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A Quantitative Analysis of the Relationships between O_3 Sorption and Its Acute Effects on Plant Leaves Using Image Instrumentation

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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.

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.[1,2] Entry of O_3 into a leaf through the stomata causes various effects on leaves.[3-6] 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.[7-8] 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,[9,10] 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.

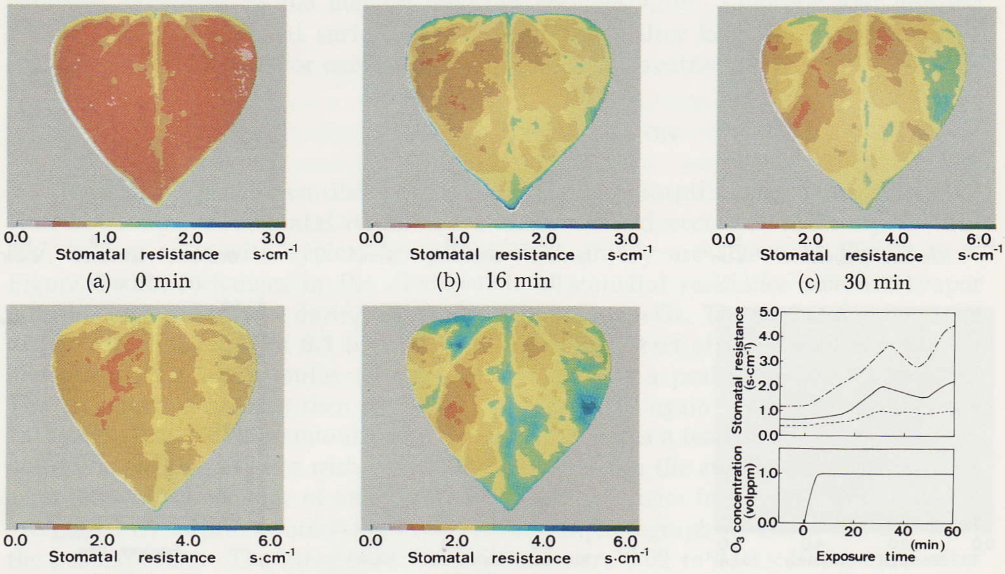
MATERIALS AND METHODS

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.[9,10] 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 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 $2.37\pm0.05\times10^{-3}$ $cal\cdot cm^{-2}\cdot s^{-1}$, $2.23\pm0.01\times10^{-2}$ $cal\cdot cm^{-2}\cdot s^{-1}$, ca. 25 klx, and 1.5 ± 0.1 $s\cdot cm^{-1}$, except at the leaf edges.

Instrumentation system.[9,10] 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 PD1401X 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 $256^H\times240^V$ pixels, 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 the beginning of fading of vegetal pigments. Radiation and boundary layer resistance to heat transfer were measured within precisions of 1×10^{-4} $cal\cdot cm^{-2}\cdot s^{-1}$ and 0.1 $s\cdot cm^{-1}$, 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.

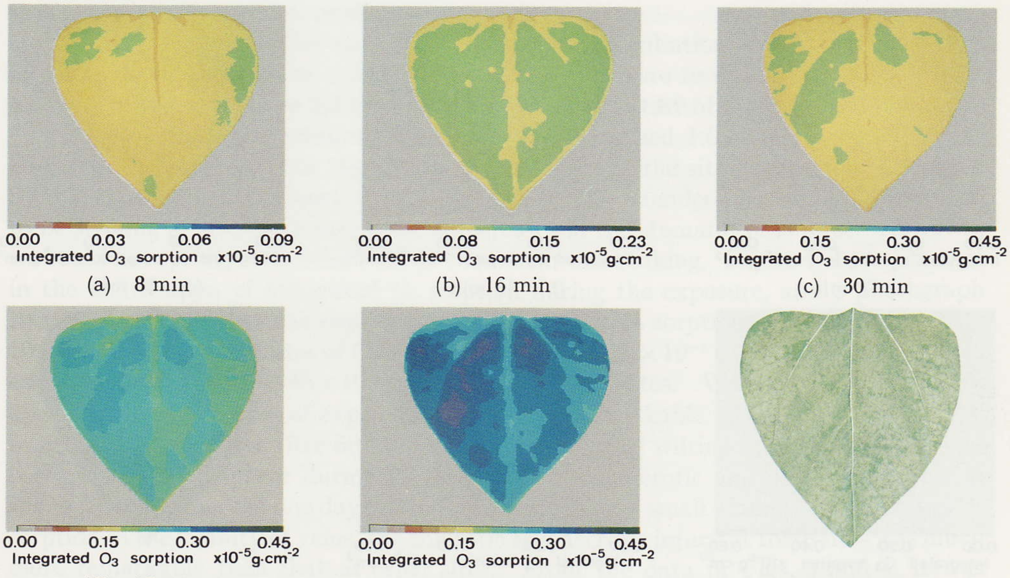
Experimental methods. An intact leaf of a test plant grown in a phytotron was attached to a thick plastic sheet and was kept horizontally on the fixing apparatus in the chamber. After the plant was sufficiently acclimatized to the given conditions, O_3 exposure to plant was carried out for 60 minutes. 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 tem-



(a) 0 min (b) 16 min (c) 30 min

(d) 46 min (e) 60 min (f)

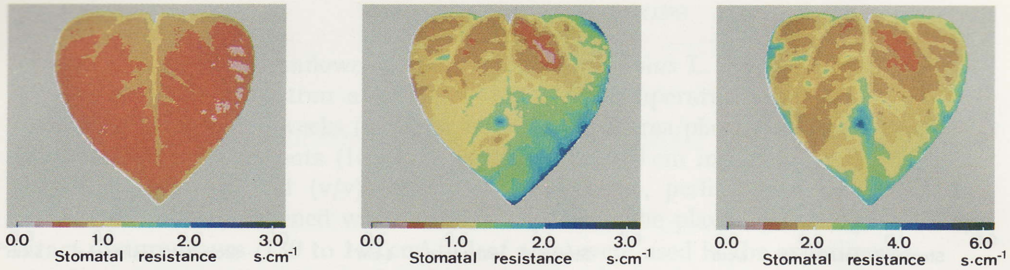
Fig. 1 Changes in distribution of stomatal resistance to water vapor diffusion on a leaf during exposure to ca. 1 volppm O_3. (a) to (e) show distribution patterns of the stomatal resistance at given periods of exposure. (f) shows changes with time of maximum (— — —), minimum (-----) and mean (——) stomatal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



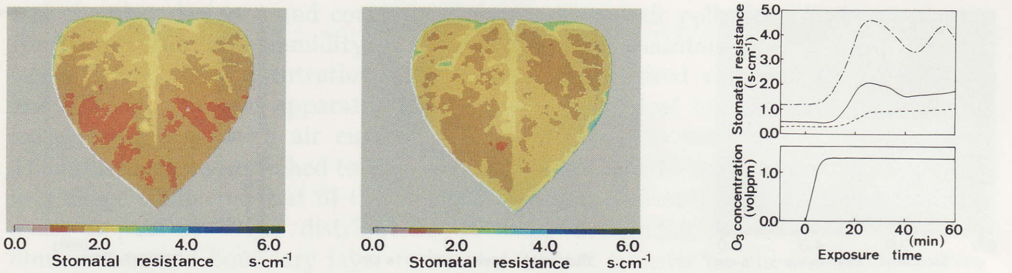
(a) 8 min (b) 16 min (c) 30 min

(d) 46 min (e) 60 min (f) visible injury

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.

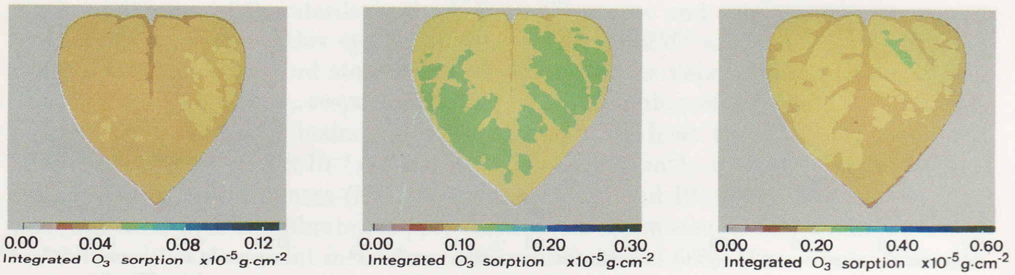


(a) 0 min (b) 16 min (c) 30 min

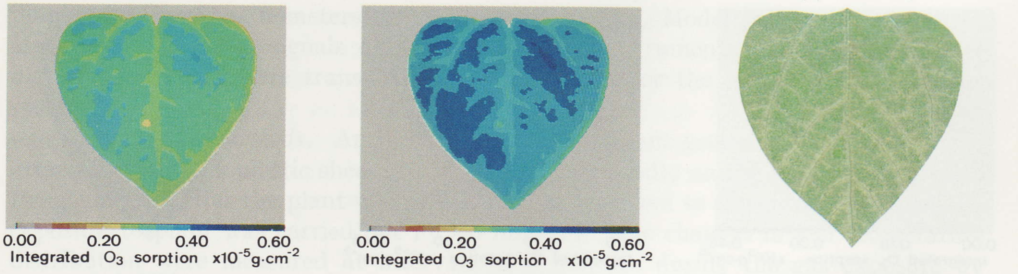


(d) 46 min (e) 60 min (f)

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.



(a) 8 min (b) 16 min (c) 30 min



(d) 46 min (e) 60 min (f) visible injury

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.

perature, according to the method reported previously.[9,10] 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 1.2 $s \cdot cm^{-1}$ 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 4.5 $s \cdot cm^{-1}$ 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 0.04×10^{-5} $g \cdot cm^{-2}$ after 8 minutes of O_3 exposure, 0.12 to 0.20×10^{-5} $g \cdot cm^{-2}$ after 30 minutes exposure and 0.25 to 0.39×10^{-5} $g \cdot cm^{-2}$ 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 1.2 $s \cdot cm^{-1}$ before the start of O_3 exposure and began to increase after a few minutes of exposure, and reached 1.0 to 3.8 $s \cdot cm^{-1}$ 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 0.05×10^{-5} $g \cdot cm^{-2}$ after 8 minutes of O_3 exposure, 0.14 to 0.25×10^{-5} $g \cdot cm^{-2}$ after 30 minutes exposure and 0.30 to 0.45×10^{-5} $g \cdot cm^{-2}$ 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 data 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.[11,12] Several investigators have evaluated a stomatal response to O_3 by using the stomatal resistance determined with a resistance porometer,[3,4,13] 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.[14] Another available method for evaluating stomatal response to O_3 is a stomatal impression method.[11,15] 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.[10]

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.[15-17] 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.[18-20] However, since O_3 sorption depends on not only stomatal conditions, but also leaf boundary conditions at local sites,[12,21] 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.[10] They also suggest 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.

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<和文抄録>

画像計測による植物葉の O_3 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，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，葉の局所部位により不規則であり，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，他の生理的な要因の違いにも依存することを示唆している．