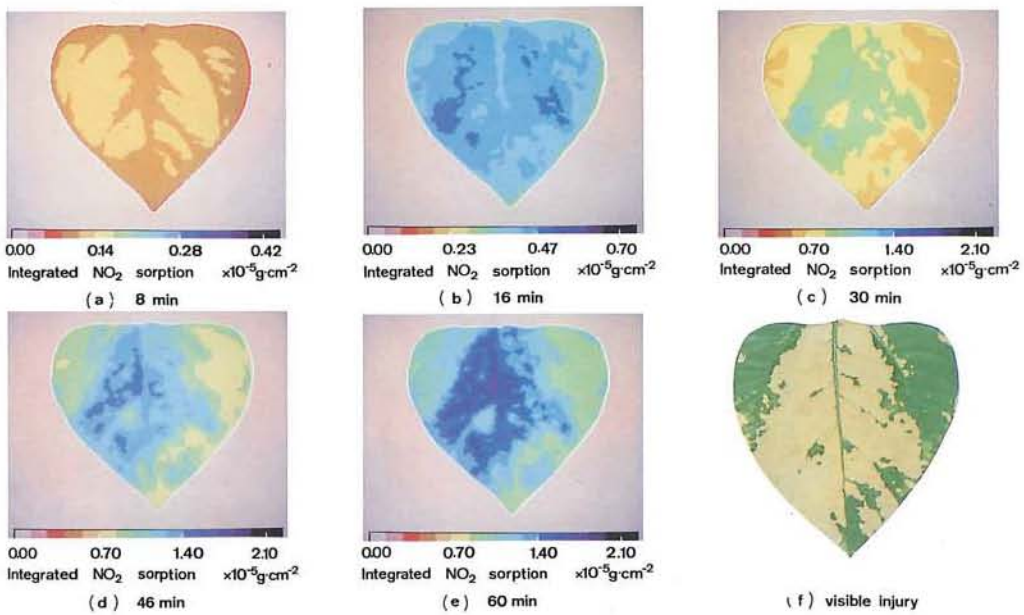

Fig. 4 Changes in distribution of integrated NO_2 sorption during the exposure in Fig. 3 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated NO_2 sorption at given periods of exposure. (f) shows distribution pattern of the visible injury.

exposure and 0.83 to 1.85×10^{-5} g·cm^{-2} after 60 minutes. Visible injuries progressed successively with water-soaking, wilting, fading of pigments and necrosis or chlorosis in a manner similar to that seen in the images with SO_2 exposure. Water-soaking began to appear at the sites with the smallest stomatal resistance after 30 minutes exposure, and saturation occurred at the sites with small stomatal resistances after 40 minutes. It was observed that successive wilting began at the same sites after 50 minutes. The injuries in Fig. 4, (f) which occurred one day later, reached the stage of necrosis and chlorosis. There was a tendency for the visible injury to occur at sites where the integrated NO_2 sorption at the end of 60 minutes exposure was over a threshold value of ca. 1.2×10^{-5} g·cm^{-2}. The differences in stomatal response at local sites were not dependent on those in the integrated NO_2 sorption.

Stomatal responses to SO_2 or NO_2 are complex; Several investigators have shown that the stomatal responses vary with plant species, culture conditions and gas concentrations(Majernik and Mansfield 1970, 1972, Unsworth 1972, Kondo and Sugahara 1978, Furukawa *et al.* 1979, Omasa *et al.* 1979). Recently, by using the image instrumentation method, we have noticed that the stomatal responses to SO_2 vary at different sites on a leaf, and that the stomata behaved randomly(Omasa *et al.* 1980, 1981a). In the present study, we recognized that the stomatal response to NO_2 at local sites is also random. We further noticed that differences in stomatal response at local sites are not dependent on those in the integrated SO_2 or NO_2 sorption. These results suggest that there are differences in the stomatal sensitivity to SO_2 and NO_2 at local sites on a leaf.

Some investigators have attempted to analyze quantitatively the relationship between the amount of SO_2 or NO_2 sorption and the degree of visible injury from the standpoint of one leaf or of a whole plant(Thomas and Hill 1935, Bressan *et al.* 1978). However, they did not pay attention to differences in gas sorption and the visible injury at local sites. It is a characteristic of SO_2 and NO_2 visible injuries, which reached the stage of necrosis and chlorosis, that an injured leaf is generally separated into two areas, a healthy area and an injured one. In the present study, we examined the relationship between integrated sorption and visible injury at local sites all over the leaf surface, and showed that the characteristic injuries were caused by the slight differences in these vicinities of the threshold value of the integrated sorption.

In the present experiments, differences in the integrated SO_2 or NO_2 sorption at local sites were caused by differences in the stomatal resistance because the boundary layer resistance was kept constant all over the leaf surface, and the SO_2 or NO_2 concentration at the gas-liquid interface in the substomatal cavity was assumed to be zero volppm(Omasa and Abo 1978, Omasa *et al.* 1979, Omasa 1980). Under the usual exposure conditions in the field and growth chamber, however, the differences in the gas sorption at local sites depend on both boundary layer and stomatal resistances. Therefore, it can be said that the characteristic injuries of SO_2 or NO_2 are caused by differences in the boundary layer and stomatal resistances at local sites.

We sincerely wish to thank Messrs. Naka Ito and Takeshi Yamada of the University of Tsukuba for their assistance in these experiments, and the members of the Engineering Division who maintained the equipment and cultivated the plants used in the experiments.

This study was published in Environment Control in Biology(Omasa *et al.* 1981b).

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植物の画像計測

(2) 植物葉の SO_2 あるいは NO_2 収着と急性影響との関係

大政謙次[1]・橋本　康[2]・相賀一郎[1]

本論文では，先に開発した画像計測手法を用いて，SO_2 あるいは NO_2 に被暴した植物葉に生じる気孔反応や可視害等の急性影響とガス収着量との関係を定量的に検討した。その結果は，以下のようであった。

1） SO_2 あるいは NO_2 暴露に伴い，気孔は閉鎖する傾向があった。しかし，その挙動は，葉の局所部位により異なり，不規則であった。これらの局所部位における気孔反応の違いは，ガス暴露期間中の積算ガス収着量の違いには依存しなかった。このことは，SO_2 あるいは NO_2 に対する気孔の感受性が葉の局所部位により異なることを示唆している。

2） SO_2 あるいは NO_2 に被暴した葉において，可視害は，積算ガス収着量がしきい値を越える領域に発現する傾向があった。そして，障害葉は，正常な領域と可視害域に分離されるという特徴があった。この特徴ある可視害の発現は，葉の局所部位における気孔抵抗や葉面境界層抵抗などの SO_2 あるいは NO_2 収着を支配する要因の違いにより生じることが示唆された。

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Image instrumentation of plants exposed to air pollutants (3) Relationships between O_3 sorption and its acute effects on plant leaves

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We examined the relationships between O_3 sorption and the acute effects of the O_3 on sunflower leaves, such as changes in stomatal response and visible injury, using an image instrumentation method. The results obtained were as follows.

1) Changes in stomatal response to O_3 varied randomly at different sites on a leaf, and were not dependent on the integrated O_3 sorption at these sites. This result suggests that there are differences in stomatal sensitivity to O_3 among local sites on a leaf.

2) The degree of visible injury at local sites on a leaf, which reached chlorosis and necrosis, was not related to the integrated O_3 sorption at these sites. This result suggests that the differences in the degree of the visible injury among local sites on a leaf are dependent on differences not only in factors related to O_3 sorption, but also in other physiological factors among these sites.

Key words: Image — Leaf temperature — O_3 sorption — Acute effects — Stomatal response — Leaf injury

Ozone is a major air pollutant of photochemical oxidant, which is produced in the atmosphere by a series of photochemical reactions involving nitrogen oxides and gaseous hydrocarbones (Demerjian et al. 1974, Hecht et al. 1974). Entry of O_3 into a leaf through the stomata causes various effects on leaves (Dugger and Ting 1970, Heath 1975, Horsman and Wellburn 1976, Manning and Feder 1976). The degree of the effects varies at different sites on a leaf. For example, chlorotic and necrotic visible injuries caused by O_3, which are conspicuous symptoms of the acute effect, are generally characterized by numerous discrete lesions scattered over all or a large portion of the leaf surface. Depending on the plant species and the severity of the injury, the lesions range from small superficial spots to a large necrotic area (Hill *etal.* 1961, 1970). Therefore, to better understand the effects of O_3 sorption, it is necessary to define relationships between local gas sorption, factors governing the sorption, and the degree of the gas sorption effects at local sites.

As described in the preceding papers (Omasa *et al.* 1981a, b), we have developed an instrumentation method for studying the distribution of the sorption of air pollutants, transpiration, and stomatal diffusion resistance of leaves using a thermal infrared image instrumentation system, and have examined quantitatively the relationships between the acute

effects, such as changes in stomatal response and visible injury, of SO_2 or NO_2 on a leaf and gas sorption. However, the relationships between O_3 sorption and its effects all over the leaf surface have not been clarified.

We therefore made the present study to elucidate the relationships between the distribution patterns of O_3 sorption and its acute effects such as changes in stomatal response and visible injury on a leaf.

Materials and methods

Plant materials

Sunflower plants (*Helianthus annuus* L. cv. Russian Mammoth) were grown in a phytotron at 25/20° C day/night temperature and 70%RH under natural light for 6 to 7 weeks (1,800 to 2,500 cm^2 leaf area/plant and 20 to 25 leaves/plant) after sowing in pots (10 cm in diameter and 20 cm in height). The pot was filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss and fine gravel which was moistened with nutrient solution. The plants were irrigated daily. Intact mature leaves (130 to 140 cm^2 in leaf area) were used in the experiments.

Environment control system (Omasa *et al.* 1981 a, b)

O_3 was introduced into the environment control chamber designed and constructed for studies of air pollution effects on plants. Air temperature and humidity in the chamber were maintained at 25.0 ± 0.1° C and 62 ± 1%RH. O_3 concentrations were kept at the desired values of ca. 1.2 volppm and 1.0 volppm. An apparatus for fixing an intact leaf horizontally and fans for maintaining a uniform air current on the leaf surfaces were set in the chamber. The intact leaf was attached to a thin plastic sheet (20×20 cm^2) cut out geometrically in a shape similar to that of the leaf (cut area; ca. 100 cm^2), and was placed on the fixing apparatus. The distributions of shortwave radiation, longwave radiation, illumination and boundary layer resistance to heat transfer on the leaf surface were maintained at $2.37 \pm 0.05 \times 10^{-3}$ cal·cm^{-2}·s^{-1}, $2.23 \pm 0.01 \times 10^{-2}$ cal·cm^{-2}·s^{-1}, ca. 25klx, and 1.5 ± 0.1 s·cm^{-1}, except at the leaf edges.

Instrumentation system (Omasa *et al.* 1981 a, b)

Distributions of integrated O_3 sorption and stomatal resistance to water vapor diffusion were evaluated from the leaf temperature distribution, measured by using a thermal infrared image instrumentation system. The system was calibrated by a blackbody source (Electro Optical Industries, Models PD1410X and D254) with chromel-constantan thermocouples which were traceable to the National Bureau of Standards in the U.S.A. The error and resolution in measuring leaf temperature using this system were ±0.1° C and $256^H \times 240^V$ pixels, and errors in evaluating sorption and stomatal resistance to water vapor diffusion were within ca. 10% and 0.3s·cm^{-1}, respectively, until the beginning of fading of vegetal pigments. Radiation and boundary layer resistance to heat transfer were measured within precisions of 1×10^{-4} cal·cm^{-2}·s^{-1} and 0.1s·cm^{-1}, respectively, using this system and electric balances (Mettler, Models PK 16 and PL 3000). Air temperature was measured with a calibrated copper-constantan thermocouple of 0.1 mm diameter, humidity with a dew-point instrument (EG and G, Model 660), O_3 concentration with a chemiluminescent O_3 analyzer (Kimoto, Model 806), and shortwave radiation or illumination with radiometers or a photometer (Eko, Model MS-42 and LI-COR, Model LI-185). The signals detected with these instruments were converted into digital signals and were transmitted to a computer for the image instrumentation system.

Experimental methods

An intact leaf of a test plant grown in a phytotron was attached to a thin plastic sheet and

was kept horizontally on the fixing apparatus in the chamber. After the plant was sufficiently acclimatized to the given conditions, O_3 exposure to plant was carried out for 60 minutes. The changes in leaf temperature distribution were measured at intervals of 2 minutes during the gas exposure by using the image instrumentation system, and the measured data were filed on magnetic tapes. The changes in distributions of stomatal resistance to water vapor diffusion and integrated O_3 sorption were evaluated from the filed image data of the leaf temperature, according to the method reported previously (Omasa *et al.* 1981a, b). Chlorotic and necrotic visible injury on the leaf surface was photographed after keeping the plant under lighting in the chamber for one day after the exposure treatment.

Results and discussion

Relationships between distribution patterns of O_3 sorption and its acute effects, such as changes in stomatal response and chlorotic and necrotic visible injury on a leaf, in two cases with typical symptoms of O_3 injury are shown in Figs. 1 to 4. Figure 1 shows changes in the distribution of stomatal resistance to water vapor diffusion on a leaf surface during exposure to ca. 1 volppm O_3. The stomatal resistances, distributed in a range of 0.3 to 1.2s·cm^{-1} before the start of O_3 exposure, began to increase after a few minutes of exposure and showed a peak after ca. 30 minutes. The stomatal resistances then decreased, but increased again thereafter and reached 1.0 to 4.5s·cm^{-1} after 60 minutes of exposure. There was a tendency for the phenomenon to be striking at sites with faster initial increases in the stomatal resistance, such as at sites in the vicinity of veins. Figure 2 shows changes in the distribution of the integrated O_3 sorption during the exposure, and a photograph (f) taken one day after the gas exposure. The integrated O_3 sorptions were 0.02 to 0.04 $\times$ 10^{-5}g·cm^{-2} after 8 minutes of O_3 exposure, 0.12 to 0.20 $\times$ 10^{-5}g·cm^{-2} after 30 minutes exposure and 0.25 to 0.39 $\times$ 10^{-5}g·cm^{-2} after 60 minutes. Visible injuries such as water-soaking, leaf wilting and fading of vegetal pigments did not appear during O_3 exposure. The chlorotic and necrotic injuries in Fig. 2 (f) were observed one day later. Irrespective of differences in the O_3 sorption among local sites, the degree of injuries was fairly uniform all over the upper leaf surface. From the data in Figs. 1 and 2, it was also shown that changes in the stomatal response at local sites were not dependent on the integrated O_3 sorption at these sites.

Figure 3 shows another case of changes in the distribution of stomatal resistance to water vapor diffusion on a leaf surface during exposure to ca. 1.2 volppm O_3. The stomatal resistances were 0.3 to 1.2s·cm^{-1} before the start of O_3 exposure and began to increase after a few minutes of exposure, and reached 1.0 to 3.8s·cm^{-1} after 60 minutes of exposure. Then the stomatal resistances at the sites fluctuated with time. Like the phenomenon observed in Fig. 1, there was a tendency for the phenomenon to be striking at sites with faster initial increases in the stomatal resistance. However, we could not specify sites where the phenomenon was striking. Figure 4 shows changes in the distribution of integrated O_3 sorption during the exposure, and a photograph (f) taken one day after gas exposure.The integrated O_3 sorptions were 0.03 to 0.05 $\times$ 10^{-5}g·cm^{-2} after 8 minutes of O_3 exposure, 0.14 to 0.25 $\times$ 10^{-5}g·cm^{-2} after 30 minutes exposure and 0.30 to 0.45 $\times$ 10^{-5}g·cm^{-2} after 60 minutes. Water-soaking began to appear after 50 minutes of exposure, and became remarkable at the sites with a large integrated O_3 sorption after 60 minutes. However, leaf wilting and fading of vegetal pigments did not appear during O_3 exposure. The chlorotic and necrotic injuries in Fig. 4 (f) were observed one day later. Irrespective of the small amount of integrated O_3 sorption in the vicinity of veins, the chlorotic and necrotic injury at these sites was much more remarkable than that at other sites. From the

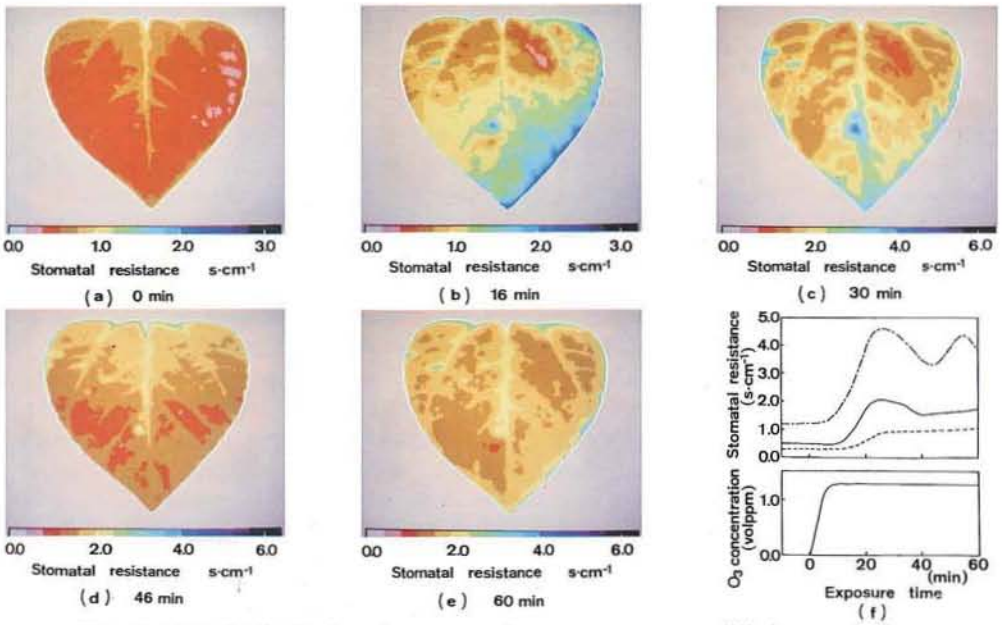

Fig. 1 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 1 volppm O_3. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (— - —), minimum (- - - - -) and mean (——) stomatal resistance and O_3 concentration during the exposure. Environmental conditions: air temperature, 25.0° C; humidity, 62%RH; short-wave radiation, 2.37×10^{-3} cal·cm^{-2}·s^{-1}; longwave radiation, 2.23×10^{-2} cal·cm^{-2}·s^{-1}; illumination, 25klx; boundary layer resistance to heat transfer, 1.5 s·cm^{-1}.

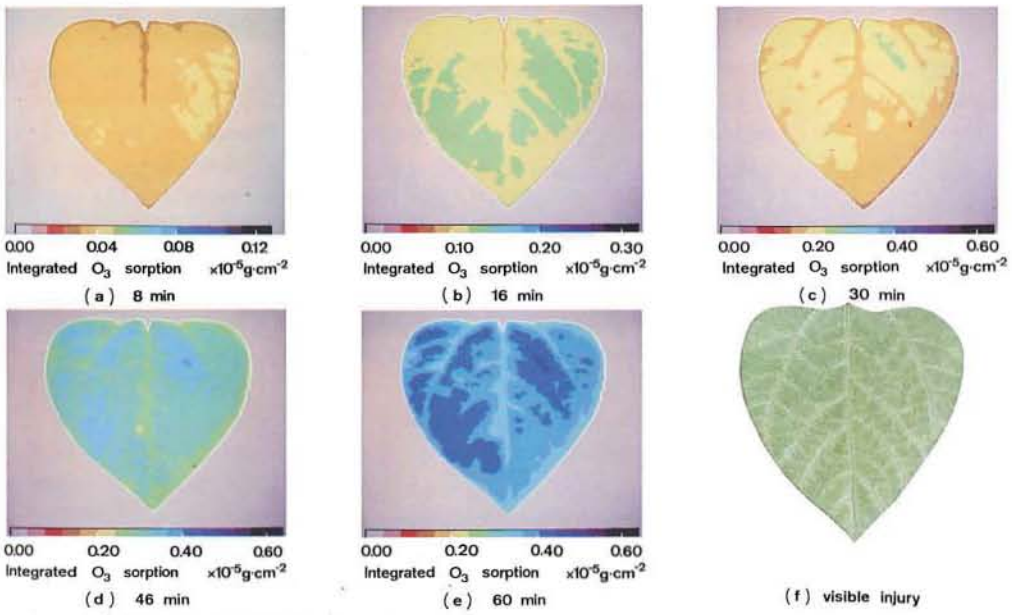

Fig. 2 Changes in distribution of integrated O_3 sorption during the exposure in Fig. 1 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated O_3 sorption at given periods of exposure. (f) shows the distribution pattern of the visible injury.

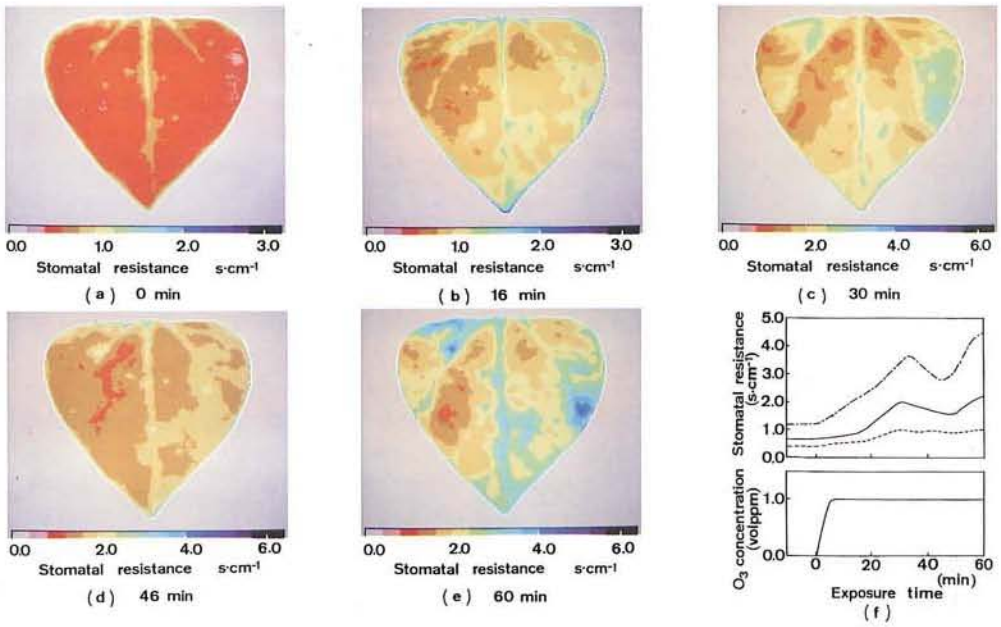

Fig. 3 Another case of changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 1.2 volppm O_3. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (— - —), minimum (- - - - -) and mean (——) stomatal resistances and O_3 concentration during the exposure. Environmental conditions were the same as those in Fig. 1.

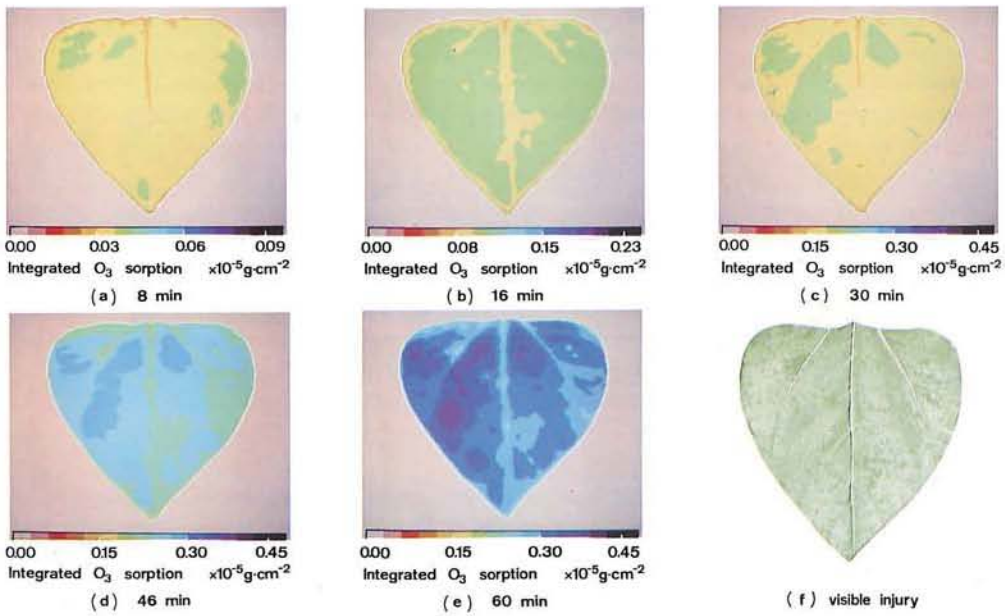

Fig. 4 Changes in distribution of integrated O_3 sorption during the exposure in Fig. 3 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated O_3 sorption at given periods of exposure. (f) shows distribution pattern of the visible injury.

deta in Figs. 3 and 4, it was also shown that changes in the stomatal response at local sites were not dependent on the integrated O_3 sorption at these sites.

Stomatal resistance is generally accepted as an indicator of the degree of stomatal opening under usual growing conditions, except in cases of water-soaking and wilting (Meidner and Mansfield 1968, Monteith 1973). Several investigators have evaluated a stomatal response to O_3 by using the stomatal resistance determined with a resistance porometer (Dugger and Ting 1970, Heath 1975, 1980), while Evans and Ting pointed out that the stomatal resistance was influenced by destruction of the palisade parenchyma and upper epidermis resulting from O_3 exposure (Evans and Ting 1974). Another available method for evaluating stomatal response to O_3 is a stomatal impression method (Lee 1965, Meidner and Mansfield 1968). However, these methods are not effective for evaluating the stomatal response and the relationship between the response and O_3 sorption all over a leaf surface. In the present study, by using our image instrumentation method, we showed that changes in stomatal response to O_3 varied randomly at different sites on a leaf, and were not dependent on the integrated O_3 sorption at these sites. This result suggests that there are differences in the stomatal sensitivity to O_3 among local sites on a leaf similar to those observed in experiments with SO_2 or NO_2 exposure (Omasa *et al.* 1981b).

Several investigators have reported that resistance of plant leaves to O_3 was dependent on stomatal conditions such as the number and opening of the stomata (Lee 1965, MacDowall 1965, Engle and Gabelman 1966). They have reported a positive correlation between the degree of O_3 damage and the stomatal opening, but others have failed to confirm these conclusions (Dugger *et al.* 1962, Menser *et al.* 1963, Ting and Dugger 1968). However, since O_3 sorption depends on not only stomatal conditions, but also leaf boundary conditions at local sites (Monteith 1973, Omasa *et al.* 1979), the resistance or susceptibility of plant leaves to O_3 must be evaluated by elucidating relationships between stomatal condition, O_3 sorption and leaf damage at local sites all over a leaf surface. In the present study, we took two cases with typical symptoms of O_3 injury and examined the relationship between the integrated O_3 sorption and the chlorotic and necrotic visible injury at sites all over a leaf surface, and showed that the degree of the visible injury was not correlated with the integrated sorption, irrespective of the patterns in the symptoms. These results differ from those obtained in the preceding study on SO_2 or NO_2 exposure, where there was a tendency for visible injury to occur at sites where the integrated SO_2 or NO_2 sorption was over a threshold value (Omasa *et al.* 1981b). They also suggest that the differences in the degree of the visible injury among local sites on a leaf are dependent on defferences not only in factors related to O_3 sorption, but also in other physiological factors among these sites.

We sincerely wish to thank Messrs. Naka Ito and Takeshi Yamada of the University of Tsukuba for their assistance in these experiments, and the members of the Engineering Division who maintained the equipment and cultivated the plants used in the experiments.

This study was published in Environment Control in Biology (Omasa *et al.* 1981c).

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植物の画像計測

(3) 植物葉の O_3 収着と急性影響との関係

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本論文では，先に開発した画像計測手法を用いて，O_3 に被暴したヒマワリ葉に生じる気孔反応や可視害等の急性影響とガス収着量との関係について検討した。その結果は，以下のとおりであった。

1） O_3 に対する気孔反応は，葉の局所部位によらず不規則であり，また，これらの局所部位における積算 O_3 収着量には関係しなかった。このことは，O_3 に対する気孔の感受性が葉の局所部位により異なることを示唆している。

2） ネクロシスやクロロシスの段階に進行した葉の局所部位における可視害の程度は，これらの部位での積算 O_3 収着量には関係しなかった。このことは，葉の局所部位での可視害の程度の違いが，O_3 収着に関係する要因だけでなく，他の生理的要因の違いにも依存することを示唆している。

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Image instrumentation of plants exposed to air pollutants (4) Methods for automatic evaluation of the degree of necrotic and chlorotic visible injury

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Methods for automatic evaluation of degree of necrotic and chlorotic visible injury caused by air pollution were examined using a multi-spectral image instrumentation system.

The spectral characteristics of the injured parts of SO_2-and NO_2-injured leaves at bands of 0.45, 0.55 and 0.67 μm differed from those of healthy parts. The gray levels of the injured parts at those bands were clearly separated from those of the healthy parts. Therefore, we separated a spectral image of an injured leaf into injured parts and healthy parts using a threshold gray level value, and evaluated the degree of SO_2 or NO_2 visible injury of a leaf from the determining ratio of the injured area to the leaf area. The most effective wavelength for the separation was 0.67 μm.

The spectral characteristics of the injured parts of O_3-injured leaves at bands of 0.45, 0.55 and 0.67 μm also differed from those of the healthy parts. However, the above method was not useful because the gray levels of injured parts at these bands were distributed over a wide range. Therefore, we used a mean value of gray levels of the spectral image of an injured leaf as an indicator for the evaluation. The mean value of gray levels related to content of total chlorophylls, which were major components of plant pigments faded by air pollution. The correlation was highest in the case of band ratio of 0.55 μm/0.90 μm.

In polluted areas, air pollutants rarely exist alone; instead, the air environment consists of a complex mixture of phytotoxic gases. We can evaluate the degree of visible injury by such mixed pollutants using both methods mentioned above.

Key words: Image — Air pollution — Necrotic and chlorotic visible leaf injury — Automatic evaluation

Necrotic and chlorotic visible leaf injuries are used as an important index for evaluating the effects of air pollutants on plants in polluted areas and laboratories because they are the most conspicuous symptoms of the effects (Jacobson and Hill 1970, Japan Society of Air Pollution 1973, Mudd and Kozlowski 1975). Discrimination of the symptoms for the evaluation of the visible injuries and quantification of the degree have been performed by visual observation (Chester 1959, Jacobson and Hill 1970, Japan Society of Air Pollution 1973). However, these methods are not objective because the results depend upon the persons performing the discrimination and quantification. Therefore, methods of objective evaluation which can automatically process many samples must be developed (Mortensen and Weisberg 1980, Omasa *et al.* 1980, Omasa and Aiga 1984). We proposed a method using an image instrumentation

system in the previous report (Omasa *et al.* 1980). In the present paper,. we examined more minutely methods adapted to automatic evaluation of the degree of visible injuries.

Materials and methods

Plant materials

Sunflower plants (*Helianthus annuus* L. cv. Russian Mammoth) were grown in an environment-controlled greenhouse (Aiga *et al.* 1982) at 25/20 °C day/night temperature and 70% RH under natural light for 4 to 6 weeks (1,500 to 2,500 cm^2 leaf area/plant and 15 to 25 leaves/plant) after sowing in pots. The pots were filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss and fine gravel which were moistened with nutrient solution. The plants were irrigated daily.

Gas exposure

Test plants were exposed to 2 volppm SO_2, 8 volppm NO_2 or 1 volppm O_3 for 1 hour in an environment-controlled chamber (Aiga *et al.* 1982). Air temperature, humidity and illumination in the chamber were maintained at 25°C, 60% RH and 30 klx. After the exposure, the plants were returned to the greenhouse and grown for ca. 1 to 3 days. Thereafter, the fading of vegetal pigments at the injured parts did not progress.

Image instrumentation and processing

After healthy and injured leaves were cut away, spectral reflection images of these leaves or healthy and injured parts (20×20 mm^2) of these leaves were immediately measured through various interference filters (central wavelength μm (half-band width μm); 0.45 (0.03), 0.55 (0.01), 0.67 (0.01), 0.78 (0.01), 0.90 (0.01)) under constant lighting with a silicon vidicon camera. The detected signals from the camera were converted into 8-bit (256 gray levels) by a video A/D converter, transmitted to a computer through a video I/O interface and analyzed by the computer (Omasa *et al.* 1983). The resolution of the obtained leaf image was ca. 0.8 mm.

Determination of chlorophyll

After spectral reflection images of healthy and injured parts of leaves were measured, these parts were cut out, and then homogenized in 80% acetone, respectively. After centrifuging, the absorption spectra of the supernatant solution were measured with spectrophotometer. Chlorophyll content was determined using absorption coefficient of Mackinney (Mackinney 1941).

Results and discussion

Figure 1 shows photographs of typical acute SO_2-, NO_2- and O_3-injured leaves of sunflower plants, these reach to the necrosis and chlorosis stage. It was observed that large areas of clearly visible injury occurred on the SO_2- or NO_2-injured leaf, whereas numerous discrete, not clearly visible injury occurred on the O_3-injured leaf.

Figure 2 shows spectral characteristics with the average gray levels of healthy, SO_2-injured and NO_2-injured parts. The spectral characteristics of the injured parts at bands of 0.45, 0.55 and 0.67 μm differed from those of the healthy parts. The gray levels of the injured parts at these bands were clearly separated from those of the healthy parts. Therefore, we can separate a spectral image of a injured leaf into injured parts and healthy parts using a threshold gray level value, and can evaluate the degree of SO_2 or NO_2 visible injury of a leaf from the determining ratio of the injured area to the leaf area. Table 1 shows the mean value and standard error of gray

levels of all SO_2- and NO_2-injured and healthy parts at each band of 0.45, 0.55 or 0.67 μm. The most effective wavelength for the separation is 0.67 μm because the difference between the mean values of the healthy parts and the injured parts is the greatest and the standard error of the healthy parts is smallest. Figure 3 shows an example of evaluation of degree of visible injury in

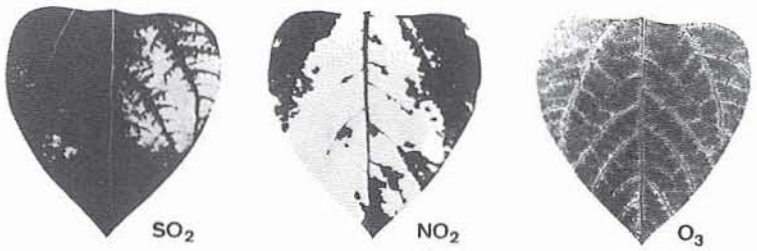

Fig. 1 Photographs of typical acute SO_2-, NO_2- and O_3-injured leaves of sunflower plants.

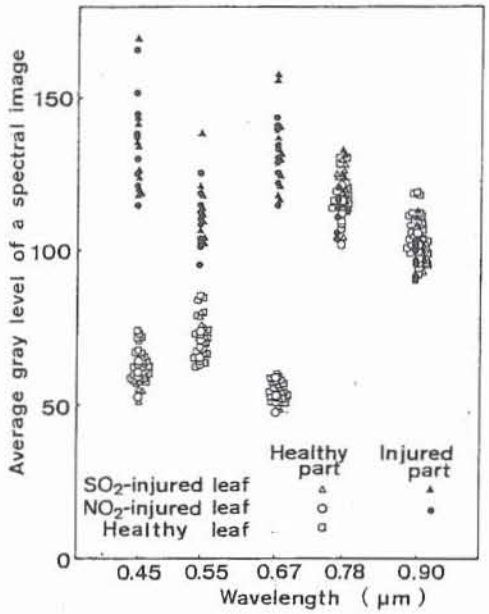

Fig. 2 Spectral characteristics with the average gray levels of healthy, SO_2-injured and NO_2-injured parts of sunflower leaves

Table 1 Mean value and standard error of gray levels of all SO_2- and NO_2- injured and healthy parts at each band of 0.45, 0.55 or 0.67 μm in Fig. 2.

Wavelength	Healthy part		Injured part	
μm	Mean value	Standard error	Mean value	Standard error
0.45	61.3	6.2	134.4	18.9
0.55	71.9	7.1	111.9	11.7
0.67	53.5	3.7	132.3	15.5

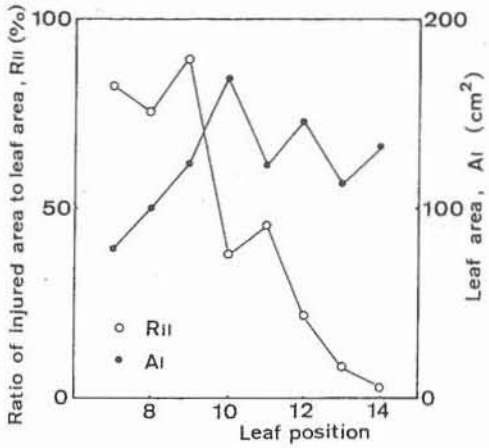

Fig. 3 An evaluation of degree of visible injury in different leaf positions of a sunflower plant exposed to SO_2.

different leaf positions of a sunflower plant exposed to SO_2. A threshold value for the separation, G_{sh} was given by $G_{av}+4G_{se}\simeq 70$, where G_{av} is the mean value of the gray levels of the injured parts at the 0.67 μm band (= 53.5) and G_{se} is the standard error (= 3.7). The degree of visible injury was greater at lower leaf positions. This result suggests that the leaf resistance to SO_2 is smaller in low positions than in high positions.

Figure 4 shows spectral characteristics with the average gray levels of healthy and O_3-injured parts. The spectral characteristics of the injured parts at bands of 0.45, 0.55 and 0.67 μm differed from those of the healthy parts. However, the above method is not useful because the gray levels of injured parts at these bands were distributed over a wide range. Therefore, we have exmained an evaluation method using mean value of gray levels of the spectral image of an injured leaf as an indicator. Table 2 shows the correlation coefficient and standard error which indicate the relationship between total chlorophyll contents of healthy and O_3-, SO_2- and NO_2-injured parts, C_{hl} ($\mu g\cdot cm^{-2}$) and the average gray levels of the spectral images, G_{av} or the band ratios of the images, B_r. The correlation was highest in the case of band ratio of 0.55/0.90, where the correlation coefficient was -0.95 and the regression line was given by $C_{hl}=-69.6B_r+82.6$ ($\mu g\cdot cm^{-2}$). The standard error in evaluating total chlorophyll content according to this equation was 4.2 $\mu g\cdot cm^{-2}$. The visible injuries were caused by fading of plant pigments. Chlorophylls are major components of the faded pigments. Therefore, we can evaluate the degree of O_3 visible injury of a leaf by determining the ratio of the total chlorophyll content of the exposed leaf to the average content of the healthy leaves. Figure 5 shows an example of evaluation of degree of visible injury in different leaf positions of a sunflower plant exposed to O_3. The average content of the healthy leaves was 36.1 $\mu g\cdot cm^{-2}$ and the standard error was 4.3 $\mu g\cdot cm^{-2}$. The degree of visible injury was greater at lower leaf positions similar to SO_2 exposure.

In polluted areas, air pollutants rarely exist alone; instead, the air environment consists of a complex mixture of phytotoxic gases. We can evaluate the degree of visible injury by such mixed pollutants using both methods mentioned above. It is a future problem to develop evaluation methods including automatic discrimination of symptoms of visible injuries caused by air pollution, sickness, insect and so on.

We sincerely wish to thank Mr. Taketoshi Ino of the Ehime University for his assistance in these experiments, and the members of the Engineering Division who maintained the equipment and cultivated the plants used in the experiments.

This study was published in Technological and Methodological Advances in Measurement (Omasa *et al.* 1983).

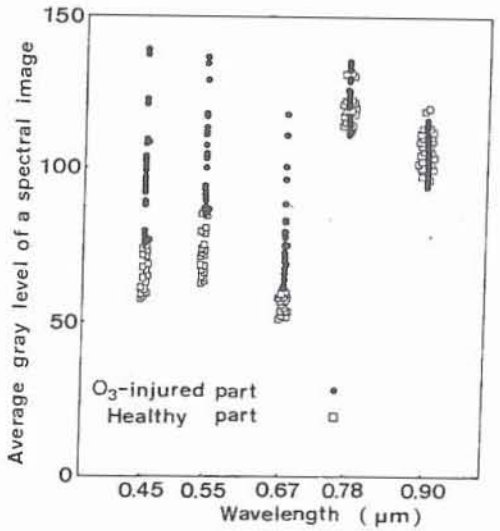

Fig. 4 Spectral characteristics with the average gray levels of healthy and O_3-injured parts of sunflower leaves.

Table 2 Correlation coefficient and standard error which indicate the relationship between total chlorophyll contents of healthy and O_3-, SO_2- and NO_2-injured parts and the average gray levels of the spectral images or the band ratios of the images.

Wavelength of image or band ratio	Correlation coefficient	Standard error
0.45 μm	−0.91	5.6 $\mu g \cdot cm^{-2}$
0.55	−0.89	6.1
0.67	−0.87	6.7
0.78	−0.09	—
0.90	0.03	—
0.45/0.90	−0.93	5.1
0.55/0.90	−0.95	4.2
0.67/0.90	−0.88	6.4
0.78/0.90	−0.20	—
0.45/0.78	−0.91	5.7
0.55/0.78	−0.91	5.8
0.67/0.78	−0.86	6.9
0.45/0.67	0.16	—
0.55/0.67	0.60	10.9
0.45/0.55	−0.78	8.5

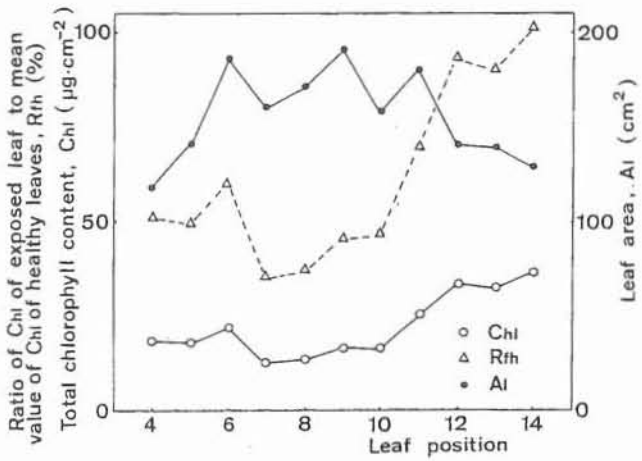

Fig. 5 An evaluation of degree of visible injury in different leaf positions of a sunflower plant exposed to O_3.

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植物の画像計測
(4) 壊疽状可視害の程度の自動評価手法

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本論文では，マルチスペクトル画像計測システムを用いて，大気汚染により生じた植物葉の壊疽状可視害の程度の自動評価手法について検討した。その結果は，以下の通りであった。

正常葉及びSO_2あるいはNO_2被害葉の正常領域と被害領域のスペクトル特性を比較したところ，0.45，0.55，0.67 μm の帯域において，正常領域と被害領域のスペクトル特性は異なっていた。このことから，SO_2及びNO_2被害葉の可視害の程度は，スペクトル画像を閾値濃度により正常領域と被害領域に二値化し，葉面積に対する被害領域の面積比を求めることにより評価できることがわかった。使用されるスペクトル帯域は，正常領域と被害領域の濃度平均値の差が大きく，正常領域の標準誤差が小さい0.67 μm が最も有効である。

他方，O_3被害葉も，0.45，0.55，0.67 μm の帯域において，正常領域と被害領域のスペクトル特性は異なっていたが，SO_2やNO_2被害葉のように被害領域と正常領域に二値的に分かれるのではなく，斑点状の可視害として現れるために被害領域の面積比を指標とした評価よりは，スペクトル画像の濃度平均値を用いた方が有効であることが分かった。スペクトル帯域は，クロロフィル含有量と最も相関の高い0.55/0.90（μm）のバント比の場合が有効である。

なお，複合汚染による可視害の評価には，上記の二つの方法を併用することが有効である。

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Res. Rep. Natl. Inst. Environ. Stud., Jpn., No.66, 1984.

Image instrumentation of plants exposed to air pollutants (5) Evaluation of early visible leaf injury by polarized spectral reflection image

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We developed a method for evaluating the visible leaf injury using an image instrumentation system for measuring and analyzing the polarized spectral reflection image. Since this method corrected the error caused by the leaf uneveness and the difference of distance from the light source at local sites on the leaf, we could exactly evaluate not only the necrotic and chlorotic visible injury but also the early visible injury such as water-soaking and wilting.

Key words: Image — Air pollution — Water-soaking — Wilting — Evaluation method —Polarized spectral reflection.

Acute leaf injuries caused by air pollutants progress successively with water-soaking, wilting and fading of pigments, and reach to the necrosis and chlorosis stage (Jacobson and Hill 1970, Mudd and Kozlowski 1975). Methods for objective evaluation of the injuries in the necrosis and chlorosis stage have been reported (Omasa *et al.* 1980, 1983, 1984). However, methods for evaluating the early visible injuries such as water-soaking and wilting have not been developed.

Light reflected from the object's surface is polarised. The characteristics of the polarised light vary with properties of the object's surface. Therefore, the polarized light from the leaf surface involves many informations concerned with properties of leaf surface. Eguchi et al. (1982) recently reported a method for evaluating acute leaf injury using reflectance of the polarized reflection image. However, this method had large error in the evaluation because the reflectance at local sites on the leaf, which was influenced by leaf unevenness and a distance from the light source, was not corrected. In addition, the relationships between the leaf surface conditions and their reflectances were not shown.

We, therefore, made the present study to develop an evaluation method, which was not influenced by leaf uneveness and a distance from the light source, using an image instrumentation system for measuring and analyzing the polarized spectral reflection image. We also examined the relationships between the polarized spectral reflection image and the leaf surface conditions.

Materials and methods

Plant materials

Sunflower plant (*Helianthus annuus* L. cv. Russian Mammoth) was grown in an environment-controlled greenhouse (Aiga *et al.*, 1982) at 25/20°C day/night temperature and 70% RH under natural light for 5 weeks after sown in a pot. The pot was filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss and fine gravel, which was moistened with nutrient solution. The plant was irrigated daily. A mature leaf (ca. 150 cm^2 leaf area) of the intact plant was used in the experiment.

Instrumentation and environment control systems

Figure 1 shows a diagram of our image instrumentation system for measuring and analyzing the polarized spectral reflection image. An intact leaf was attached to a thin plastic sheet (20×20 cm^2) cut out geometrically in a shape similar to that of the leaf (cut area; ca. 100 cm^2), and was horizontally placed on a fixing apparatus set in an environment-controlled chamber (Aiga *et al.*, 1982). Air temperature and humidity in the chamber was maintained at 25°C and 65% RH. SO_2 concentration was kept at the desired value of ca. 2 volppm for 1 hour. The metal halide lamps and a tungsten lamp through the heat absorbing glass filters were used as a light source, and the illumination was ca. 40 klx. The tungsten lamp was set in an angle of 30 degrees to the leaf surface, and the polarized reflection image from the leaf surface was measured with a TV camera (chalnicon) set in an angle of −30 degrees through a polarized light filter and an interference filter (central wavelength 0.67 μm and half-band width 0.01 μm). The polarized light filter was adjusted to a slit angle where the polarized reflection from the water-soaked leaf surface was the largest. The measured image was converted into 8-bit digital signals by a video A/D converter, transmitted to a computer through a video I/O interface and analyzed by the computer (Omasa *et al.*, 1983).

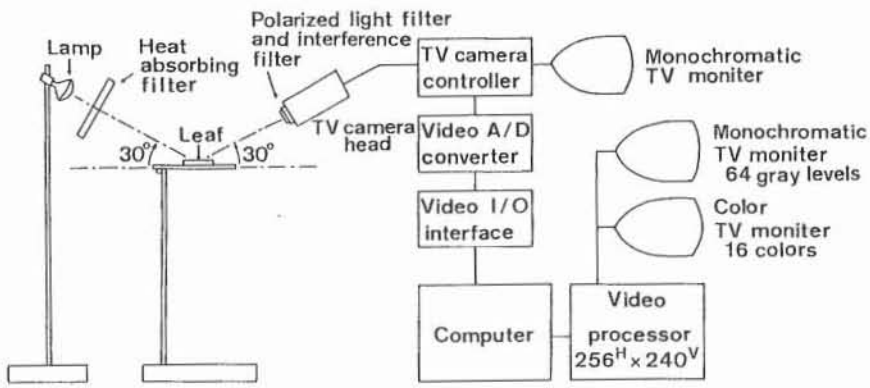

Fig. 1 Diagram of image instrumentation system for measuring and analyzing the polarized spectral reflection image.

Image processing

Let S be a polarized reflection image, and S_{ij} be the gray level at pixel (i, j) in S. In order to correct the influences of leaf uneveness and the distance from the light source, the rate of increase of the reflection, $P_{ij}(t)$ caused by the air pollutant exposure is given by

$$P_{ij}(t) = \{S_{ij}(t) - S^N_{ij}\} / S^N_{ij} \tag{1}$$

where S^N_{ij} is gray level at pixel (i, j) of a healthy leaf before the exposure and t is time after starting the exposure.

When the injured area isdefined by $P_{ij}(t) \geqq P_{sh}$, ratio of the injured area to the leaf area, $K(t)$ is expressed by

$$K(t) = \Sigma n_{ij}(t) / A, \quad n_{ij}(t) = \begin{cases} 1: P_{ij}(t) \geqq P_{sh} \\ 0: P_{ij}(t) < P_{sh} \end{cases} \tag{2}$$

where P_{sh} is the threshold value and A is number of pixels in the leaf. Furthermore, the rate of increase of the average reflection at the injured area, $X(t)$ is given by

$$X(t) = \{\Sigma n_{ij}(t) \mathrm{P}_{ij}(\mathrm{t})\} / \Sigma n_{ij}(t) \tag{3}$$

Results and discussion

Figure 2 shows frequency distributions in $\bar{P}_{ij}(0)$ of an original image of a healthy leaf at $t =$ 0 minutes and $P_{ij}(0)$ of an image corrected by Eq. (1), where; S^N_{ij} in Eq. (1) was given by $S_{ij}(t)$ at t $= -10$ minutes, i.e., 10 minutes before the exposure; $\bar{P}_{ij}(0)$ was defined by the following equation in order to compare with $\bar{P}_{ij}(0)$.

$$\bar{P}_{ij}(0) = \{S_{ij}(0) - \bar{S}(0)\} / \bar{S}(0) \tag{4}$$

where $\bar{S}(0)$ is the average gray level of $S_{ij}(0)$. The distribution in $P_{ij}(0)$ was improved to 1/5 of that in $\bar{P}_{ij}(0)$ because the error caused by the leaf uneveness and the difference of distance from the light source at local sites on the leaf was corrected. A threshold value for dividing the injured area from the healthy area, P_{sh} was given by $P_{sh} = 20\%$, where $K(0)$ determined from Fig. 2 was within 1%.

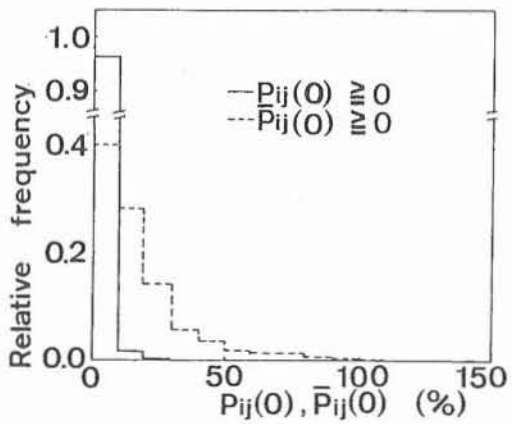

Fig. 2 Frequency distributions in $\bar{P}_{ij}(0)$ of an original image of a healthy leaf and $P_{ij}(0)$ of an image corrected by Eq.(1).

The changes in $K(t)$ and $X(t)$ of a leaf after the exposure to ca. 2 volppm SO_2 are seen in Fig. 3; $K(t)$ began to increase according to the appearance of the water-soaking at 50 minutes after starting the exposure and reached to a constant value after overshooting at 70 minutes; $X(t)$ kept on increasing after the injury began to appear. Figure 4 shows typical microphotograph in each stage of the visible leaf injury. The leaf injury progressed successively with water-soaking (B), wilting (C) and fading of pigments, and reached to necrosis and chlorosis stage (D). However, there was the mixture of these stages, especially water-soaking and wilting, in the leaf. Figure 5

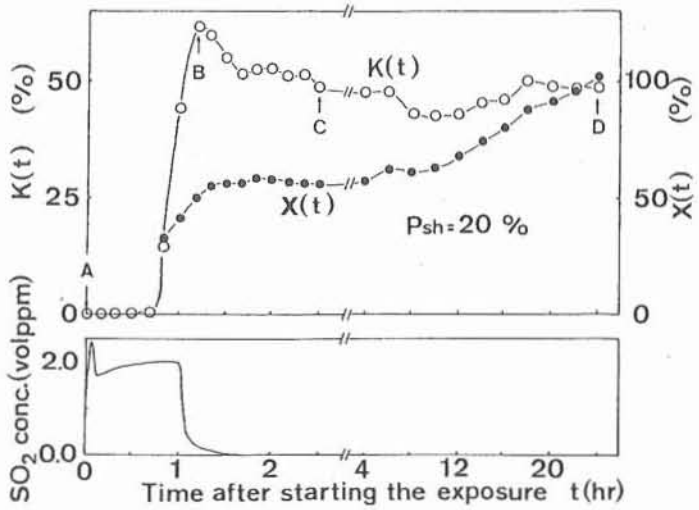

Fig. 3 Changes in $K(t)$ and $X(t)$ of a leaf after the exposure to ca. 2 volppm SO_2. Typical stage of visible leaf injury: A, healthy; B, water-soaking; C, wilting; D, necrosis and chlorosis.

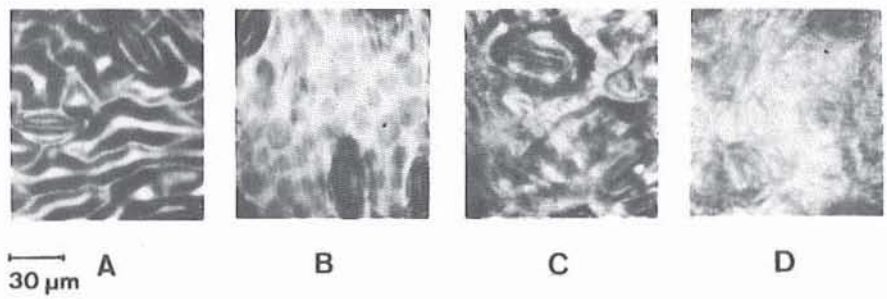

Fig. 4 Microphotograph in each stage (A, B, C, or D) shown in Fig. 3.

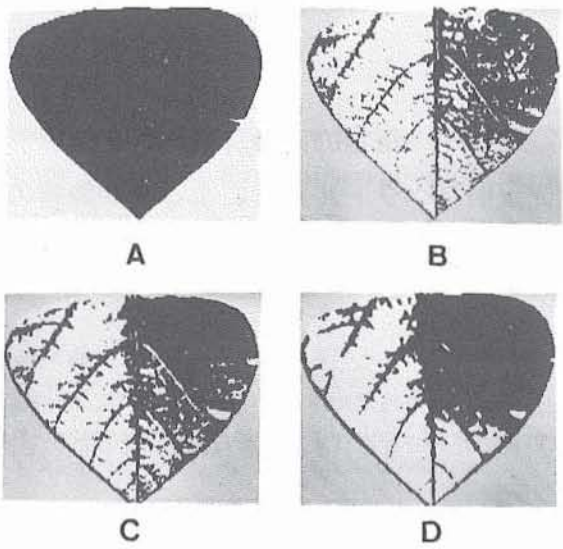

Fig. 5 Two-valued image of the leaf divided into healthy and injured areas by $P_{sh}=20\%$ in each stage (A, B, C, or D) shown in Fig. 3.

shows changes in the two-valued image of the leaf divided into healthy and injured areas by $P_{sh}=20\%$. Although the slight water-soaking and wilting area recovered its health, the serious area progressed with fading of pigments, and reached to the necrosis and chlorosis stage.

The above-mentioned results denotes that this method is effective for evaluating not only the necrotic and chlorotic visible injury but also the early visible injury such as water-soaking and wilting.

We sincerely wish to thank Mr. Taketoshi Ino of the Ehime Univesity for his assistance in these experiments, and the members of the Engineering Division who maintained the equipment and cultivated the plants used in the experiments.

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植物の画像計測

(5) 偏光反射スペクトル画像による初期障害の評価

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偏光反射スペクトル画像計測システムを用いて可視害を評価する手法を開発した。この手法は，葉面の各部位の反射角や光源からの距離の違いにより異なる反射特性を補正したので，壊疽状可視害だけでなく，水滲や萎れといった初期の可視害を正確に評価できる。

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Image instrumentation of plants exposed to air pollutants (6) Light microscope system for direct observation of stomatal movements of intact plants

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In order to observe the stomatal response of intact plants to environmental changes under growing conditions, we developed a remote-control image instrumentation system with a light microscope. This system is composed of: (1) a light microscope with a wide working distance (13 mm) at high magnification (ca. 1,600-fold magnification on a TV monitor); (2) a movable microscope stage designed to permit the passage of conditioned air along both sides of a fixed leaf and for illuminating from above and below; (3) a high-sensitivity SIT camera (S20 type spectral response) and a monochromatic TV monitor with high resolution and small distortion, used to observe the microscope image in a separate room (stomata can be observed with single reflected or transmitted light above ca. 0.1 mW/cm^2), (4) remote controllers to adjust camera sensitivity, microscope focus and movement of the visual field of the microscope image from the separated observation room. This system solved the problems encountered with an ordinary light microscope in observing stomatal movement of intact growing plants. This system also can be used to observe many intact stomata because of its easy and rapid operation. Furthermore, the stomatal aperture and the ratio of transpiration from the cuticle to that from the stomata can be accurately determined using this system.

Key words: Observation of stomatal movements - Intact plants - Image instrumentation - Light microscope - Remote control.

Stomatal movement is greatly influenced by the plant's environment (Meidner and Mansfield 1968, Burrows and Milthorpe 1976, Raschke 1979, Pospišilová and Solárová 1980, Jarvis and Mansfield 1981a). The stomatal conductance or resistance, measured using porometers (Meidner and Mansfield 1968, Meidner 1981) and a thermal image instrumentation system (Omasa et al. 1981a, 1983a) has been used to evaluate the stomatal response of intact leaves to environmental changes of growing plants (Meidner and Mansfield 1968, Kaufmann 1976, West and Gaff 1976, Omasa et al. 1981b, c). However, direct observation of the stomatal movement of growing intact plants has been very difficult (Meidner and Mansfield 1968, Meidner 1981). Although the scanning electron microscope (Turner and Heichel 1977, Shiraishi et al. 1978) and the light microscope in which a piece of leaf is immersed in water or liquid paraffin (Monzi 1939, Stålfelt 1959, Meidner 1981) can provide a clear image at high magnification, observation of intact stomata under their growing conditions is impossible. An

ordinary light microscope can provide a clear image of the large stomata of an intact plant at low magnification with transmitted or reflected light. Heath (1959) obtained a clear image of a large stoma below 400-fold magnification with transmitted light from a condenser and a dry objective corrected for the lack of a cover glass. However, observation of the small stomata, which requires high magnification, was very difficult (Heath 1959). Furthermore, observation with an ordinary light microscope under a plant's growing conditions poses some problems (Meidner and Mansfield 1968). First, visual observation under weak light is very difficult. Second, the environment of the lower side of the leaf can not be controlled because the leaf is directly held on the microscope stage; the environment is also affected by human manipulation of the microscope. And third, the working distance, that is, the distance between the leaf and the objective during the observation is very narrow at high magnification, thus subjecting the leaf to the danger of sticking to the objective during focusing and destroying the plant's environment between the leaf and the objective.

We, therefore, developed a remote-control image instrumentation system using a new light microscope with a wide working distance at high magnification and an SIT camera with high sensitivity, and observed the stomatal response of intact plants to environmental changes under their growing conditions using this system.

Materials and methods

Plant materials

Sunflower (*Helianthus annuus* L. cv. Russian Mammoth) and tomato (*Lycopersicon esculentum* Mill. cv. Fukuju No.2) plants were grown in an environment-controlled greenhouse (Aiga et al. 1982) at 25/20°C day/night temperature and 70% RH under natural light for 5 to 6 weeks after being sown in pots. Broad bean plants (*Vicia faba* L. cv. Otafuku) were grown at 20/15°C day/night temperature and 70% RH for 6 to 7 weeks. The pots were filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss, and fine gravel, which was moistened with nutrient solution. The plants were irrigated daily. After the test plants had been moved to an environment-controlled chamber (Aiga et al. 1982) and acclimatized to the new conditions, mature leaves of the intact plants were used in the experiments.

Remote-control image instrumentation system with a light microscope

Fig. 1 shows a diagram of our remote-control image instrumentation system with a light microscope and Fig. 2 a photograph of the system. This system has a light microscope with a wide working distance (ca. 13 mm) at high magnification (a 50x objective, 1.5x and 2x amplifiers and a TV adapter lens; ca. 1,600-fold magnification on a TV monitor) (Bausch & Lomb, MicroZoom), an SIT camera with high sensitivity of S20 type spectral response, image resolution above 600 TV lines, distortion within ±2% and shading within 20% (Hamamatsu TV, Model C1000-12) as a detector of the microscope image, a monochromatic TV monitor with image resolution of ca. 1,000 TV lines and distortion within 3%(Chuomusen, Model MD2002A) and remote controllers for adjusting camera sensitivity, microscope focus, and movement of the microscope stage.

Observation of stomatal movements of intact plants under their growing conditions

The microscope with the SIT camera head was set in the environment-controlled chamber. Air temperature in the chamber was maintained at 20.0 ± 0.1°C (broad bean plants) or 25.0 ± 0.1°C (sunflower and tomato plants) and humidity was kept at 70 ± 1% RH. Illumination (irradiance) from fluorescent lamps was varied with SCR electric manipulators and that from

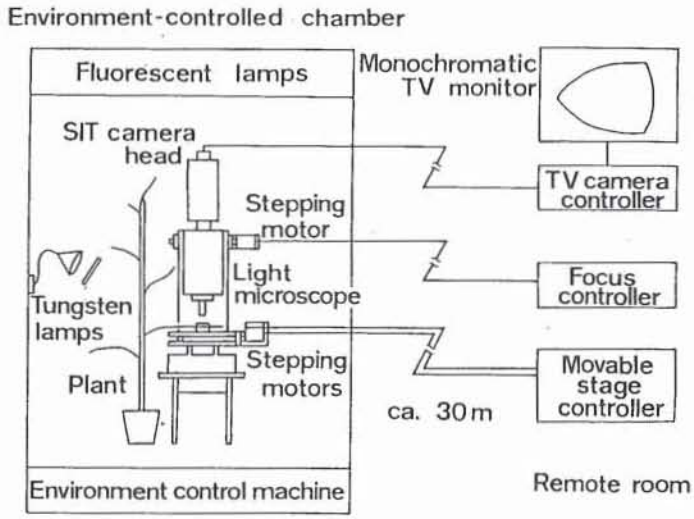

Fig. 1 Diagram of the remote-control image instrumentation system with a light microscope.

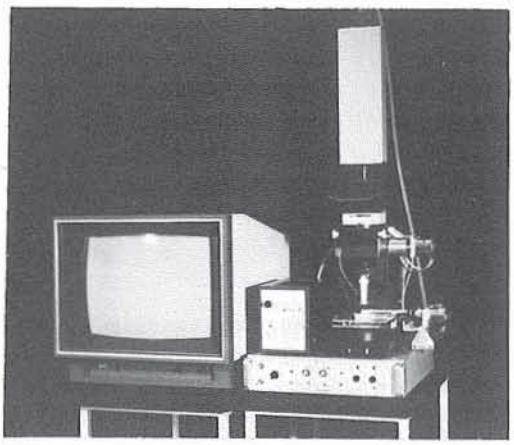

Fig. 2 Photograph of the image instrumentation system.

tungsten lamps with autotransformers through the heat-absorbing glass filters. Illumination was measured with a photometer/radiometer (LI-COR, Model LI-185).

Fig. 3 shows a schematic cross-sectional view of the microscope stage for holding a leaf of the intact plant. The leaf (C) was held on a ring (F, 30 mm in inner diameter, 10 mm wide and 10 mm high) fixed to a remote-control movable stage (G) by a holding ring (E, the same diameter and width as F, 3 mm in height) in order to have the conditioned air pass under the surface of the leaf. Furthermore, since the center of the movable stage was cut out in a circle 30 mm in diameter and the distance between the movable stage and a plate (H) fixed on a base (K) was kept at 10

mm, the same temperature and humidity were maintained on both sides of the leaf. The movable stage and the plate were made of transparent acrylic resin in order to allow light from the environment to enter. A shade cover (B) was not used except in the observation with the transmitted light. Although the observation was usually carried out with light from the environment, a halogen lamp (L) was sometimes used as a supplementary light for the observation with the transmitted light.

The microscope image of the leaf held on the movable stage was observed on the TV monitor in the separate observation room. Camera sensitivity, microscope focus, and the movement of the visual field of the microscope image were adjusted with the remote controllers in the separate room. The knobs for focusing and moving the stage were driven by the stepping motors. The stomatal image displayed on the TV monitor was photographed with black and white film.

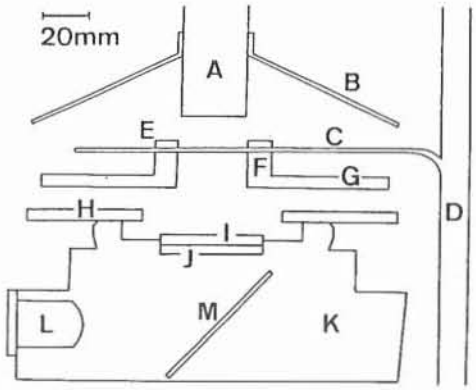

Fig. 3 Schematic cross-sectional view of the microscope stage for holding an intact leaf. A, objective; B, shade cover; C, leaf; D, stem; E, holding ring; F, ring fixed to remote-control movable stage; G, remote-control movable stage; H, plate; I, heat absorbing glass filter; J, diffusing filter; K, base; L, halogen lamp; M, mirror.

Experiment 1

The performance of this instrumentation system was examined. We observed the reflection and transmission images of the stomata of intact sunflower plants under various types of illumination from above and below in order to examine the sensitivity of the system. We also checked a test chart for the microscope (Toppan Printing, Toppan resolution test target) to examine the resolution of the system.

Experiment 2

This system was then used for continuous observation of the stomatal response of intact broad bean plant to illumination change under its growing conditions. An intact leaf of the test plant was held on the microscope stage and illuminated at 30 klx (11.9 $mW \cdot cm^{-2}$). After the leaf had been sufficiently acclimatized to the new conditions, the illumination was changed from 30 to 2 klx (0.5 $mW \cdot cm^{-2}$) and then from 2 to 20 klx (7.7 $mW \cdot cm^{-2}$). The microscope image was observed with the reflected light.

Experiment 3

The relationships between stomatal apertures of sunflower, broad bean, and tomato plants and their stomatal conductances were examined. An intact leaf of the test plant was held on the microscope stage under constant illumination. After the leaf had been sufficiently acclimatized

to the new conditions, ca. 35 stomata in an area ca. 15 mm in diameter of the leaf were randomly observed with the reflected light, and then the stomatal conductance of the area was quickly measured with a porometer (LI-COR, Model LI-1600). This procedure was repeated for the same area after the illumination was changed.

Results and discussion

The system was first evaluated and Fig. 4 shows microphotographs of an intact stoma observed with reflected and transmitted light using this system. The stomatal image was clear at high magnification (ca. 1,600-fold magnification on the TV monitor). The stoma was observed with reflected light and then rapidly observed with transmitted light, and the stomatal aperture was found to be the same for both. Although this system could provide stomatal images with a mixture of reflected and transmitted lights, the clearest image was obtained with the single reflected or transmitted light. The stomata could be observed with single reflected or transmitted light above ca. 0.1 $mW \cdot cm^{-2}$ [environment illumination ; ca. 2 klx (0.5 $mW \cdot cm^{-2}$) with reflected light ca. 0.5 klx (0.5 $mW \cdot cm^{-2}$) with transmitted light]. If the observation with the single light of 0.1 $mW \cdot cm^{-2}$ is done by naked eye through the eyepiece instead of the SIT camera, the eye must be sufficiently acclimatized in a dark room. Judging from the microphotograph (Fig. 5) of the test chart measured using this system, we could tell that the resolution of the microscope image was within 1 μm.

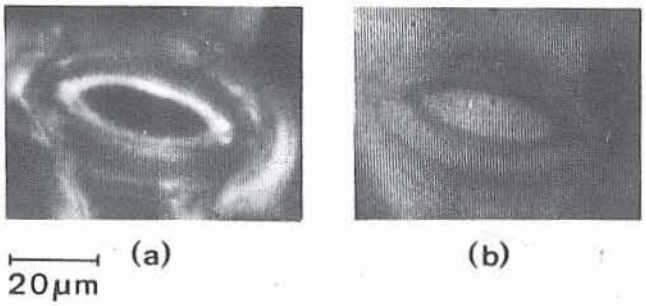

Fig. 4 Microphotographs of an intact stoma observed with reflected or transmitted light using the image instrumentation system. (a) reflection image; (b) transmission image.

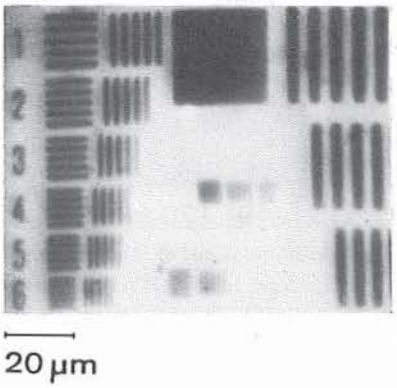

Fig. 5 Microphotograph of test chart measured using the image instrumentation system.

When this system was used for continuous observation of the stomatal movement of growing intact plant, we obtained microphotographs like those in Fig. 6 which show the response of an intact stoma of the adaxial epidermis of broad bean plant to illumination change. The illumination was changed from 30 to 2 klx at 0 min (a) and from 2 to 20 klx at 20 min (e). The movement of the central pore of the stoma could be continuously observed. Fig. 7 shows changes in the width (l_a) and degree of aperture (k_l) of the stomatal pore shown in Fig. 6; l_a began to decrease within 5 min (b) after lowering the illumination (30 to 2 klx) and became 0 μm after ca. 15 min (d). It began to increase again within 15 min after raising the illumination (2 to 20 klx), and after 180 min (i), had recovered to ca. 75% of the value before the illumination change. The degree of the stomatal aperture (k_l) was expressed by the ratio l_a/l_{bmax}, where l_{bmax} was the length (l_b) of the stomatal pore of an opened stoma, that is, the maximum value of l_b ($l_{bmax} = 28.3$ μm).

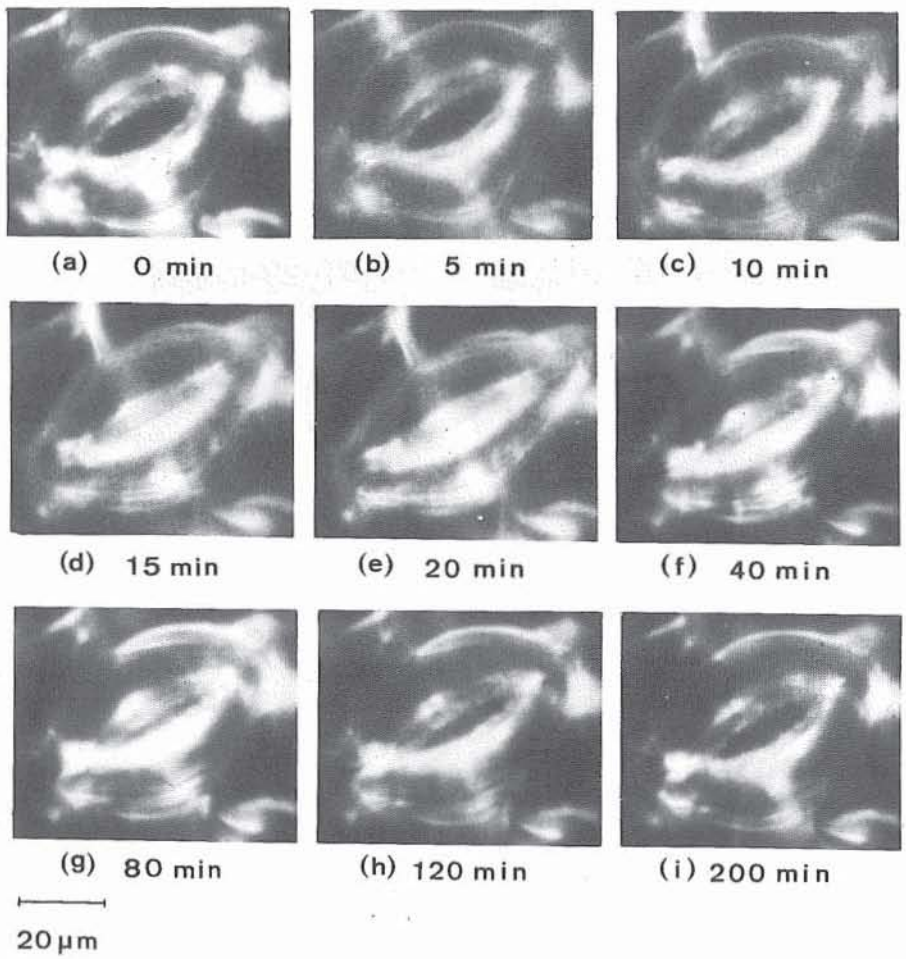

Fig. 6 Microphotographs of responses of an intact stoma of an adaxial epidermis of a broad bean plant to illumination change. The time after the first illumination change is shown under the photographs. The illumination was changed from 30 to 2 klx at 0 min (a) and from 2 to 20 klx at 20 min (e).

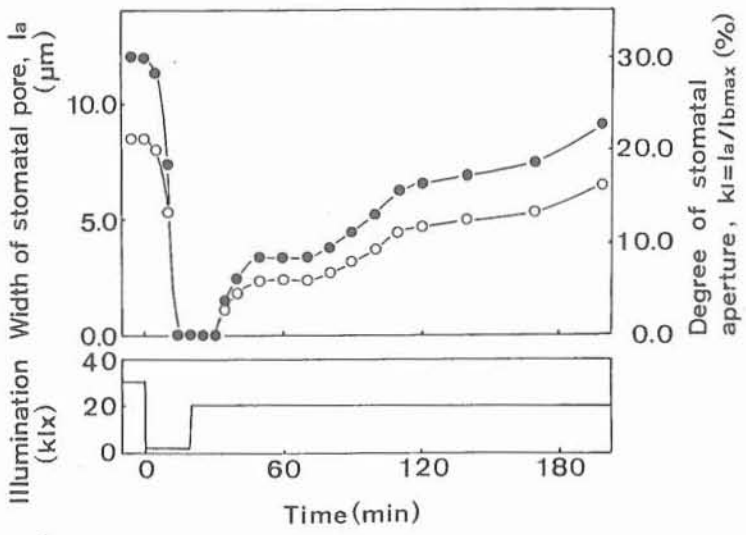

Fig. 7 Changes of the aperture of the stoma shown in Fig. 6. ○, width of stomatal pore (l_a); ●, degree of stomatal aperture expressed by l_a/l_{bmax} (k_l), where $l_{bmax} = 28.3$ μm.

This system was used to analyze the relationship between the stomatal aperture and the stomatal conductance. Fig. 8 shows the relaionships between l_a's of the stomata of the adaxial or abaxial epidermis of the various intact plants and their stomatal conductances (g_s). There was a positive correlation between l_a and g_s measured in the same area of the leaf. However, the regression curves varied with the kind of plant and epidermis. The maximum values of l_a and g_s also varied. Since all regression curves were concentrated near the origin of the coordinate axes, the transpiration from the cuticle of these plants was negligible in comparison with that from the stomata. Table 1 shows the density of the stomata (n_s) and the mean value of l_{bmax} [$E(l_{bmax})$] in the same areas as Fig. 8. From Fig. 8 and Table 1, we could see that g_s was dependent not only upon l_a but also upon n_s. The maximum values of g_s of the sunflower plants with large n_s and $E(l_{bmax})$ were larger than those of the broad bean and tomato plants with small n_s or $E(l_{bmax})$. Fig. 9 shows the relationships between the degree of stomatal aperture (k_l) and g_s obtained fromFig.8 and Table 1. From the ratio $E(l_a)$ / $E(l_{bmax})$, where $E(l_a)$ was the mean value of l_a, k_l could be calculated and was found to decrease at a given value of g_s in the order of: tomato adaxial epidermis > broad bean adaxial epidermis > broad bean abaxial epidermis > tomato abaxial epidermis > sunflower adaxial epidermis > sunflower abaxial epidermis.

To overcome the difficulty of directly observing the stomatal movement of intact plants under their growing conditions, we developed a new remote-control image instrumentation system with a light microscope and used it for continuous observation of the stomatal response to environmental changes and for analysis of the relationship between stomatal aperture and conductance. This system is composed of (1) a light microscope with a wide working distance (13 mm) at high magnification (ca. 1,600-fold magnification on a TV monitor); (2) a movable microscope stage designed to permit the passage of conditioned air along both sides of a fixed leaf and for illuminating from above and below; (3) an SIT camera with high sensitivity (S20 type spectral response) and a monochromatic TV monitor with high resolution and small

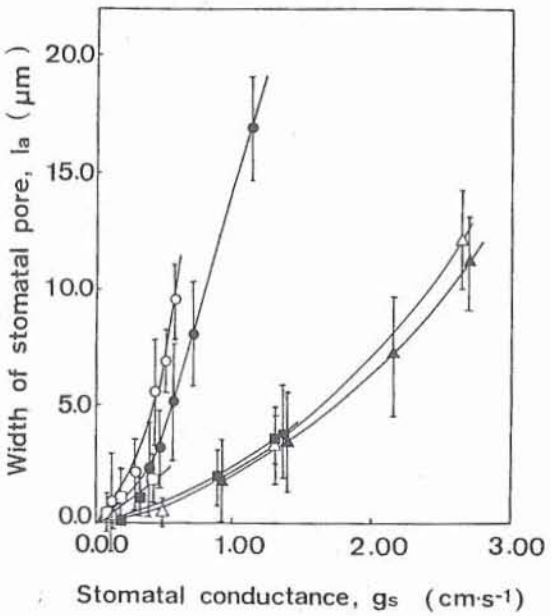

Fig. 8 Relationships between l_a's of the stomata of adaxial or abaxial epidermis of various intact plants and their stomatal conductances (g_s). Symbols represent mean values of the l_a and vertical bars indicate ± standard error. ○, broad bean adaxial epidermis; ●, broad bean abaxial epidermis; △, sunflower adaxial epidermis; ▲, sunflower abaxial epidermis; □, tomato adaxial epidermis; ■, tomato abaxial epidermis.

Table 1 Density of stomata (n_s) and mean value of l_{bmax}[$E(l_{bmax})$] in the same areas as Fig. 8

Plant species	Kinds of epidermis	Density of stomata (n_s)	Mean value of l_{bmax} ($E(l_{bmax})$)
		pieces/mm^2	μm
Sunflower	adax	86.6	30.1
	abax	76.8	35.9
Broad bean	adax	17.7	31.1
	abax	34.7	31.9
Tomato	adax	24.8	11.6
	abax	77.8	14.5

distortion, to allow observation of the microscope image in a separate room (the stomata can be observed with single reflected or transmitted light above ca. 0.1 mW/cm^2), and (4) remote controllers for adjusting camera sensitivity, microscope focus, and visual field movement of the microscope image. Thus, we were able to solve the problems of the ordinary light microscope in observing the stomatal movement of intact plants under growing conditions. This system is also effective for observing many intact stomata because of its easy and rapid operation. Furthermore, the stomatal aperture and the ratio of the transpiration from the cuticle to that from the stomata can be accurately determined with this system.

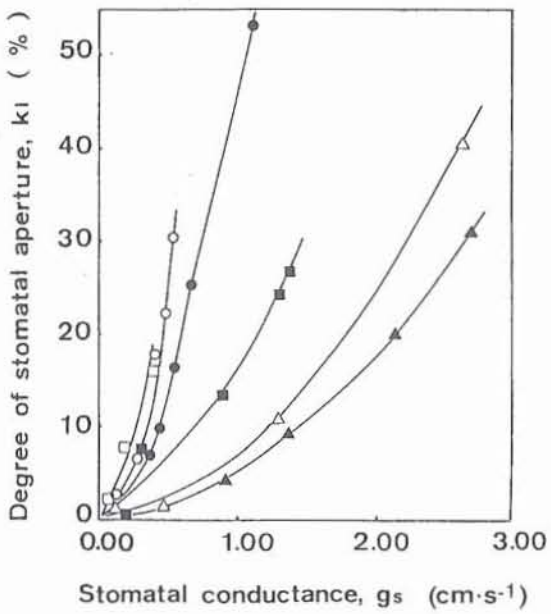

Fig. 9 Relationships between the degree of the stomatal aperture (k_l) and g_s obtained from Fig. 8 and Table 1. Symbols are the same as those in Fig. 8.

We wish to thank the members of the Engineering Division, The National Institute for Environmental Studies, who maintained the equipment and cultivated the plants used in these experiments.

This study was published in Plant & Cell Physiology (Omasa et al. 1983b)

Appendix

l_a, width of a stomatal pore; l_b, length of a stomatal pore; l_{bmax}, l_b in the case of an opened stoma, i.e., maximum value of l_b; g_s, stomatal conductance; n_s, density of the stomata; $E(l_a)$, mean value of l_a; $E(l_{bmax})$, mean value of l_{bmax}; k_l, degree of the stomatal aperture expressed by l_a / l_{bmax} or $E(l_a) / E(l_{bmax})$; working distance, distance between the leaf and the microscope objective during observation; RH, relative humidity; SIT, silicon intensifier target.

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植物の画像計測

(6) 生育している植物の気孔開閉運動の直接観察のための光学顕微鏡システム

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植物の気孔開閉運動を，その生育している環境を破壊することなく観察するための光学顕微鏡システムを開発した。このシステムは，(1) 高倍率（TV モニタ上で1600倍）で広い作動距離（13mm）をもつ光学顕微鏡，(2) 固定された葉の両側が周辺大気と同じ環境条件に保たれ，また，上方及び下方からの照明を可能にした可動式のステージ，(3) 微弱光下での観察を可能にするための SIT カメラ，(4) 別室で観察するための高解像，低歪みの TV モニタ，また，カメラの感度や顕微鏡のフォーカス，視野などを遠隔で調節するためのコントローラなどで構成される。このシステムにより，簡単に，生育している植物の気孔開閉運動の連続観察を行うことができた。

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An evaluation of chlorophyll content of leaves based on the spectral reflectivity in several plants

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The effective characteristics of spectral reflectivity for estimating chlorophyll content of leaves were investigated experimentally in eight species. Using regression equation between chlorophyll content and reflectivity ratio rNI′/rG′, where rNI′ and rG′ are the spectral reflectivity at 800nm and 550nm, respectively, the chlorophyll content of the leaves was estimated in May to December. The obtained value showed the standard error of about 5$\mu g \cdot cm^{-2}$ which is about 10% of the mean chlorophyll content of all leaves (sample size: 147) examined, irrespective of plant species and seasons.

Keywords: Chlorophyll content of a leaf, spectral reflectivity, deciduous trees, evergreen trees, sweet potato

The content of chlorophyll in leaves which is a principal photosynthetic pigment, may be an important index of physiological activity of leaves. It is known that chlorophyll content of leaves decreases to the demage of air and/or soil pollutions (Knudson et al. 1977, Sakaki and Kondo 1981). It is very usefull and convenient as a remote sensing technique if such decreases can be evaluated without destroying leaf samples.

The authors (Aoki et al. 1980) have reported that the chlorophyll content of leaves in camphor tree, oleander and Japanese viburnum can be detected by the use of reflectivity ratios, such as rNI′/rG′ and rNI′/rR′ where rNI′, rR′ and rG′ are the spectral reflectivity at 800nm, 630nm and 550nm, respectively. They also showed that the estimation error was about 10% of the chlorophyll content of healthy leaves and that the error is induced mainly by the thickness of leaves, and that the variation of leaf water content makes little error if the shortage of leaf water content is not so severe.

The aim of the present paper is to clarify the most effective characteristics of spectral reflectivity for estimating the chlorophyll content of leaves of various plant species in different seasons.

Materials and Methods

Species of plants tested were selected from three categories, that is the evergreen tree, the deciduous tree and the herbaceous plants. As the evergreen trees, camphor tree (*Cinnamomum camphora* Sieb.)Japanese viburnum(*Viburnum awabuki* K. Koch), (oleander *Nerium indicum* Mill), Japanese holly oak (*Quercus phillyraeoides* A. Gray) and Japanese privet (*Ligustrum japonicum Thunb.*) were selected. As the deciduous trees, poplar (*Populus nigra L.*), plane tree (*Platanus orientalis L.*) and Japanese zelkova (*Zelkova serrata* Makino) were chosen, and sweet potato (*Ipomea batatas Lam*) was chosen as the herbaceous plant. The spectral reflectivity of the leaves was measured at the wavelengths of 450nm, 550nm, 600nm, 630nm, 680nm and 800 nm using Cary 17DX Spectralphotometer (Varian Co. Ltd.).The procedure of the measurements was the same as reported before (3). The obtained spectral reflectivities in % were expressed as rB′, rG′, rY′, rR′, rFR′ and rNI′, respectively. Total 42 kinds of reflection characteristics which include the above mentioned six kinds of spectral reflectivities, the reciprocals of them and the ratios between both reflectivities in all combinations among them were chosen as an independent variable.

The measurements were repeated in late May, early July, late August and late December 1981 to investigate the seasonal changes in the reflectivity. The sample size of measurements in each month is shown in Table 1. Additional measurements were made for 25 leaves in camphor tree, 25 leaves in Japanese viburnum and 20 leaves in oleander in July, and 30 leaves in Japanese viburnum in August.

Table 1 Sample sizes of measurements in each month.

plant species	sample sizes of measurement				
	May	July	Aug.	Dec.	(sum)
camphor tree	4	5	5	5	19
Japanese viburnum	4	5	5	5	19
oleander	4	5	5	5	19
Japanese holly oak	5	5	5	5	20
Japanese privet	–	–	–	5	5
poplar	5	5	5	5	20
plane tree	5	5	5	–	15
Japanese zelkova	5	5	5	–	15
sweet potato	–	5	5	5	15
(sum)	32	40	40	35	147

Results

Fig. 1 shows the relationship between reflectivity ratios of rNI′/rG′ and rNI′/rR′ and chlorophyll content of leaves. For Japanese viburnum in August, reflection characteristics such as rNI′/rG′, rNI′/rY′, rNI′/rR′, rB′/rG′, rB′/rY′, rB′/rR′, rG′ and 1/rG′ showed very high correlation with chlorophyll content with correlation coefficient (r) of above 0.8, although the other 34 reflection characteristics showed poor correlation.

The relation between reflectivity ratio rNI′/rG′ and chlorophyll content showed the highest

correlation (r=0.98) for seven species of plant (sample size, n=35) in December (Fig. 2). There was a little difference in the relation among species. Table 2 shows the list of correlation coefficient between several kinds of reflection characteristics and chlorophyll content for camphor tree, oleander and Japanese viburnum in July using 20-25 leaves in each leaves in each species. The numerals in Table 2 show that the reflectivity ratios rNI'/rB' and rNI'/rFR' correlated poorly (r=0.01-0.07) with chlorophyll content, while other reflection characteristics showed high correlation (r>0.8). The correlation coefficient for rNI'/rG' obtained by 40 samples in 8 species (r=0.95) showed similar value to that obtained in each species of camphor tree (r=0.98), oleander (r=0.94) and Japanese viburnum (r=0.95).

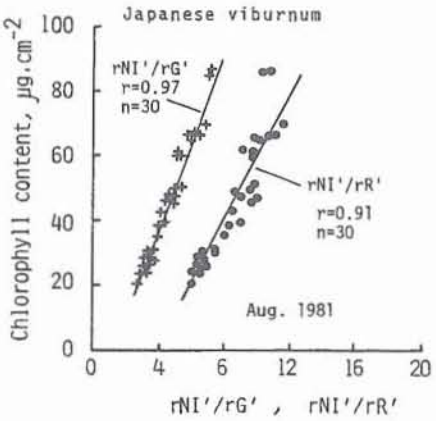

Fig. 1 Relation between reflectivity ratios (rNI'/rG' and rNI'/rR') and chlorophyll content of leaves in Japanese viburnum in August 1981. rNI', rR' and rG' are the spectral reflectivity at 800nm, 630nm and 550nm, respectively. n is the sample size, and r is the correlation coefficient. +: rNI'/rG' ●: rNI'/rR'

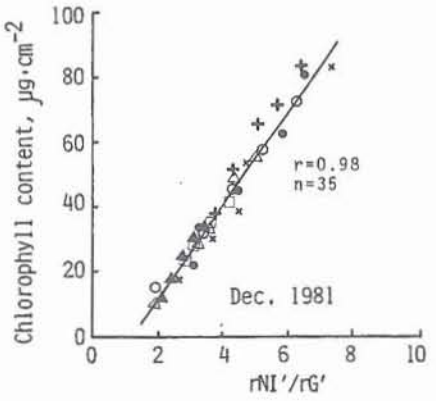

Fig. 2 Relation between reflectivity ratio rNI'/rG' and chlorophyll content of leaves in 7 species examined in December 1981. Symbols in the figure show the species as follows: ○: oleander △: camphor tree ×: Japanese viburnum +: Japanese holly oak ●: Japanese privet △: poplar □: sweet potato

Table 2 Correlation coefficients for the relation between characteristics of spectral reflectivity and chlorophyll content of leaves in camphor tree, oleander and Japanese viburnum and 8 species in July. Species are shown in Table 1. n is the sample size, and r is the correlation coefficient. Correlation coefficients tested in 9 species (n=147, see Table 1) examined in May, July, August and December 1981 are also shown. rNI', rFR', rR', rY', rG' and rB' are the spectral reflectivity at 800nm, 680nm, 630nm, 600nm, 550nm and 450nm, respectively.

reflectivity ratio	camphor tree	oleander	Japanese viburnum	8 species*	9 species**
	$n = 25$	$n = 20$	$n = 25$	$n = 40$	$n = 147$
rNI'/rB'	0.68	0.13	0.23	0.36	0.44
rNI'/rG'	0.98	0.94	0.95	0.95	0.95
rNI'/rY'	0.95	0.89	0.89	0.87	0.89
rNI'/rR'	0.92	0.83	0.84	0.80	0.83
rNI'/rFR'	0.70	0.18	0.30	0.42	0.51
rB'/rG'	0.94	0.95	0.97	0.80	0.76
rB'/rY'	0.93	0.93	0.93	0.84	0.77
rB'/rR'	0.90	0.90	0.90	0.82	0.71
rG'	−0.94	−0.92	−0.91	−0.84	−0.83
1/rG'	0.96	0.94	0.94	0.91	0.90

*camphor tree, Japanese viburnum, oleander, Japanese holly oak, poplar, plane tree, Japanese zelkova, sweet potato (see Table 1).

**The eight species above mentioned and Japanese privet were mesured (see Table 1).

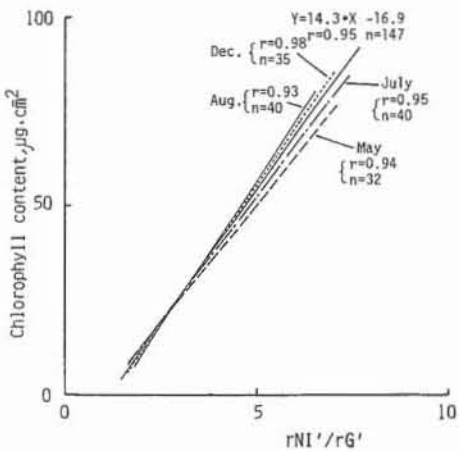

Fig. 3 Seasonal changes in the relationship between reflectivity ratio rNI'/rG' and chlorophyll content of leaves tested in 7-8 species. Species tested are shown in Table 1. The regression line obtained for all samples (n=147) tested in 9 species and different months is also shown in the figure, where Y and X are the chlorophyll content in $\mu g \cdot cm^{-2}$ and rNI'/rG', respectively. The correlation coefficient (r) was 0.95 and the standard estimation error (se) was 5.0 $\mu g \cdot cm^{-2}$.

Fig. 3 shows the seasonal changes of regression line between rNI′/rG′ and chlorophyll content examined in 7-8 species (4-5 leaves in each species; n=32-40; see Table 1). The regression lines in August and December when the leaves matured, showed slightly larger slope than those in May and July when the leaves were still younger or unmatured. However, the calculated correlation coefficient of rNI′/rG′ examined for 147 samples including data obtained by 9 species in different months was 0.95, which was the largest among all the reflection characteristics tested (see Table 2). Therefore, the reflectivity ratio rNI′/rG′ seems to be the most usefull reflection characteristics for evaluating chlorophyll content of leaves in any plant species tested. Following correlation equation between chlorophyll content (Y, $\mu g \cdot cm^{-2}$) and rNI′/rG′ (X) is applicable:

$$Y = 14.3X - 16.9 \quad (1)$$

$$r = 0.95$$

$$n = 147$$

$$se = 5.0\ \mu g \cdot cm^{-2}$$

The standard estimation error (se) was 5.0 $\mu g \cdot cm^{-2}$ chlorophyll, and the error was equivalent to about 10% of the mean chlorophyll content of all leaves examined.

Discussion

The structures of transverse section of lamina are quite different among plant species tested. However, the chlorophyll content of leaves in different species could be evaluated accurately by the same correlation equation (1), irrespective of maturity of leaves. This finding suggests that the evaluation of chlorophyll content of forest canopies composed of several plant species can be performed with high reliability by a remote sensing technique using infrared color photography, multi-spectral scanner.

The above mentioned results are applicable to a single leaf. It is more important to develop the remote sensing technique for evaluating chlorophyll content of plant canopies of herbes and trees in larger area under field condition. The farther examination should be performed in this respect.

Acknowledgements

The authors thank to Mr. Minoru Nishida, who was a student of College of Agriculture, University of Osaka Prefecture (present address: Atsugi Technical Developmental Center, Asia Air Survey Co. Ltd., Atsugi, Yokohama) for his help in the experiments. The research was supported financially by "The Special Research Project on Environmental Sciences; Basic Research on Analysis and Evaluation of Environmental Information by Remote Sensing" supported by Grants and Aid for Scientific Research, The Ministry of Education, Culture and Science, Japan.

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分光反射特性に基づく数種植物の葉内クロロフィル含量の推定

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9種類の植物葉のクロロフィルの含量を遠隔探査法により推定するために適した分光反射特性を実験的に明らかにしようとした。

rNI'/rG'の比反射率（rNI'及びrG'はそれぞれ800nm及び550nmにおける分光反射率）から5月～12月にわたり葉内クロロフィル含量の推定を行った結果，植物の種類及び時期のいかんにかかわらず5 $\mu g \cdot cm^{-2}$*leaf*（標本数147枚の葉のクロロフィル含量の平均値の約10％に相当する）の推定標準誤差で推定しえた。

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Phytotrons in the National Institute for Environmental Studies

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Phytotrons in the National Institute for Environmental Studies (NIES) were introduced. The feature of these facilities was described from the standpoint of engineering. Furthermore, an experiment for analyzing the sorption of air pollutants by plant community was shown.

Key words: Phytotron — Controlled Greenhouse — Growth Cabinet — Environment Simulator — Plant Community — Environmental Study

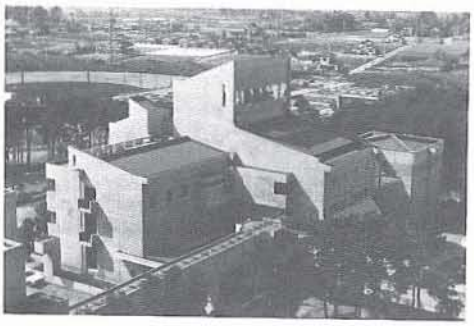
The first-period Phytotron (Phytotron I) in the National Institute for Environmental Studies (NIES) was built in December, 1975, and the second-period Phytotron (Phytotron II) was completed in August, 1981 in order to obtain basic data for planning the environmental policy.These facilities belong to the great and new Phytotron in the world. The Phytotron I has controlled greenhouses and growth cabinets for the air pollutant exposure, and the Phytotron II includes simulators to analyze the plant-environment system. Outlines of these facilities and their technical features are described in this paper.

Outlines of facilities

Overall views of the Phytotron I and II are shown in Photo 1. Fig. 1 is room layouts in the Phytotrons. The Phytotron I is a building consisting of three floors above the ground and a penthouse (total area 3340 m^2). In the southern area of each floor, the following facilities are arranged three-dimensionally; six controlled greenhouses (growth room 40 m^2) for the study of correlations between environmental factors such as temperature and humidity and plant reactions under the natural light, and four growth cabinets (4 m^2, double chamber system) for studying the effect of air pollutants on plants under the natural light. Nine growth cabinets of the artificial-light type (4 m^2) are also included for studying the influence of air pollutants on plants.

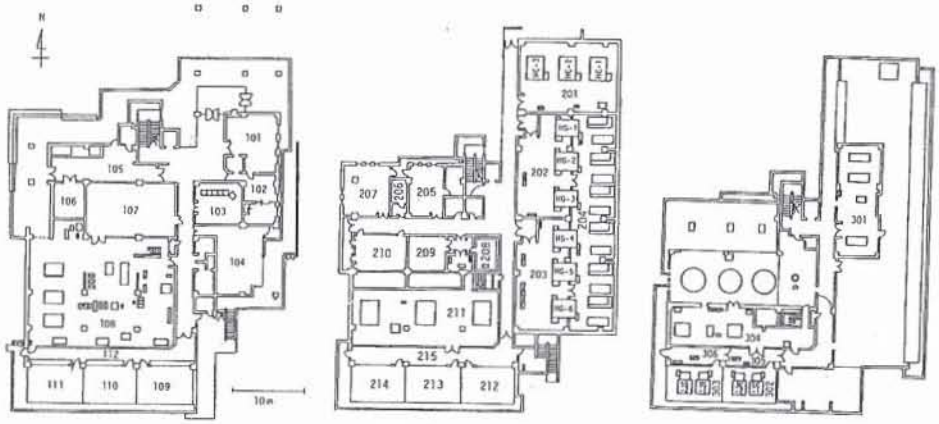
On the other hand, the Phytotoron II is a building consisting of three floors above the ground, one floor under the ground, and a penthouse (total area 2090 m^2). This Phytotron is provided with two simulators (growth room 6 m^2) for reproducing plant community's environments (light, air, and soil), in order to examine the effect of the environmental deterioration on plants, the transfer phenomenon of the plant-environment system, and the improvement capacity of environment by plants; also, there are three growth cabinets (11 m^2)

(a) Phytotron I.



(b) Phytotron II.

Photo 1 Overall views of the NIES Phytotron.



1F

101; managing room
102; office
103; control room
104; seminar room
105; preparation room for plant materials
106; storage
107; transformer room
108; central machinary room
109-111; growth room of controlled greenhouse
112; air conditioned corridor

2F

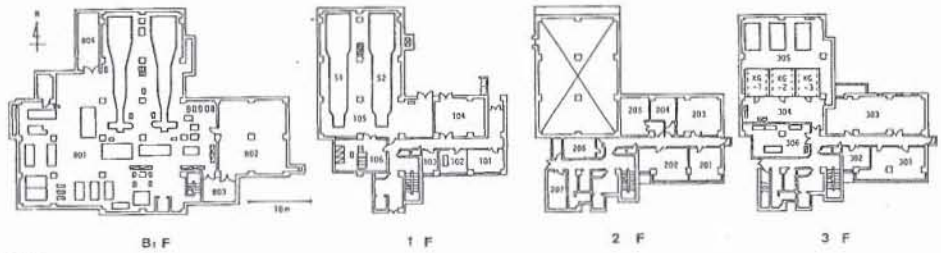
201-203; growth cabinet room
204; machinary room
205; laboratory
206; dark room
207; laboratory
208; cold room
209; dark laboratory
210; seminar room
211; machinary room
212-214; growth room of controlled greenhouse
215; air conditioned corridor

3F

301; machinary room
302-303; growth cabinet room (natural-light type)
304; machinary room
305-306; air conditioned corridor
SC1-2; growth cabinet (natural-light type)
SG1-2; growth cabinet for air pollutant exposure (natural-light type)

HC1-3; growth cabinet (artificial-light type)
HG1-6; growth cabinet for air pollutant exposure (artificial-light type)

(a) Phytotron I.



B1F

B01; central machinary room
B02; transformer room
B03-04; utility

1F

101; managing room
102; control room
103; storage
104; preparation room for plant materials
105; simulator room
106; simulator control room
S1-2; simulator for analyzing the plant-environment system

2F

201; seminar room
202; laboratory
203; sterile room
204-205; dark laboratory
206; cold room
207; storage

3F

301; laboratory
302; instrumentation room
303; chamber room
304; front area of growth cabinet
305-306; machinary room
307; storage
KG1-3; growth cabinet

(b) Phytotron II.

Fig. 1 Room layouts in the NIES Phytotron.

for performing various growth and physiological experiments under the artificial light.

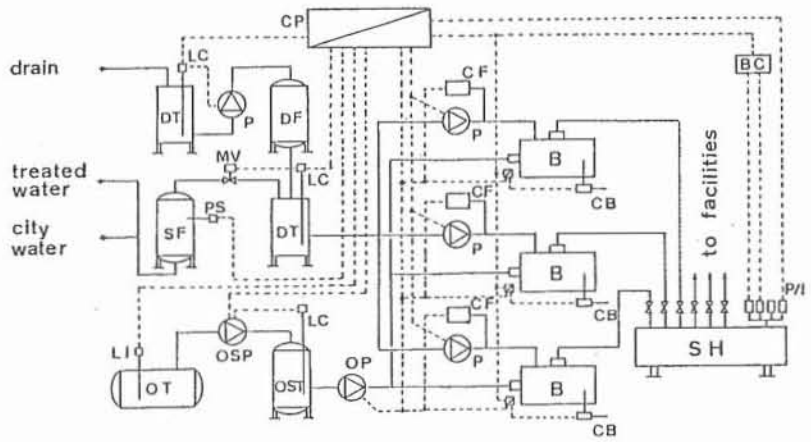
In addition, as relative facilities, an energy center supplies heating and cooling sources such as steam and cold water, and a storehouse provides gases used for the experiment. Furthermore, there are equipments for supplying brine as a cooling source in each Phytotron.

Supply of energy and gas

Energy center

The sources of heating and cooling except brine are supplied from the energy center to various facilities in the NIES. The total amount of the sources supplied from the energy center are estimated at 1.27×10^7 kcal/h for the heating source and at 8.35×10^6 kcal/h for the cooling source. These sources must be supplied stably without interruption throughout the year to the facilities for biological experiments and intermittently to the facilities for physical experiments.

Fig. 2 shows a system diagram of the steam supply system in the energy center. The steam to the facility is supplied by three flue boilers that uses kerosene as fuel. The water consumption is reduced by using the recovery drain for the boiler water. The load reduction on the softner is also performed by reprocessing the waste water from the facility. The number of operated boilers is determined according to the load fluctuation and the steam pressure supplied to the facility is maintained 5 kg/cm^2. The steam is used in the facility after the heat exchange or the pressure reduction.



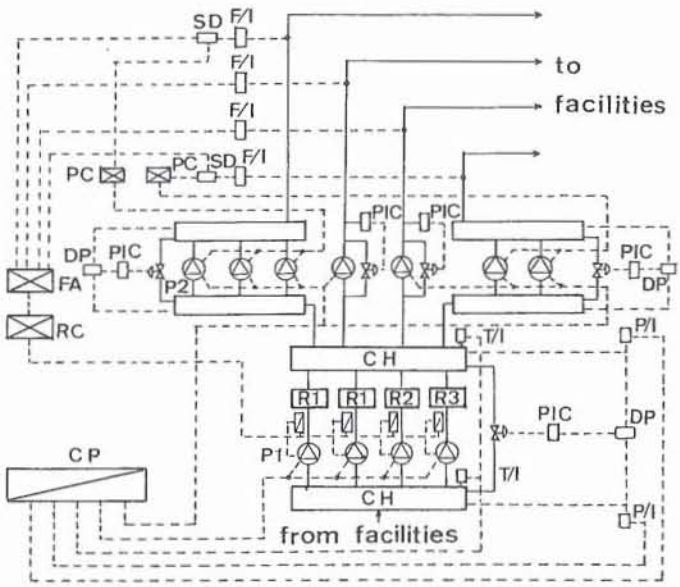
B:	boiler (5,388,000 kcal/hx3)	OP:	feed oil pump
BC:	boiler controller	OSP:	oil transfer pump
CB:	continuous blow down controller	OST:	fuel oil service tank
CF:	continuous chemical feeder	OT:	fuel oil storage tank
CP:	control panel	P:	feed pump
DF:	drain filter	P/I:	pressure transducer
DT:	drain tank	PS:	water pressure switch
LC:	level controller	SF:	softner
LI:	level indicator	SH:	steam header
MV:	motor valve		

Fig. 2 Steam supply system in the energy center.

Fig. 3 shows a system diagram of the cold water supply system in the energy center. Three turbo-refrigerators and an absorption refrigerator are equipped to supply the cold water to the facility. In order to respond to the load fluctuation a closed circulation system is introduced and the number of operated refrigerators is determined according to the load. Since the recycled water is used for cooling these refrigerators in a similar manner as the boiler water, the control of pH and electroconductivity becomes easy. The temperature of cold water supplied to the facility is 7°C.

Energy supply system in Phytotron

The energy supply sytem in Phytotron's central machinary room converts the steam and the cold water supplied from the energy center according to the purpose of the application and supplies to the controlled greenhouse, the growth cabinet, and the simulator for analyzing the plant-environment system. The system also provides brine as a cooling source for these equipments.

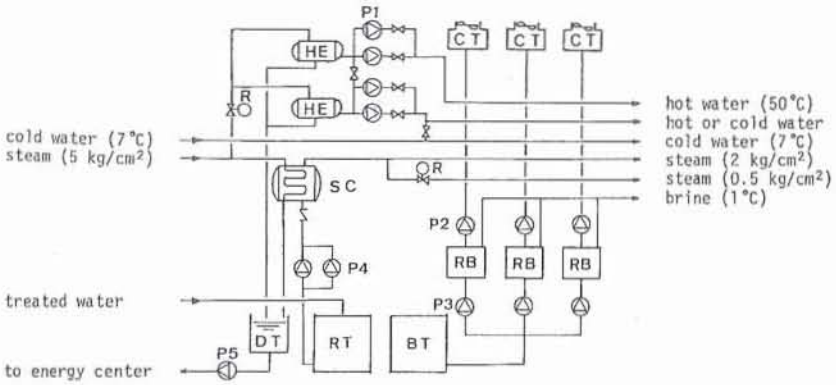


CH; cold water header
CP; control panel
DP; differential pressure sensor
FA; flow rate adder
F/I; flow rate transducer
P1; primary cold water pump (4,050 l/min x 2, 10,000 l/min x 2)
P2; secondary cold water pump
(1,870 l/min x 3, 3,410 l/min x 2, 2,350 l/min, 870 l/min)
PC; secondary cold water pump controller
P/I; pressure transducer
PIC; pressure controller
R1; turbo-refrigerator (1,210,000 kcal/h x 2)
R2; turbo-refrigerator (3,024,000 kcal/h)
R3; absorption refrigerator (3,024,000 kcal/h)
RC; refrigerator controller
SD; signal distributer
T/I; thermal transducer

Fig. 3 Cold water supply system in the energy center.

Fig. 4 shows a system diagram of the energy supply system in Phytotron II. The steam supplied from the energy center is converted into hot water by a heat exchanger and is provided to the simulator and the growth cabinet as a heating source. The steam for humidification is generated by a steam converter using the treated water, which does not contain any harmful substance to plants, and then is supplied to the simulator at a pressure of 2 kg/cm^2. It is also supplied to the growth cabinet after the pressure is reduced to 0.5 kg/cm^2. As a cooling source, besides the cold water from the energy center, the brine is provided for the simulator and the growth cabinet, which have a wide temperature and humidity control range, by three refrigerators. In the brine supply system, a brine tank is used to absorb the load variation in the day and the experiment as a accumulator, and the number of operated refrigerators is

determined according to the brine temperature in the tank. High control accuracy, which was required for air temperature and humidity in the simulator and the growth cabinet, was incorporated in the design of the control system of heating and cooling sources. As a result, hot water can be supplied at ±0.1°C, brine at ± 0.1°C, and steam at ±0.1 kg/cm^2.

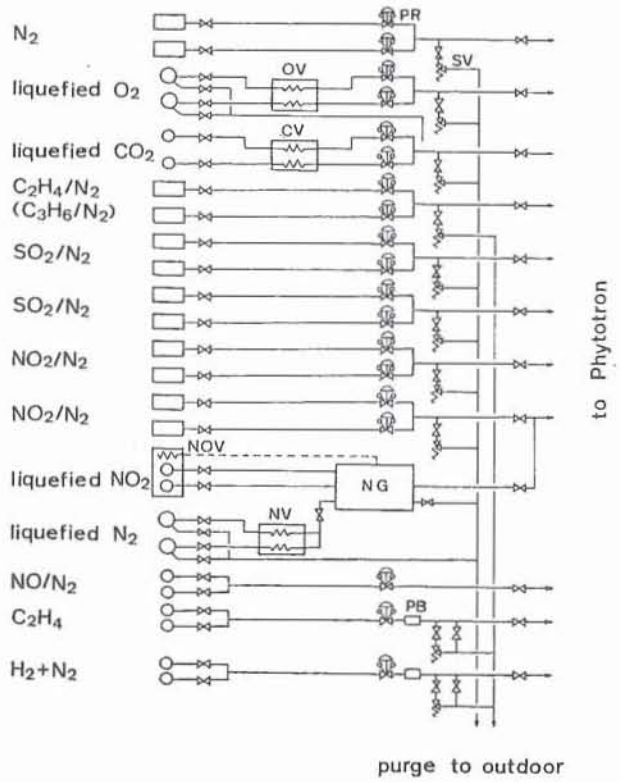


BT;	brine tank	P4;	feed pump
CT;	cooling tower	P5;	drain pump
DT;	drain tank	R;	pressure reducing valve
HE;	heat exchanger (369,000 kcal/h x 2)	RB;	reciprocal refrigerator (244,000 kcal/h x 3)
P1;	hot water pump	RT;	water tank
P2;	cooling water pump	SC;	steam converter (683 kg/h)
P3;	brine pump		

Fig. 4 Energy supply system in Pnytotron II.

Gas supply system

A gas storehouse is provided in order to stably supply large amount of gas used in the facility for biological experiements. Fig. 5 shows a system diagram of the gas supply system in the gas storehouse. The gas used for the plant experiment should be carefully controlled in order to increase the reliability of the experiment. We use gases loaded in various storing containers for SO_2, NO_2, NO, or HC (C_2H_4, C_3H_6). These gases have high traceability in concentration and quality. As the storing container, a cylinder (47 *l*), a set of cylinders (47 *l*× 10) and a container (175 *l*) are selected according to the amount of the use and the storage conditions. Two series of containers are always connected to each supply line to support longterm and continuous experiments; the switchover is automatically performed according to the reduction of the pressure in the supply line. In addition, NO_2 can be supplied by diluting liquefied NO_2 with N_2.

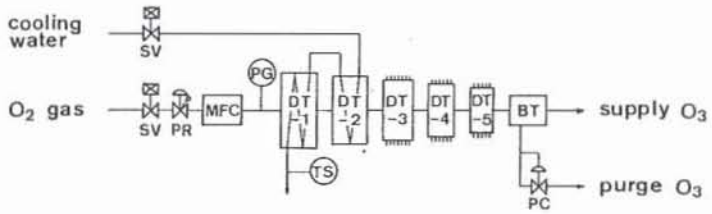


CV;	CO_2 vaporizer	OV;	O_2 vaporizer
NG;	NO_2 mixer	PB;	backfire protector
NOV;	NO_2 vaporizer	PR;	pressure reducing valve
NV;	N_2 vaporizer	SV;	safety valve

Fig. 5 Gas supply system.

On the other hand, O_3, which cannot be supplied from the container is made from O_2 by a O_3 generator that utilize silent discharge. Fig. 6 shows a system diagram of the O_3 generator. The principal causes of fluctuation in concentration of O_3 generated by silent discharge are the change in flow of the supplied O_2, the change in pressure within the discharge tube, and the change in discharge voltage. Therefore, in this equipment, the O_3 flow rate is regulated by mass-flow controllers, the pressure within the discharge tube by a pressure control valve, and the discharge voltage by a stabilizer for power supply. By these counterplans, a stable supply of constant concentration O_3 is possible. The concentration of O_3 from the generator can be selected by combining discharge tubes with different capacities and adjusting the primary voltage of each discharge tube.

The other manipulation gas, CO_2 is produced from liquefied CO_2 by a vaporizer.



BT;	buffer tank	PG;	pressure gauge
DT;	discharge tube	PR;	pressure reducing valve
MFC;	mass flow controller	SV;	solenoid valve
PC;	pressure control valve	TS;	thermal limit switch

Fig. 6 System diagram of O_3 generator.

Operation system

The Phytotron is continuously operated day and night and some experiments are continued for several months. Therefore, we should have some attentions to the design, the maintenance, and the operation.

The ultra high-voltage substation receives electricity from two lines to guard against accidents such as the falling of a thunderbolt. Furthermore, an independent electric power plant secures all powers for the facility and one third of the lighting for one's living during the failure of commercial power supply. Also, by closely managing the operation corresponding to fluctuation in the load of boilers and refrigerators, spare machines may be kept a stand-by condition. For equipment that may frequently fail such as the pump system, two parallel series are provided.

The operation is divided into three parts; daily monitoring, checking, and repair. The monitoring function is concentrated into a central contorl panel, where there are emergency alarms (buzzers and lamps) for the power system and each control system. When an emergency occurs, the failure point can be located by tracing the alarm. Also, the steam pressure, the cold water temperature, the brine temperature, the gas leakage, the gas pressure, etc. are being monitored at many locations.

The check of the equipment is performed on an hourly, daily, weekly, monthly, semiannually, or annually basis depending on piece of the equipment. Furthermore, an overhaul is annually performed to maintain the normal operation of the equipment. The trouble is repaired on occasion. The adjustment of equipments and the correction of each sensors are performed from time to time.

Controlled greenhouse

Overall view of the controlled greenhouse in Phytotron I was shown in Photo 1. In the natural-light type facility, the light intensity should come near that in the natural world. Therefore, every growth room of the controlled greenhouse fronts on the south to effectively catch the sunlight. The room area is designed to be 40 m^2, which is relatively large for this type of greenhouse, in order to reduce the ratio of a area, where the light transmission decreases due to

the side glass, to the entire room area. Furthermore, to improve the spatial distribution of air temperature and humidity, the entrance side wall is glazed, and the air-conditioned corridor is installed. In addition, the influence of heat transfer is reduced by the pair glass.

Table 1 is a list of the basic performance of the controlled greenhouse. Photo 2 shows a general view of the growth room, and Fig. 7 shows a sectional view. The air from the growth room passes the air return duct and is mixed with fresh air in the mixing box. Furthermore, the air, after the dust is removed by the filter, is regulated to the desired value of temperature and humidity by air conditioners such as the heat exchanger and the humidifier, and then is blown out from the floor to the growth room through the sending duct and the buffer chamber by the blower. The floor with porous plates and the buffer chamber permitted to uniformly blow out the air from the whole floor surface. Also, the structural design of the room such as the gradient of the ceiling glass, the location of the air return port, etc. considers spatial distributions of wind velocity, temperature and humidity in the room and the heat transfer from glass surfaces. In addition, the roof is equipped with sprinklers in order to reduce the thermal load caused by solar radiation in summer.

Fig. 8 shows a system diagram of the temperature and humidity control system for the greenhouse. The PID controller is used to regulated the opening of the modutrol motor with the proportioning relay for the heat exchanger and the humidifier. The temperature and humidity conditions in the growth room can be optionally selected according to the object of the experiment. Fig. 9 shows an exmaple of the program control of temperature and humidity. Temperature and humidity can be regulated to accuracy within ± 1°C and ± 3%RH under transient conditions.

Table 1 Characteristics of the controlled greenhouse.

		characteristics
temperature	range	day 15–35°C night 10–30°C
	deviation	±1°C (max)
	distribution	±2°C (max)
humidity	range	50–70%RH
	deviation	±3%RH (max)
	distribution	±6%RH (max)
wind	mean velocity	0.5 m/s
	distribution	±0.35 m/s (max)
ventilation		500 m^3/h

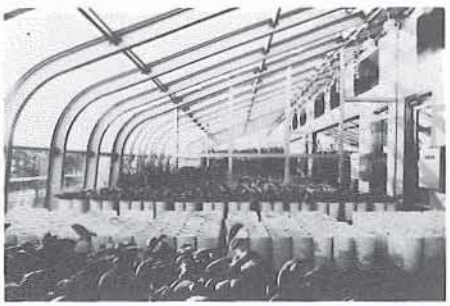
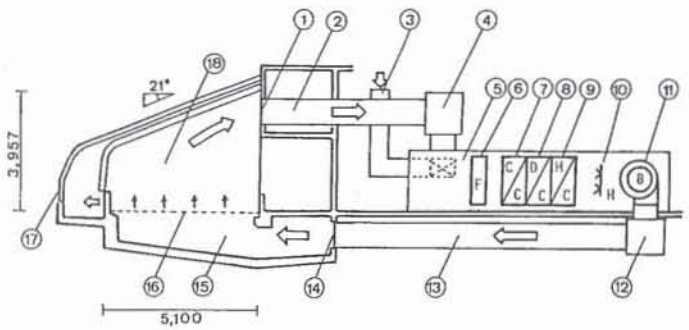
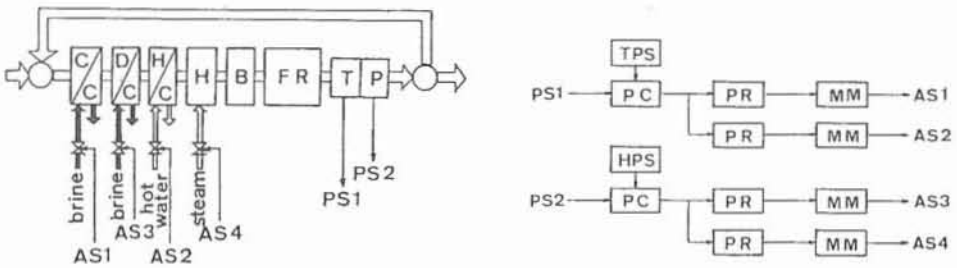


Photo 2 General view in the growth room of the controlled greenhouse.



1; air return port
2; air return duct
3; fresh air inlet
4; buffer chamber for return air
5; mixing box
6; filter
7; cooling coil
8; dehumidifying coil
9; heating coil
10; steam jet unit
11; blower
12; air sending chamber
13; air sending duct
14; blowout position
15; buffer chamber
16; floor with porous plates
17; ladder
18; growth room

Fig. 7 Sectional view of the controlled greenhouse.



(a) diagram of the air flow.

(b) diagram of the signal.

B; blower
C/C; cooling coil
D/C; dehumidifying coil
FR; growth room
H; steam jet unit
H/C; heating coil
T; resistance thermometer

P; psychrometer
HPS; program set station for humidity
MM; modutrol motor
PC; PID controller
PR; proportioning relay
TPS; program set station for temperature

Fig. 8 System diagram of the temperature and humidity control system for the greenhouse.

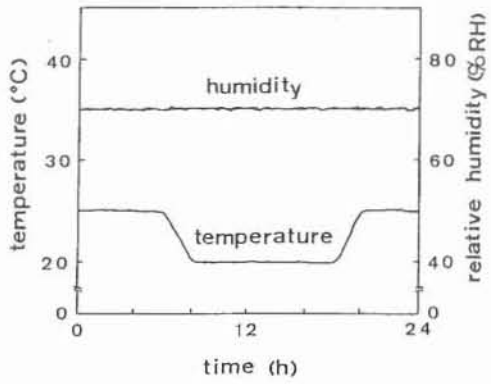


Fig. 9 An example of the program contorl of temperature and humidity in the greenhouse.

Growth cabinet for air pollutant exposure

As mentioned above, various growth cabinets of artificial-light type and natural-light type are included in Phytotron I and II. Here the growth cabinet for exposure to mixed air pollutants in Phytotron I is introduced.

Photo 3 shows a general view of the gas exposure cabinet. Table 2 is a list of the basic performance of the cabinet. The feature of this cabinet is that it can keep the gas concentration to the legal standard value for a long time under various conditions of air temperature, humidity and light intensity in order to analyze the effect of the air pollutant on plants.

Fig. 10 shows a sectional view of the cabinet, which is composed of a fresh air processing unit, a chamber to perform the gas exposure experiment on plants, and an exhaust processing unit. The fresh air for ventilation is introduced into the chamber through an air conditioner and both filters of active carbon and manganese in the fresh air processing unit. The frequency of ventilation can be adjusted by D1~4 and A1~2 dampers, being variable from 0 to 280 times/h in order to remove reaction products generated by chemical or photochemical reactions of the air pollutants in the chamber.

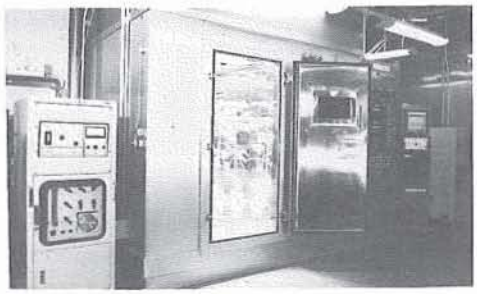
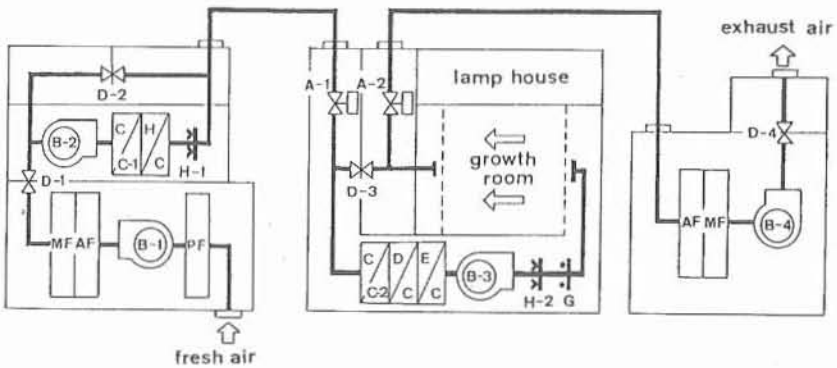


Photo 3 General view of the growth cabinet for air pollutant exposure.

Table 2 Characteristics of the growth cabinet for air pollutant exposure.

		characteristics
temperature	range	15–40°C
	deviation	±0.1°C (max)
	distribution	±0.3°C (max)
humidity	range	50–80%RH
	deviation	±1%RH (max)
	distribution	±2%RH (max)
gas concentration	range	refer to range of gas analyzers. (Table 3)
	deviation	SO_2 ±3.2 ppb (at 0.05 ppm) NO_2 ±0.6 ppb (at 0.05 ppm) O_3 ±0.2 ppb (at 0.01 ppm) CO_2 ± 1.4 ppm (at 450 ppm) HC ±10 ppb (at 0.2 ppm)
wind	mean velocity	0.2 m/s
	distribution	±0.1 m/s (max)
lighting		max 40 klx
ventilation		0–2,800 m^3/h



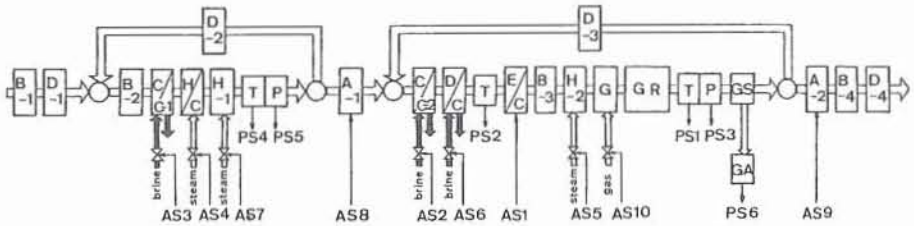
(a) fresh air processing unit. (b) chamber. (c) exhaust processing unit.

A;	automatic damper	E/C;	electric heating coil
AF;	active carbon filter	G;	gas jet unut
B;	blower	H;	steam jet unit
C/C;	cooling coil	H/C;	heating coil
D;	manual damper	MF;	manganese filter
D/C;	dehumidifying coil	PF;	pre-filter

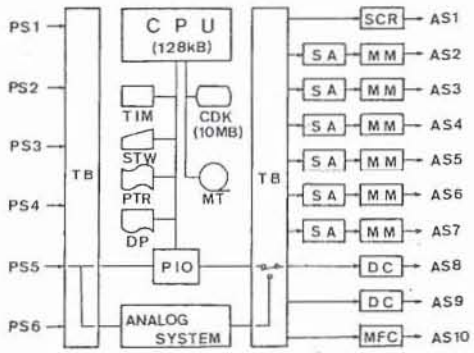
Fig. 10 Sectional view of the growth cabinet for air pollutant exposure.

Fig. 11 shows a system diagram of the control system for the cabinet. This system is controlled by a computer with analog PID controllers as a support system. For the ventilation, the fresh air is introduced through the fresh air processing unit and is mixed with the circulated air in the chamber. The mixed air is cooled and dehumidified by brine coils, and then air temperature and humidity in the growth room is regulated to the desired value by an electric heater and a steam humidifier. The gas supplied from the gas storehouse or the O_3 generator is

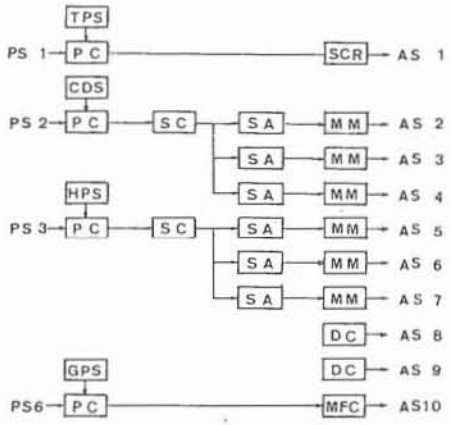
manipulated by the mass flow controller, according to instructions from the computer or the analog controller. It is necessary to carefully select the gas analyzer used for the feedback sensor from the standpoint of reliability, responsibility, and sensitivity. Table 3 shows a list of the performance of the gas analyzer obtained from the operation.The analyzer is always calibrated by the standard gas diluted with standard air using mass flow controllers. Fig. 12 shows an example of the program control of gas concentration under mixed conditions of SO_2, NO_2 and O_3.



(a) diagram of the air flow.



(b) diagram of the computer control system.



(c) diagram of the analog back up system.

A; automatic damper
B; blower
C/C; cooling coil
D; manual damper
D/C; dehumidifying coil
E/C; electric heatiang coil
G; gas jet unit
GA; gas analyzer
GR; growth room
GS; gas sampling unit
H; steam jet unit
H/C; heating coil
P; psychrometer
T; resistance thermometer
CDK; cartridge disk
CPU; central processing unit
DC; damper controller
DP; dot printer
MFC; mass flow controller
MM; modutrol motor
MT; magnetic tape recorder
PIO; process I/O interface
PTR; paper tape reader
SA; servo-actuator
SCR; SCR electric manipulator
STW; system typewriter
CDS; set station for cooling and dehumidifying base
GPS; program set station for gas concentration
HPS; program set station for humidity
PC; PID controller
SC; signal converter
TPS; program set station for temperature

Fig. 11 System diagram of the growth cabinet for air pollutant exposure.

Table 3 Characteristics of gas analyzers.

	NO, NO_2	SO_2	O_3	CO_2	HC
principle	chemilumi-nescence	pulse fluorescence	chemilumi-nescence	NDIR	FID
range (ppm)	0.05–10.0	0.1–10.0	0.01–2.0	500–2,000	1–50
noise (ppb)	2	4	0.5	1 (ppm)	10
zero drift (ppb/day)	0.25	1.0	0.4	1 (ppm/day)	20
span drift (ppb/day)	0.5	1.0	0.5	2	20
response time (min)	2.0	3.5	1.0	0.1	0.1

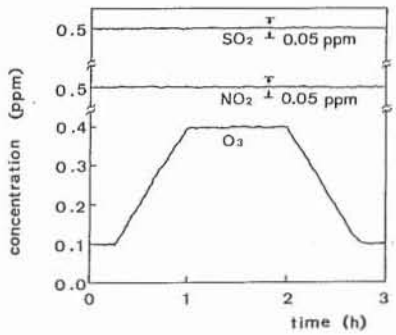
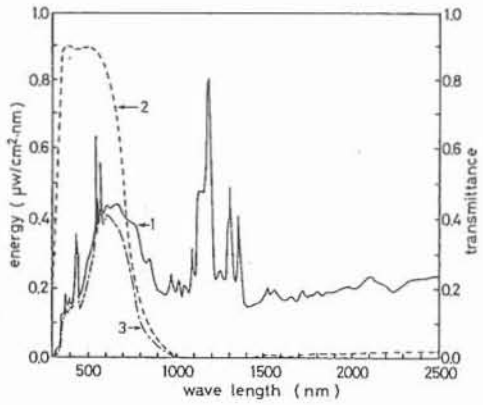


Fig. 12 An example of the program contorl of SO_2, NO_2 and O_3 in the growth cabinet.

The artificial light source of the cabinet is described. Since the sunlight changes at every moment, the artificial light source should be used to obtain the reproducibility of experiments. Among lamps sold at present, the xenon lamp and the special metal halide lamp approximate to sunlight in the spectrum in the range of visible light. Metal halide lamps (400 W × 24 lamps) with the emission spectrum of tin halide are used in the cabinet. Since the infrared emission from the lamp is unnecessary for the growth of plants and may cause abnormal increase in plant temperature, the phosphide glass containing iron oxide is used as the heat-absorbing filter. Fig. 13 shows spectral characteristics of the lighting in the growth cabinet. The infrared emission is mostly removed by the filters. The wall surface of the cabinet is made to increase the light intensity by stainless steel. The air inside the lamp house is maintained ca. 30° C in order to protect the lamp and the heat-absorbing glass filter.

Simulator for analyzing plant-environment system

A general view of the simulator for analyzing the plant-environment system is shown in Photo 4, and a list of basic performance of the simulator in Table 4. This simulator can reproduce the plant community environment as a light-atmosphere-plant community-soil system. Particularly, it is possible to change the spatial distribution of temperature, humidity, and wind velocity in height with three kinds of the profile units. Also, spectrum and intensity in the lighting can be freely changed.

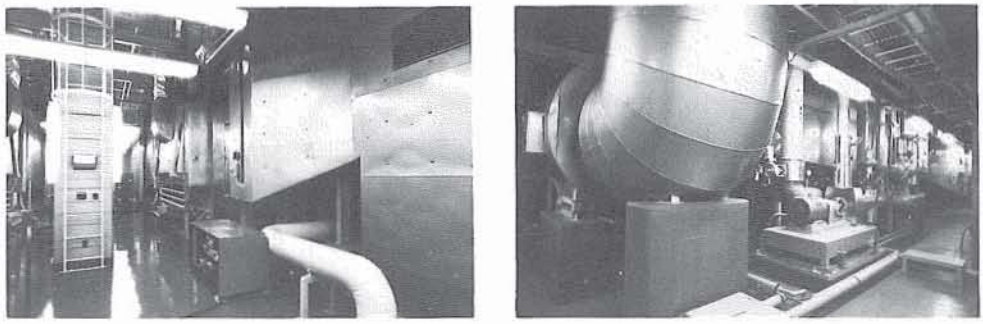


1; Characteristics of the metal halide vapor lamp (Yoko-lamp; Toshiba).
2; Characteristics of the heat-absorbing filter.
3; Spectral characteristics of the lighting in the growth cabinet.

Fig. 13 Spectral characteristics of the lighting in the growth cabinet.

Table 4 Characteristics of the simulator for analyzing the plant-environment system.

		characteristics
atmospheric environment		
temperature	range	10–35°C
	deviation	±0.1°C (max)
	distribution	±0.3°C (max)
	stratification	10 stages, $t_{max} - t_{min} \leqq 10°C$
humidity	range	30–80%RH
	deviation	±0.1°C (max) (dew point)
	distribution	±0.2°C (max) (dew point)
	stratification	10 stages, $h_{max} - h_{min} \leqq 50\%RH$
wind	range	0.1–2.7 m/s
	deviation	±0.1%FS (max) (revolution of blower)
	distribution	±3% (max)
	turbulence intensity	±3% (max)
	stratification	10 stages, $V_{max}/V_{min} \leqq 8$
gas concentration	range	refer to range of gas analyzers (Table 3)
	deviation	SO_2 ±3.2 ppb (at 0.05 ppm)
		NO_2 ±0.6 ppb (at 0.05 ppm)
		O_3 ±0.2 ppb (at 0.01 ppm)
		CO_2 ±1.4 ppm (at 450 ppm)
		HC ±10 ppb (at 0.2 ppm)
solar simulator		
lighting	range	4–60 klx (initial)
	deviation	±0.1%FS (max) (SCR output)
	distribution	±10 klx (at 60 klx)
soil environment		
temperature	range	−5–+35°C (brine temperature)
	deviation	±0.1°C (brine temperature)
ventilation	range	50–250 m^3/h
	deviation	±2 m^3/h



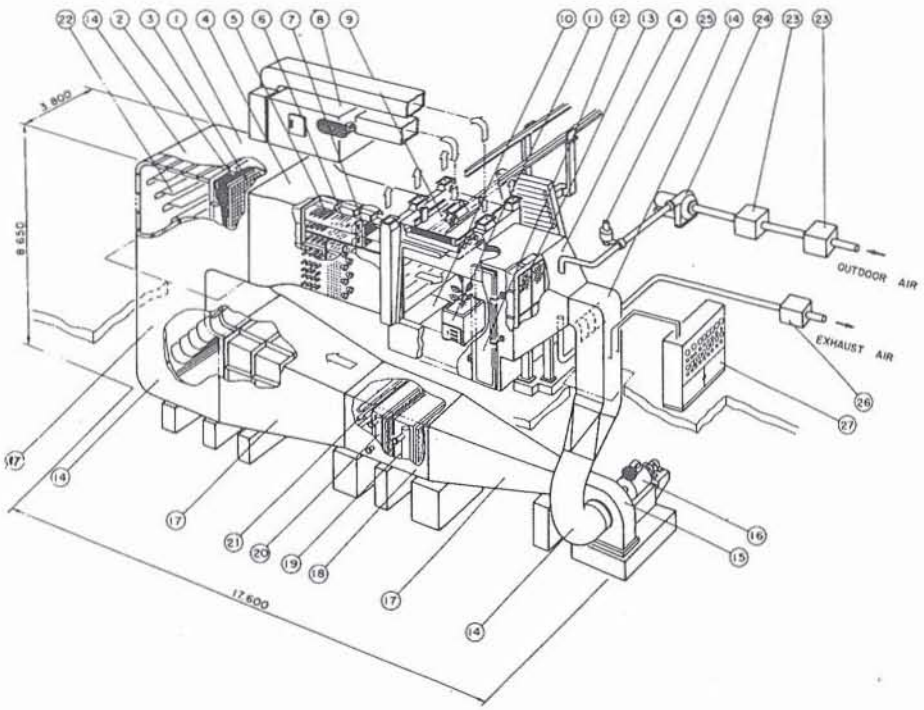
(a) upper tunnel. (b) lower tunnel.

Photo 4 General view of the simulator for analyzing the plant-environment system.

Fig. 14 shows a configuration of the simulator. This simulator is a special wind tunnel of the low wind velocity and circuit type. The main air conditioner is installed in the lower tunnel and the profile units for wind velocity, air temperature and humidity are equipped in the upper tunnel. The growth room is also installed in the upper tunnel. On its upper and side surfaces, the solar simulator, which can automatically regulate light spectrum and intensity, are mounted. The soil environment control unit is installed on the lower surface of the room. Viewing the air stream within the equipment, the air circulated from the growth room comes to the main blower through the contraction cone. The fresh air is mixed with the circulated air in the cone and the air pollutant in the corner. The air sent from the main blower comes to the main air conditioner through the diffuser. After the air temperature and the humidity is decreased basic conditions for forming temperature and humidity profiles by the main air conditioner, the air is introduced into the settling chamber through the diffuser and the corner. By means of the settling chamber with screen and honeycomb and the contraction cone (contraction ratio is 1/2) a rectification flow is given and returned to the growth room through the profile units for air temperature, humidity and wind velocity. In the profile units the humidity profile is adjusted by steam humidifiers, the air temperature profile by electric heaters, and wind velocity profile by plates with slits. Each profile unit is arranged in order of humidity, temperature, and wind velocity for taking off droplets scattered by steam humidification and for maintaining the wind velocity profile.

Fig. 15 shows a system diagram of the control system for the simulator. Photo 5 shows a general view of the control room. This system is composed of a direct digital control (DDC) system and analog PID controllers that backs up the computer. The introduction of DDC system makes it possible to develop algorithms required for the profile control and the control of the solar simulator.

The air temperature and humidity profiles are formed by heating and humidifying using the profile units after decreasing to the air conditions required to make the profile by the main air conditioner. The profile unit is composed of 10 stages, each of which can be independently regulated by signals from the computer to provide any spatial distribution of temperature and humidity in height. The temperature profile unit, in which for each stage 12 electric heaters are arranged in the direction of perpendicular to the air flow, is considered to have uniform heat exchange in the stage. The humidity profile unit have 28 steam jet nozzles for each stage; these are attached to the four stage header, because it possible to continuously change the steam jet

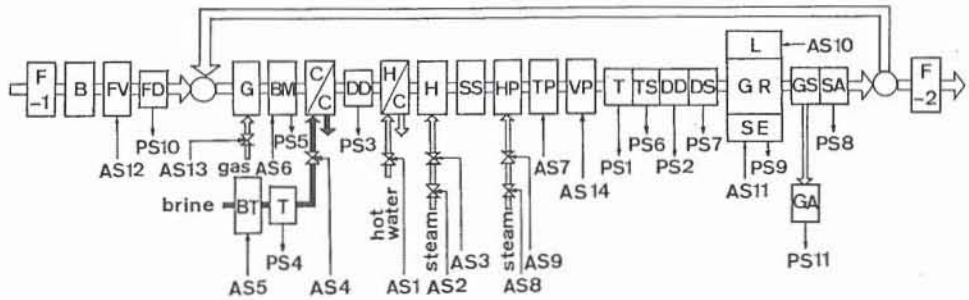


1; settling chamber
2; screen
3; honeycomb
4; contraction cone
5; humidity profile unit
6; temperature profile unit
7; velocity profile unit
8; lamp house air conditioner
9; solar simulator
10; growth room
11; soil environment contorl unit
12; door
13; dust filter
14; corner
15; main blower
16; electric motor
17; diffuser
18; main air conditioner
19; cooling coil
20; heating coil
21; humidifier
22; corner vane
23; fresh air filter
24; ventilation blower
25; ventilation control valve
26; exhaust air filter
27; gases supply system

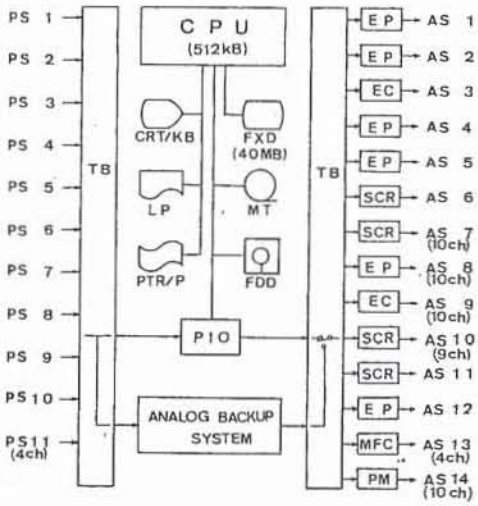
Fig. 14 Configuration of the simulator for analyzing the plant-environment system.

rate in the range of 0.2 to 12 kg/h. Fig. 16 shows an exmaple of temperature and humidity profiles formed by the profile units.

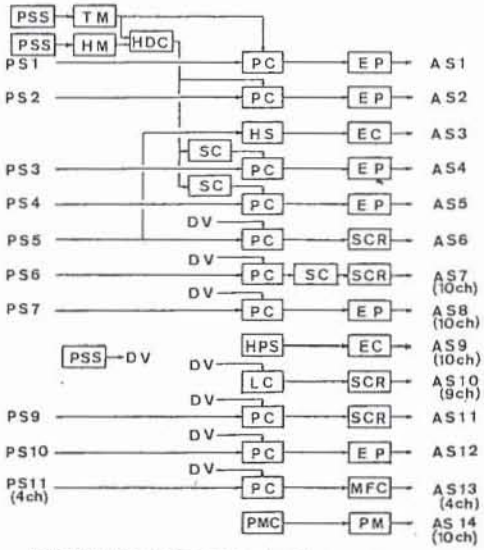
The wind velocity profile is produced by the retardation using the profile unit after preconditioning by the main blower. The velocity profile unit consists of 10 stages, each of which is made by three plates with the slit of average 50% opening. Two plates are slid to change the opening ratio within the range of 0 to 50% according to the signals from the computer. Fig. 17 shows an example of wind velocity profile.



(a) diagram of the air flow.



(b) diagram of the computer control system.



(c) diagram of the analog back up system.

B; ventilation blower
BM; main blower
BT; brine temperature controller
C/C; cooling coil
DD; dew point detector
DS; dew point detector of the humidity profile system
F; filter
FD; flow rate detector of the ventilation air
FV; ventilation rate control valve
G; gas jet unit
GA; gas analyzer
GR; growth room
GS; gas sampling unit
H; steam jet unit
H/C; heating coil
HP; humidity profile unit
L; fluorescent lamp
SA; spectrum analyzer
SE; soil environment control unit
SS; settling chamber
T; resistance thermometer
TP; temperature profile unit
TS; resistance thermometer of the temperature profile system
VP; wind velocity profile unit
CPU; central processing unit
CRT/KB; system console
EC; relay
EC; electropneumatic converter
FDD; flexible disk
FXD; fixed disk
EP; line printer
MFC; mass flow controller
MT; magnetic tape recorder
PIO; process I/O interface
PM; pulse motor
PTR/P; paper tape reader and puncher
SCR; SCR electric manipulator
HDC; signal converter from relative humidity to dew point
HM; set station of humidity
HPS; selector of the steam jet unit for the humidity profile
HS; selector of the steam jet unit
LC; solar simulator controller
PC; PID controller
PMC; pulse motor controller
PSS; program set station
SC; signal converter
TM; set station of temperature

Fig. 15 System diagram of environment control in the simulator.

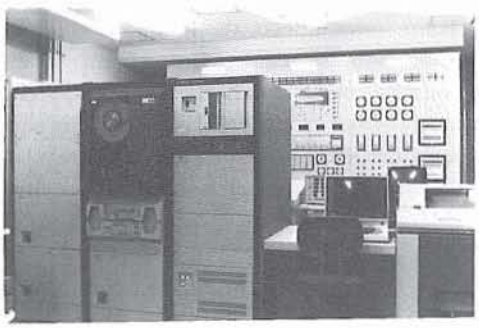
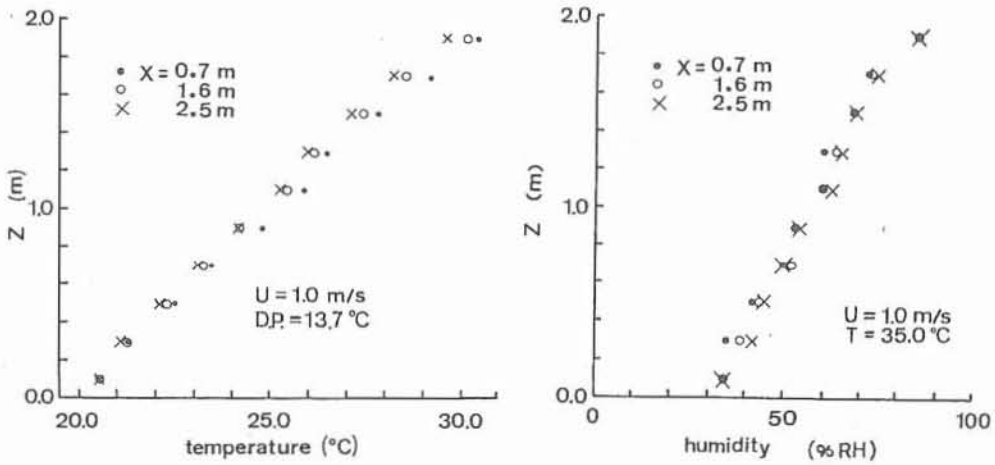


Photo 5 Control room of the simulator.



(a) temperature profile.

(b) humidity profile.

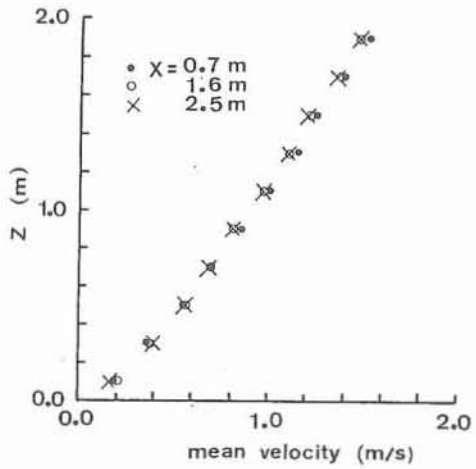
D.P.; dew point
T; air temperature
U; mean velocity
X; distance from the blowout screen of the profile unit
Z; height in the growth room

Fig. 16 An example of created profiles.

The light spectrum and the intensity in the growth room are regulated by the solar simulator, which consists of various fluorescent lamps (110 W × 224 lamps) with the SCR for power contorl. Photo 6 shows a general view of the inside of the growth room. The control system of fluorescent lamps can divide into 6 series for the upper surface and 3 series for the side in the growth room. Fluorescent lamps coated with various luminous paints are made to order. Fig. 18 shows spectral characteristics of the standard fluorescent lamps. They can be selected according to the object of experiments. The light spectrum is performed by combining these

color fluorescent lamps and by regulating their light intensity as shown in Fig. 19. Since the temperature inside the lamp house affects the luminous efficiency of the lamps, an air conditioner is installed to automatically regulate temperature of the lamp bulb wall.

Finally, the soil environment control unit is described. Six portable units (0.6 W × 0.9 D × 1.2 H m^3) with carriers are provided in the growth room. This equipment has a brine tank installed around the soil container. The soil temperature can be regulated by the control of brine temperature. Also, an automatic sprinkling unit is furnished to adjust the water content in the soil.



X; distance from the blowout screen of the profile unit
Z; height in the growth room

Fig. 17 An example of wind velocity profile.

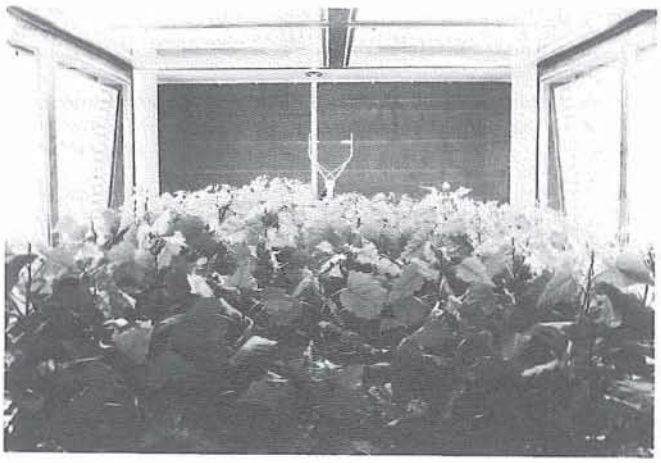


Photo 6 Growth room in the simulator.

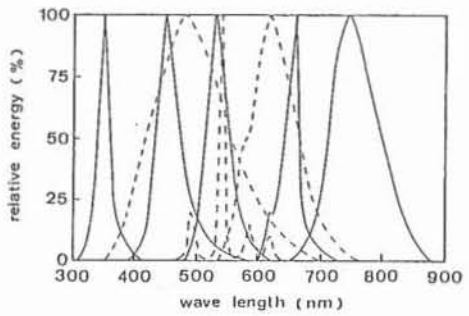


Fig. 18 Spectral characteristics of the standard fluorescent lamps.

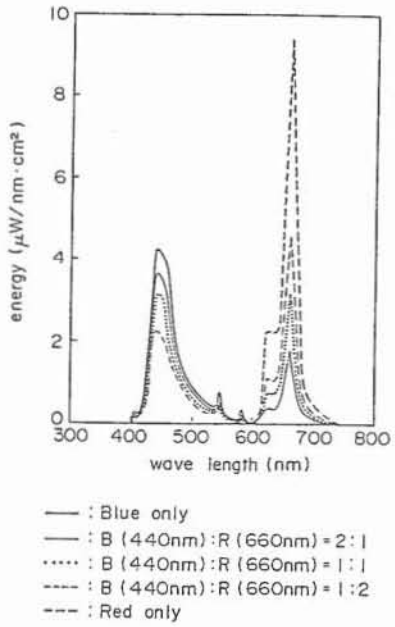


Fig. 19 Spectrum of combination lighting with red and blue fluorescent lamps.

An exmaple of the simulator experiement

Fig. 20 shows an experimental evidence on the function of plants for the purification of polluted atmosphere. Introducing 200 poplar young trees in the growth room of the simulator, we examined the absorption of O_3 and NO_2 by the plant community. Air flow from point A to point B was set at a vertical profile as shown in Fig. 20. Air temperature and humidity were maintained 25° C and 60% R H. The vertical distributions in the gas concentration at each gas sampling point were compared. A remarkable difference of the gas concentration between A and B under lighting conditions (40 klx) was noticed. The decrease of the gas concentration is resulted from the gas absorption by the plant community.

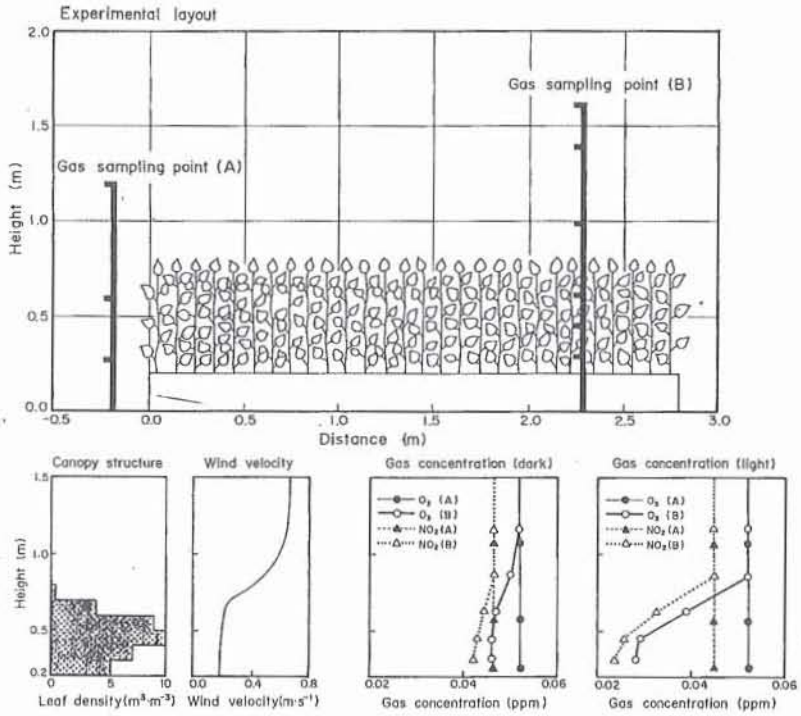


Fig. 20 An example of the experiement in the simulator.

和文抄録

国立公害研究所ファイトトロン

相賀一郎[1]・大政謙次[1]・松本茂[1]

大気汚染ガスの植物影響についての一連の研究のなかで，汚染ガス暴露実験は国立公害研究所ファイトトロンの施設で行われた。それらの実験結果の信頼性は，施設の装置特性に依存する部分が大きい。この章では，実験植物の栽培に用いられた温湿度環境を制御した実験温室及び種々の汚染ガス暴露実験が行われた環境制御キャビネットの概要，それらの装置特性及び環境制御技術と管理上の問題点について解説した。また，光，大気及び土壌についての植物群落環境を人為的に再現し，環境悪化による植物影響や植物の物質輸送現象を植物-環境系として総合的に解析し，植物による環境改善機能に関する研究を行うために新たに開発した自然環境シミュレーターの装置特性とその装置によるポプラ幼樹集団の O_3 及び NO_2 の吸収実験について述べた。

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国立公害研究所特別研究成果報告

第 1 号　陸水域の富栄養化に関する総合研究 ── 霞ヶ浦を対象域として ── 昭和51年度．（1977）

第 2 号　陸上植物による大気汚染環境の評価と改善に関する基礎的研究 ── 昭和51，52年度　研究報告．（1978）

（改　称）

国立公害研究所研究報告

第 3 号　A comparative study of adults and immature stages of nine Japanese species of the genus *Chironomus* (Diptera, Chironomidae). (1978)
（日本産ユスリカ科 *Chironomus*　属9種の成虫，サナギ，幼虫の形態の比較）

第 4 号　スモッグチャンバーによる炭化水素ー窒素酸化物系光化学反応の研究 ── 昭和52年度　中間報告．（1978）

第 5 号　芳香族炭化水素ー窒素酸化物系の光酸化反応機構と光酸化二次生成物の培養細胞に及ぼす影響に関する研究 ── 昭和51，52年度　研究報告．（1978）

第 6 号　陸水域の富栄養化に関する総合研究（Ⅱ）── 霞ヶ浦を中心として．── 昭和53年度．（1979）

第 7 号　A morphological study of adults and immature stages of 20 Japanese species of the family Chironomidae (Diptera). (1979)
（日本産ユスリカ科20種の成虫，サナギ，幼虫の形態学的研究）

第 8 号　大気汚染物質の単一および複合汚染の生体に対する影響に関する実験的研究 ── 昭和52，53年度　研究報告．（1979）

第 9 号　スモッグチャンバーによる炭化水素－窒素酸化物系光化学反応の研究 ── 昭和53年度　中間報告．（1979）

第 10 号　陸上植物による大気汚染環境の評価と改善に関する基礎的研究 ── 昭和51～53年度　特別研究報告．（1979）

第 11 号　Studies on the effects of air pollutants on plants and mechanisms of phytotoxicity. (1980)
（大気汚染物質の植物影響およびその植物毒性の機構に関する研究）

第 12 号　Multielement analysis studies by flame and inductively coupled plasma spectroscopy utilizing computer-controlled instrumentation. (1980)
（コンピュータ制御装置を利用したフレームおよび誘導結合プラズマ分光法による多元素同時分析）

第 13 号　Studies on chironomid midges of the Tama River. (1980)
Part 1. The distribution of chironomid species in a tributary in relation to the degree of pollution with sewage water.
Part 2. Description of 20 species of Chironominae recovered from a tributary.
（多摩川に発生するユスリカの研究
── 第1報　その一支流に見出されたユスリカ各種の分布と下水による汚染度との関係 ──
── 第2報　その一支流に見出された Chironominae 亜科の20種について ──）

第 14 号　有機廃棄物，合成有機化合物，重金属等の土壌生態系に及ぼす影響と浄化に関する研究 ── 昭和53，54年度　特別研究報告．（1980）

第 15 号　大気汚染物質の単一および複合汚染の生体に対する影響に関する実験的研究 ── 昭和54年度　特別研究報告．（1980）

第 16 号　計測車レーザーレーダーによる大気汚染遠隔計測．（1980）

第 17 号　流体の運動および輸送過程に及ぼす浮力効果 ── 臨海地域の気象特性と大気拡散現象の研究 ── 昭和53，54年度　特別研究報告．（1980）

第 18 号　Preparation, analysis and certification of PEPPERBUSH standard reference material. (1980)
（環境標準試料「リョウブ」の調製，分析および保証値）

第 19 号　陸水域の富栄養化に関する総合研究（Ⅲ）── 霞ヶ浦（西浦）の湖流 ── 昭和53，54年度．（1981）

第 20 号　陸水域の富栄養化に関する総合研究（Ⅳ）── 霞ヶ浦流域の地形，気象水文特性およびその湖水環境に及ぼす影響 ── 昭和53，54年度．（1981）

第 21 号　陸水域の富栄養化に関する総合研究（Ⅴ）── 霞ヶ浦流入河川の流出負荷量変化とその評価 ── 昭和53，54年度．（1981）

第 22 号　陸水域の富栄養化に関する総合研究（Ⅵ）── 霞ヶ浦の生態系の構造と生物現存量 ── 昭和53，54年度．（1981）

第 23 号　陸水域の富栄養化に関する総合研究（Ⅶ）── 湖沼の富栄養化状態指標に関する基礎的研究 ── 昭和53，54年度．（1981）

第 24 号　陸水域の富栄養化に関する総合研究（Ⅷ）── 富栄養化が湖利用に及ぼす影響の定量化に関する研究 ── 昭和53，54年度．（1981）

第 25 号　陸水域の富栄養化に関する総合研究（Ⅸ）──［*Microcystis*］（藍藻類）の増殖特性 ── 昭和53，54年度．（1981）

第 26 号　陸水域の富栄養化に関する総合研究（Ⅹ）── 藻類培養試験法によるＡＧＰの測定 ── 昭和53，54年度．（1981）

第 27 号　陸水域の富栄養化に関する総合研究（Ⅺ）── 研究総括 ── 昭和53，54年度．（1981）

第 28 号　複合大気汚染の植物影響に関する研究 ── 昭和54，55年度　特別研究報告．（1981）

第 29 号　Studies on chironomid midges of the Tama River. (1981)
Part 3. Species of the subfamily Orthocladiinae recorded at the summer survey and their distribution in relation to the pollution with sewage waters.
Part 4. Chironomidae recorded at a winter survey.
（多摩川に発生するユスリカ類の研究
── 第３報　夏期の調査で見出されたエリユスリカ亜科 Orthocladiinae 各種の記載と，その分布の下水汚染度との関係について ──
── 第４報　南浅川の冬期の調査で見出された各種の分布と記載 ──）

第 30 号　海域における富栄養化と赤潮の発生機構に関する基礎的研究 ── 昭和54，55年度　特別研究報告．（1982）

第 31 号　大気汚染物質の単一および複合汚染の生体に対する影響に関する実験的研究 ── 昭和55年度　特別研究報告．（1981）

第 32 号　スモッグチャンバーによる炭化水素－窒素酸化物系光化学反応の研究 ── 環境大気中における光化学二次汚染物質生成機構の研究（フィールド研究１）── 昭和54年度　特別研究報告．（1982）

第 33 号　臨海地域の気象特性と大気拡散現象の研究 ── 大気運動と大気拡散過程のシミュレーション ── 昭和55年度　特別研究報告．（1982）

第 34 号　環境汚染の遠隔計測・評価手法の開発に関する研究 ── 昭和55年度　特別研究報告．（1982）

第 35 号　環境面よりみた地域交通体系の評価に関する総合解析研究．（1982）

第 36 号　環境試料による汚染の長期モニタリング手法に関する研究 ── 昭和55，56年度　特別研究報告．（1982）

第 37 号　環境施策のシステム分析支援技術の開発に関する研究．（1982）

第 38 号　Preparation, analysis and certification of POND SEDIMENT certified reference material. (1982)
（環境標準試料「池底質」の調製，分析及び保証値）

第 39 号　環境汚染の遠隔計測・評価手法の開発に関する研究 ── 昭和56年度　特別研究報告．（1982）

第 40 号　大気汚染物質の単一及び複合汚染の生体に対する影響に関する実験的研究 ── 昭和56年度　特別研究報告．（1983）

第 41 号　土壌環境の遠隔計測と評価に関する統計学的研究．（1983）

第 42 号　底泥の物性及び流送特性に関する実験的研究．（1983）

第 43 号　Studies on chironomid midges of the Tama River. (1983)

Part 5. An observation on the distribution of Chironominae along the main stream in June with description of 15 new species.

Part 6. Description of species of the subfamily Orthocladiinae recovered from the main stream in the June survey.

Part 7. Additional species collected in winter from the main stream.

（多摩川に発生するユスリカ類の研究

── 第５報　本流に発生するユスリカ類の分布に関する６月の調査成績とユスリカ亜科に属する15新種等の記録 ──

── 第６報　多摩本流より６月に採集されたエリユスリカ亜科の各種について ──

── 第７報　多摩本流より３月に採集されたユスリカ科の各種について ──）

第 44 号　スモッグチャンバーによる炭化水素－窒素酸化物系光化学反応の研究．── 環境大気中における光化学二次汚染物質生成機構の研究（フィールド研究２）── 昭和54年度　特別研究中報告．（1983）

第 45 号　有機廃棄物，合成有機化合物，重金属等の土壌生態系に及ぼす影響と浄化に関する研究 ── 昭和53年～55年度　特別研究報告．（1983）

第 46 号　有機廃棄物，合成有機化合物，重金属等の土壌生態系に及ぼす影響と浄化に関する研究 ── 昭和54，55年度　特別研究報告　第１分冊．（1983）

第 47 号　有機廃棄物，合成有機化合物，重金属等の土壌生態系に及ぼす影響と浄化に関する研究 ── 昭和54，55年度　特別研究報告　第２分冊．（1983）

第 48 号　水質観測点の適正配置に関するシステム解析．（1983）

第 49 号　環境汚染の遠隔計測・評価手法の開発に関する研究 ── 昭和57年度　特別研究報告．（1984）

第 50 号　陸水域の富栄養化防止に関する総合研究（Ⅰ）── 霞ヶ浦の流入負荷量の算定と評価 ── 昭和55～57年度　特別研究報告．（1984）

第 51 号　陸水域の富栄養化防止に関する総合研究（Ⅱ）── 霞ヶ浦の湖内物質循環とそれを支配する因子 ── 昭和55～57年度　特別研究報告．（1984）

第 52 号　陸水域の富栄養化防止に関する総合研究（Ⅲ）── 霞ヶ浦高浜入における隔離水界を利用した富栄養化防止手法の研究 ── 昭和55～57年度　特別研究報告．（1984）

第 53 号　陸水域の富栄養化防止に関する総合研究（Ⅳ）── 霞ヶ浦の魚類及び甲殻類現存量の季節変化と富栄養化 ── 昭和55～57年度　特別研究報告．（1984）

第 54 号　陸水域の富栄養化防止に関する総合研究（Ⅴ）── 霞ヶ浦の富栄養化現象のモデル化 ── 昭和55～57年度　特別研究報告．（1984）

第 55 号　陸水域の富栄養化防止に関する総合研究（Ⅵ）── 富栄養化防止対策 ── 昭和55～57年度　特別研究報告．（1984）

第 56 号　陸水域の富栄養化防止に関する総合研究（Ⅶ）── 湯の湖における富栄養化とその防止対策 ── 昭和55～57年度　特別研究報告．（1984）

第 57 号　陸水域の富栄養化防止に関する総合研究（Ⅷ）── 総括報告 ── 昭和55～57年度　特別研究報告．（1984）

第 58 号　環境試料による汚染の長期的モニタリング手法に関する研究 ── 昭和55～57年度　特別研究総合報告．（1984）

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* in japanese

RESEARCH REPORT FROM
THE NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES

No. 66
国立公害研究所研究報告　第66号
(R—66—'84)

昭和59年3月31日発行

編集　国立公害研究所編集委員会
発行　環境庁国立公害研究所
茨城県筑波郡谷田部町小野川16番2

印刷　株式会社イセブ
茨城県新治郡桜村天久保2—11—20

Published by the National Institute for Environmental Studies
Yatabe-machi, Tsukuba, Ibaraki 305, Japan.