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A Quantitative Analysis of the Relationships between SO_2 or NO_2 Sorption and Their Acute Effects on Plant Leaves Using Image Instrumentation

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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.

INTRODUCTION

Sulfur dioxide and nitrogen dioxide are major air pollutants that cause various effects on plants.[1–3] 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.[1,2,4,5] However, visible injury and stomatal response, which are conspicuous symptoms of the acute effects, vary strikingly at different sites on the leaf.[6–9] 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.[8–10] 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.[9] 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.[9] 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 thick 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 $2.37 \pm 0.05 \times 10^{-3}$ $cal \cdot cm^{-2} \cdot s^{-1}$, $2.23 \pm 0.01 \times 10^{-2}$ $cal \cdot cm^{-2} \cdot s^{-1}$, ca. 25 klx, and 1.5 ± 0.1 $s \cdot cm^{-1}$ except at the leaf edges.

Instrumentation system.[9] 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_{ws_x} $(s \cdot cm^{-1})$, is expressed by

$$r_{ws_x} = \frac{2\{X_{s_x}(T_{l_x}) - \Phi X_s(T_a)\}}{W_x} - \left(\frac{\kappa}{D_w}\right)^{2/3} r_{ka_x} \qquad (1)$$

and

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

where T_l is leaf temperature (°C), T_a is air temperature (°C), $X_s(T)$ is saturated water vapor density at T °C $(g \cdot cm^{-3})$, Φ is relative humidity, W is transpiration rate $(g \cdot cm^{-2} \cdot s^{-1})$, r_{ka} is boundary layer resistance to heat transfer $(s \cdot cm^{-1})$, κ is thermal diffusivity of air $(= 0.222\ cm^2 \cdot s^{-1})$, D_w is air-water vapor diffusivity $(= 0.249\ cm^2 \cdot s^{-1})$, E_s is shortwave radiation from the environment $(cal \cdot cm^{-2} \cdot s^{-1})$, E_w is longwave radiation from the environment $(cal \cdot cm^{-2} \cdot s^{-1})$, 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 ($=1.354\times10^{-12}$ $cal\cdot cm^{-2}\cdot s^{-1}\cdot K^{-4}$), ρc_p is volumetric heat capacity of air ($=0.285\times10^{-3}$ $cal\cdot cm^{-3}\cdot{}^\circ C^{-1}$) and L is latent heat by evaporation ($=583.3$ $cal\cdot g^{-1}$). 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_{int_x} ($g\cdot cm^{-2}$), is expressed by

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

$$Q_x=\frac{2P_a}{r_{ga_x}+r_{gs_x}} \tag{4}$$

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

and

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

where Q is SO_2 or NO_2 sorption rate ($g\cdot cm^{-2}\cdot s^{-1}$), T is exposure time (s), t is time (s), P_a is SO_2 or NO_2 concentration of air ($g\cdot cm^{-3}$), r_{ga} is boundary layer resistance to SO_2 or NO_2 diffusion ($s\cdot cm^{-1}$), r_{gs} is stomatal resistance to SO_2 or NO_2 diffusion ($s\cdot cm^{-1}$) and D_g is air–SO_2 or –NO_2 diffusivity ($=0.129$ $cm^2\cdot s^{-1}$ (SO_2), $=0.155$ $cm^2\cdot s^{-1}$ (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 0.3 $s\cdot cm^{-1}$, respectively, until beginning of fading of plant pigments. The resolution of this system was $256^H\times240^V$ 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.

$$\alpha_p E_{s_x}+\epsilon E_{w_x}-2\epsilon\sigma(273.15+T_{l_x})^4+\frac{2\rho c_p(T_a-T_{l_x})}{r_{ka_x}}-LW_x=0 \tag{7}$$

where E_{s_x}, E_{w_x}, W_x, T_{l_x} and r_{ka_x} 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} $cal\cdot cm^{-2}\cdot s^{-1}$ and 0.1 $s\cdot cm^{-1}$, 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 thick 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 1.4 $s \cdot cm^{-1}$ before the start of SO_2 exposure, began to increase within 5 minutes after the start and reached 0.7 to 3.2 $s \cdot cm^{-1}$ 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.[9,10] 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} $g \cdot cm^{-2}$ after 8 minutes of SO_2 exposure, 0.31 to 0.50×10^{-5} $g \cdot cm^{-2}$ after 30 minutes exposure and 0.62 to 1.05×10^{-5} $g \cdot cm^{-2}$ 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} $g \cdot cm^{-2}$. 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 1.4 $s \cdot cm^{-1}$ before the start of NO_2 exposure, began to increase within a few minutes after the start and reached 4.8 to 17 $s \cdot cm^{-1}$ 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} $g \cdot cm^{-2}$ at 8 minutes exposure, 0.55 to 1.0×10^{-5} $g \cdot cm^{-2}$ after 30 minutes exposure and 0.83 to 1.85×10^{-5} $g \cdot cm^{-2}$ after 60 minutes. Visible injuries progressed successively with water-soaking, wilting, fading of pigments and necrosis

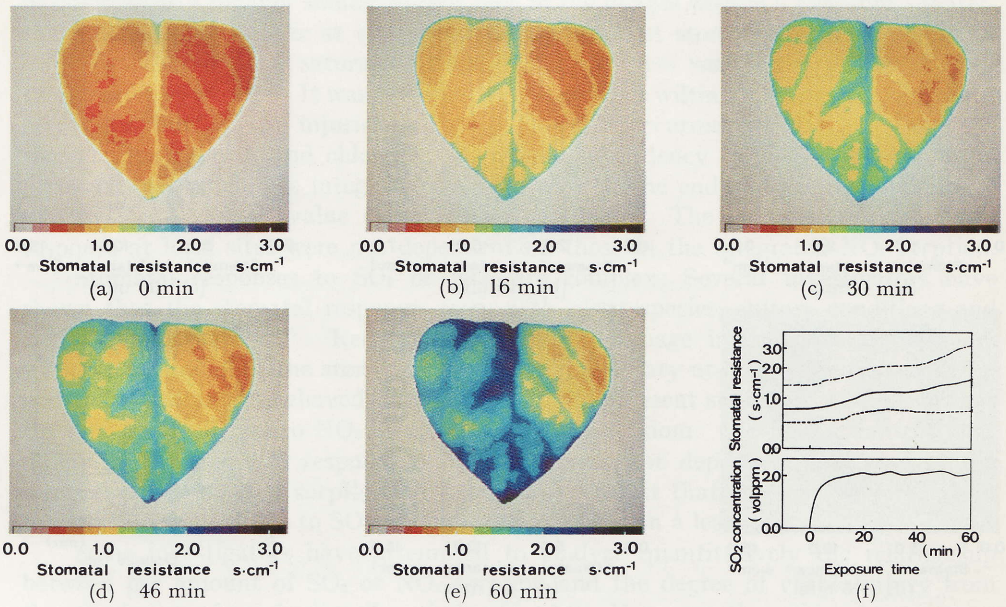

Fig. 1 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 2 volppm SO_2. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (—–—), minimum (------) and mean (——) stomatal 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, 25 klx; boundary layer resistance to heat transfer, 1.5 s·cm^{-1}.

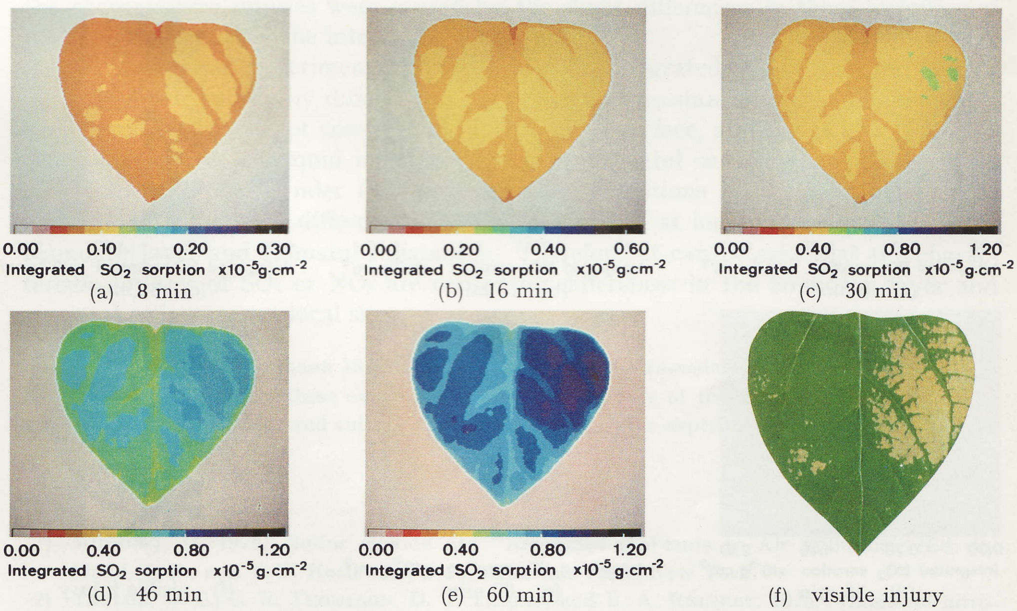

Fig. 2 Changes in distribution of integrated SO_2 sorption during the exposure in Fig. 1 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated SO_2 sorption at given periods of exposure. (f) shows the distribution pattern of the visible injury.

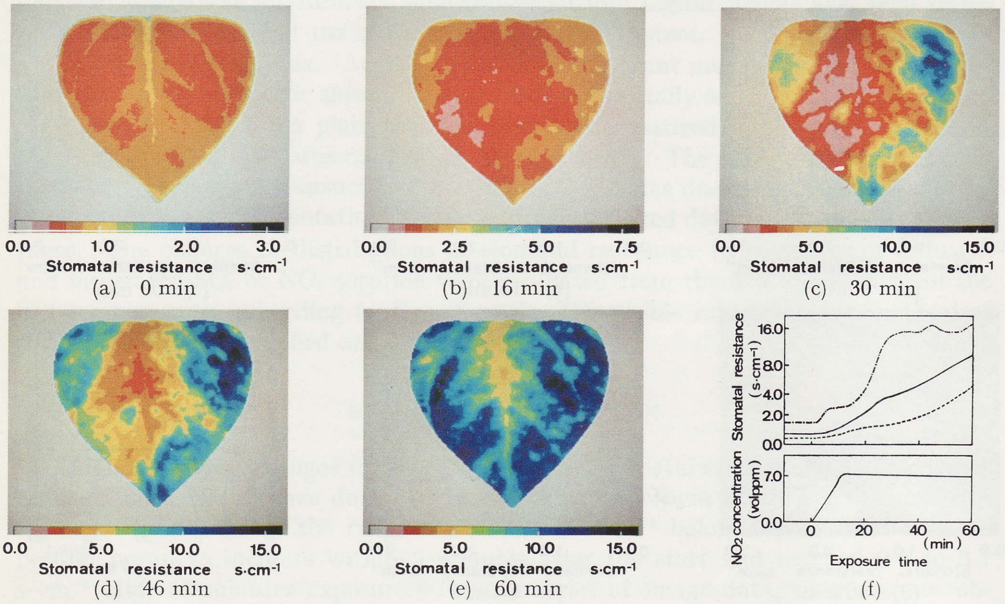

Fig. 3 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 7 volppm NO_2. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (———), minimum (-----) and mean (——) stomatal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

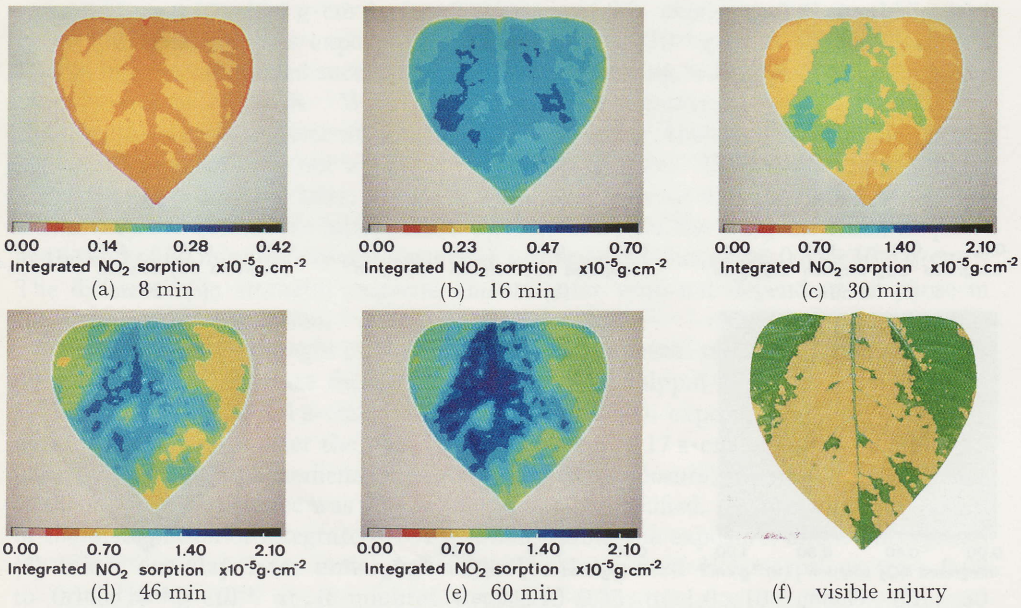

Fig. 4 Changes in distribution of integrated NO_2 sorption during the exposure in Fig. 3 and visible injury photographed one day later. (a) to (e) show distribution patterns of the integrated NO_2 sorption at given periods of exposure. (f) shows distribution pattern of the visible injury.

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. $1.2\times10^{-5}\,g\cdot cm^{-2}$. 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.[11-16] 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.[8,9] 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.[5,17] 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.[15,18,19] 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.

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<和文抄録>

画像計測による植物葉の SO_2 あるいは NO_2 収着と急性影響との関係の定量的解析

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筆者らは，既に，熱赤外画像計測システムを用いて，気孔開度の指標である水蒸気拡散に対する気孔抵抗や SO_2 あるいは NO_2 収着量の葉面分布を推定する手法について報告している．本論文では，この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，積算ガス収着量が閾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領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．