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Responses of Foliar Gas Exchanges of Poplar Clones in Relation to Resistance to Ozone

By

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Summary : Cuttings of four poplar clones (Kamabuchi, *Populus nigra* × *P. maximowiczii*; NR 84, *P. eucalyptus* NR 84; NR 6, *P. wettstein* NR 6; and OP 29, *P. charkowiensis* × *P. trichocarpa*) grown with three different nutrient conditions were exposed to ozone at concentrations of 0.25 and 0.5 ppm in a controlled environment chamber, and the relationships between differences in O_3 resistance and responses of gas exchange to O_3 were studied. Estimating O_3 resistance on the basis of visible foliar injury, NR 6 and OP 29 were more resistant than NR 84 and Kamabuchi, and also the cuttings with better nutrient condition in each clone were more sensitive. The responses of gas exchange to O_3 were tested for NR 6 and Kamabuchi. After exposure of the plants to O_3, the rate of photosynthesis and O_3 uptake decreased progressively in Kamabuchi leaves but NR 6 leaves did not show significant changes. The decrease in photosynthesis of Kamabuchi leaves did not depend on stomatal closure. The total amount of O_3 uptake was much larger in Kamabuchi than in NR 6 also O_3 uptake was greater in better nutrient conditions. The relationship between O_3 uptake and gas diffusion resistance of leaves was analyzed. The analysis may indicate that the mesophyll resistance depends partially on nutrient conditions. The results also suggest that resistant NR 6 clone could avoid O_3 stress by maintenance of higher stomatal resistance compared with sensitive Kamabuchi clone.

Introduction

Ozone is a main component of photochemical oxidants and a toxic gas to plants. The leaf is a very sensitive organ in plant regarding responses to O_3 as well as other toxic gases like SO_2 and NO_2. The responses include visible foliar injury such as necrosis or leaf abscission and invisible foliar injury involving physiological or biological lesions. In general, the visible injury has been used as a basis for determining the resistance of plant species to toxic gases. The intra- and inter-specific differences in resistance to O_3 have been well documented[5)16)20)]. In our preliminary experiment, it was observed that the resistance in poplar cuttings to O_3 differed among clones. On the other hand, it is known that the O_3 resistance also varied with the environmental factors such as soil moisture, soil nutrient, light and temperature[11)16)20)]. Prior to the occurrence of visible injury caused by O_3, the physiological changes in photosynthesis[1)3)8)9)15)], transpiration[9)18)] or stomatal aperture[2)3)7)9)13)15)] were observed, and some of the reports mention the relation between the physiological changes, particularly stomatal behavior and the resistance to O_3[2)9)15)]. However, the understanding of the mechanism of resistance to O_3 is still fragmentary.

The present paper reports the physiological foliar responses of some poplar clones grown under different nutrient conditions in relation to O_3 resistance.

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Materials and methods

(1) Plant materials

Cuttings of four poplar clones (Kamabuchi, *Populus nigra* × *P. maximowiczii;* NR 84, *P. eucalyptus* NR 84; NR 6, *P. wettstein* NR 6; and OP 29, *P. charkowiensis* × *P. trichocarpa*) were used in O_3 exposure experiment. The scions were collected at the clone banks of Kameyama Breeding Station, Oji Institute for Forest Tree Improvement, in Kameyama City and of Forest Experiment Station of Tokyo Univ. in Tanashi City in the fall, 1980, and stored in a refrigerator. The following April, they were planted into pots filled with vermiculite. One gram of powder chemical fertilizer (UDS, N-P_2O_5-K_2O 5-5-5)per pot was added on the surface of medium.

Table 1. Standard composition of nutrient solution.

Salts used	Contents (g/l)	Concentration of elements (ppm)	
$(NH_4)_2SO_4$	0.0943	N	40
$NaNO_3$	0.0911	P_2O_5	25
$Ca(NO_3)_2$	0.0293	K_2O	30
KH_2PO_4	0.0472	CaO	20
KCl	0.0261	MgO	10
$CaCl_2$	0.0193	Fe_2O_3	2
$MgSO_4 \cdot 7H_2O$	0.0615		
$FeC_6H_5O_7 \cdot 3H_2O$	0.0066		

(pH of solution=6.2)

The cuttings were grown in a green house for 2 months before O_3 exposure experiment. For 2 weeks prior to exposure, the cuttings of each clone were cultured under three different conditions of fertilization by adding 200 ml/pot/day of : (1) standard Tsutsumi's nutrient solution (Table 1), (2) one-half of the standard, and (3) no nutrients added.

(2) Ozone exposure system

The cuttings of poplar clones were set in a controlled environment chamber and exposed to O_3 under the condition of 27℃, 75% RH and 50 Klux. The lighting source consisted of mercury lamps, metal halide lamps and fluorescent lamps. The concentration of ozone in the chamber was monitored continuously by an O_3-analyzer (Monitor Labs, Model 8401) with a chemiluminescent detector, and controlled by the combination of the gas analyzer and the massflow meter regulating O_3 flow rate supplied to the chamber. The source of ozone was generated by the electrical discharge of oxygen.

(3) Determination of visible foliar injury

Visible foliar injury(necrosis) was observed after the seedlings were exposed to O_3. With these seedlings, the degree of injury was determined by the following calculation.

$$\text{Degree of injury} = \frac{1\times N_1+2\times N_2+3\times N_3+4\times N_4+5\times N_5}{5\times N}$$

(per whole plant)

N : Number of leaves($=N_0+N_1+N_2+N_3+N_4+N_5$)

0 : No injury

1 : Injury$\leqq$10% of leaf area

2 : 11%−25%

3 : 26%−50%

4 : 51%−75%

5 : 76%−100%

The degree of injury in each clone was expressed as the average value of the six to eight cuttings per clone used in an exposure experiment.

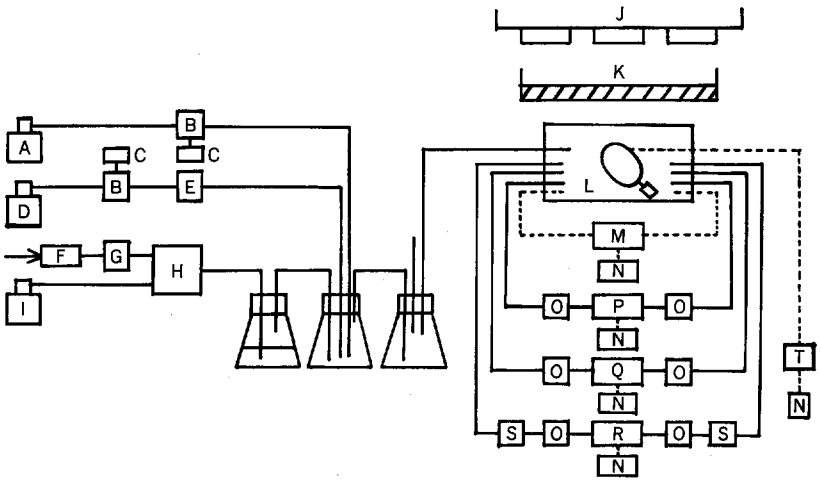

Fig. 1. Schematic diagram of the apparatus for measuring photosynthesis, transpiration and ozone uptake rate of a single leaf.

A : SO_2, B : Massflow, C : Gas control unit, D : O_2, E : Ozone generator, F : Soda lime, G : Pump, H : Mixing chamber, I : CO_2, J : Lamp, K : Water filter, L : Assimilation chamber, M : Hygrometer, N : Recorder, O : Flow meter, P : O_3 analyzer, Q : SO_2 analyzer, R : CO_2 analyzer, S : Silica gel, T : Thermocouple.

(4) Measurement of gas exchange (O_3 uptake, photosynthesis and transpiration)

The gas exchange was measured for detached mature leaves which are sensitive to O_3. The instrument for this experiment was set to measure simultaneously the rates of O_3 uptake, photosynthesis and transpiration (Fig. 1). The petiole of a single leaf was inserted into a small vessel filled with air-free water to avoid air blocks and the leaf was set in an assimilation chamber made of glass cylinder with 30 cm of length and 6 cm of diameter. Under lighting and air flow with or without O_3, the differences in concentrations of O_3, CO_2 and water vapor between the inlet and outlet of the chamber were measured for O_3 uptake, photosynthesis and transpiration, respectively. Previously, the rate of O_3 absorption and decomposition caused by the contact with walls of chamber was measured by the same system without plant materials and subtracted as a background value. The measurements were run continuously by O_3 analyzer (Monitor Labs, Model 8401), infra-red gas analyzer (Fuji Electric, Model ZSB-Z) and hygrometer (ACE, Model AR-YBL).

Results and discussion

Exposure to 0.25 and 0.5 ppm O_3 for 5 and 10 hours caused visible foliar injury. Typical symptoms were recognized as flecks with black or brown color on the adaxial surface of leaf. The flecks occurred on various parts of a leaf, i. e., along the vein or at the top and base of the leaf. As the degree of visible injury progressed, the symptom advanced from flecks to necrotic stains. The injury clearly varied with leaf age. The necrosis was observed with mature leaves much more severely than with expanding immature leaves as many investigators had reported[6)12)14)]. The degree of injury differed among clones and among

Table 2. Foliar injuries caused by 0.25 ppm and 0.5 ppm ozone.

Clones	Nutrient conditions	O_3 concentration exposed (ppm)			
		0.25		0.50	
		Exposure time (hours)			
		5	10	5	10
Kamabuchi	Standard	0.04	0.13	0.21	0.29
	One-half of standard	0.05	0.11	0.16	0.20
	No addition of minerals	0	0.06	0.04	0.07
NR 84	Standard	0.01	0.05	0.06	0.07
	One-half of standard	0.01	0.04	0.02	0.02
	No addition of minerals	0	0	0.01	0.02
NR 6	Standard	0	0	0	0.04
	One-half of standard	0	0	0	0.02
	No addition of minerals	0	0	0	0
OP 29	Standard	0	0	0.01	0.02
	One-half of standard	0	0	0.02	0.06
	No addition of minerals	0	0	0	0

nutrient conditions (Table 2). Kamabuchi and NR 84 were injured more severely than NR 6 and OP 29, and in all of those clones, the cuttings grown with the standard nutrient solution were injured most severely and the cuttings grown with tap water alone showed slight or no injury. Judging from the degree of visible foliar injury, Kamabuchi and NR 84 were sensitive to O_3, while NR 6 and OP 29 were resistant to O_3. The order of susceptibility among the clones was always same in different nutrient conditions, as the most susceptible clone in the standard nutrient condition showed the highest susceptibility in other nutrient conditions. In order to estimate the differences in O_3 resistance among poplar clones as associated with physiological foliar responses to O_3, gas exchanges in detached mature leaves of Kamabuchi (sensitive clone) and NR 6 (resistant clone) were measured. Figure 2 illustrates the typical examples of the time course in rate of photosynthesis, transpiration and O_3 uptake before, during and after exposure to O_3. In Kamabuchi, the apparent photosynthetic rate decreased markedly soon after start of O_3 exposure in any nutrient conditions, and the photosynthetic rate did not recover within 2 hours after the removal of O_3 (Fig. 2-a, b, c). Changes in O_3 uptake rate were similar to those of photosynthetic rate. However, the transpiration rate decreased gradually, and also generally the decrease in transpiration rate was detectable after the decreases in photosynthetic rate and O_3 uptake rate were apparent. All of the decreases occurred without visible injury, as observed in white pine[1]. In NR 6, on the other hand, the changes in these rates were not significant in most measured samples, except for a few examples in which the rate of photosynthesis and transpiration decresed during O_3 exposure but recovered soon after the removal of O_3, as shown in Fig. 2-d to 2-f. Rates of both O_3 uptake and photosynthesis at the starting time of O_3 exposure were much higher in Kamabuchi than those in NR 6, for the same nutrient condition (Fig. 2). The fact reflects that total amounts of O_3 uptake in Kamabuchi leaves were about 2 times of those in NR 6 leaves, although the O_3 uptake rate decreased soon after starting O_3 exposure (Table 3). Also, among different nutrient conditions, the rates of both O_3 uptake and photosynthesis and the total amount of O_3 uptake were higher

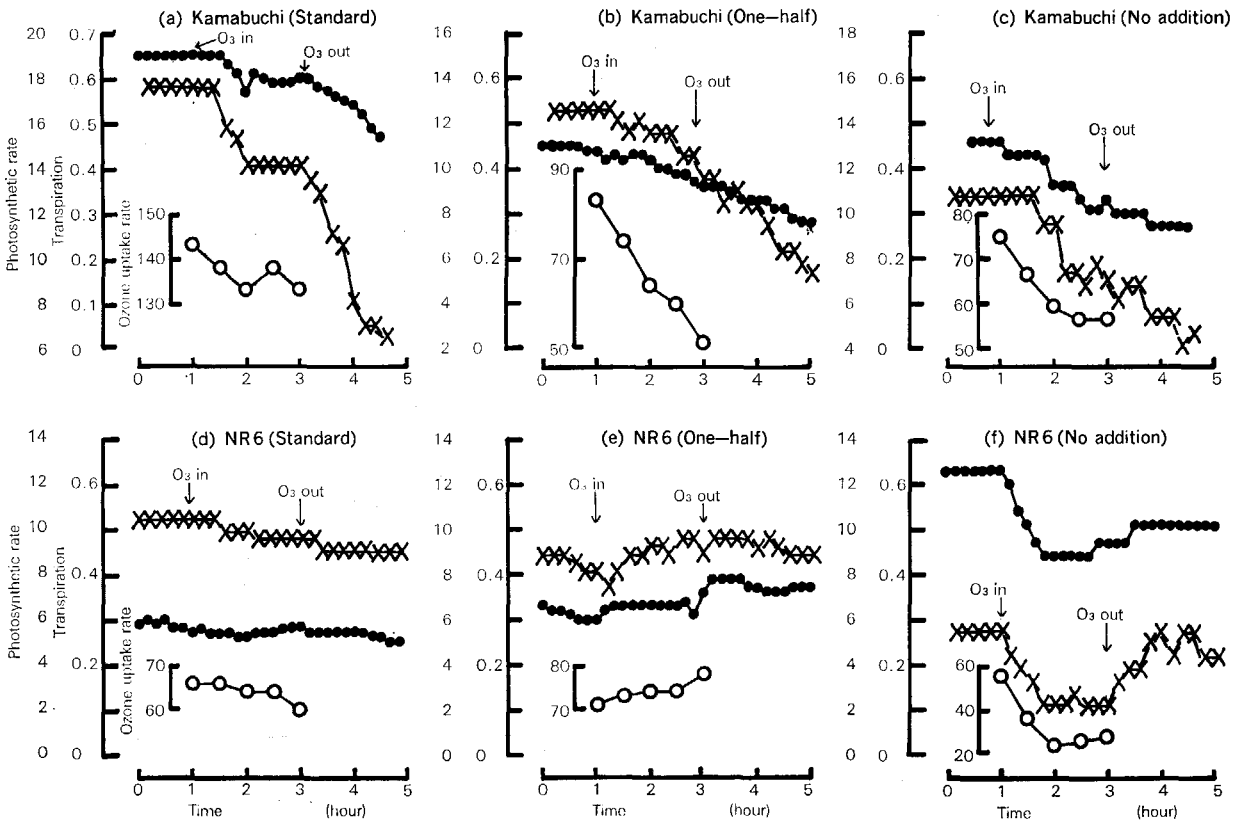

Fig. 2. Time course in rate of photosynthesis, transpiration and ozone uptake.

× : Photosynthetic rate (mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$) ● : Transpiration (g $H_2O \cdot dm^{-2} \cdot hr^{-1}$) ○ : Ozone uptake rate (μg $O_3 \cdot dm^{-2} \cdot hr^{-1}$)

Table 3. The value of $r_a + r_s$ and r_m, ozone uptake rate and amount of ozone absorbed.

Clones	Nutrient condition	$r_a + r_s$ (sec·cm^{-1})	r_m (sec·cm^{-1})	O_3 uptake rate (μg·dm^{-2}·hr^{-1})	O_3 uptake amount (μg·dm^{-2})
Kamabuchi	Standard	4.4～ 7.5	8.0～12.1	133～147	282
	One-half of standard	5.5～ 8.6	12.6～18.3	51～122	182
	No addition of minerals	6.4～ 9.6	14.8～34.5	51～100	123
NR 6	Standard	7.0～14.9	9.0～10.5	60～ 84	160
	One-half of standard	11.0～16.6	11.0～16.0	41～ 77	96
	No addition of minerals	7.2～11.6	19.0～110.0	24～ 78	68

(r_a : boundary layer resistance
r_s : stomatal resistance
r_m : mesophyll resistance)

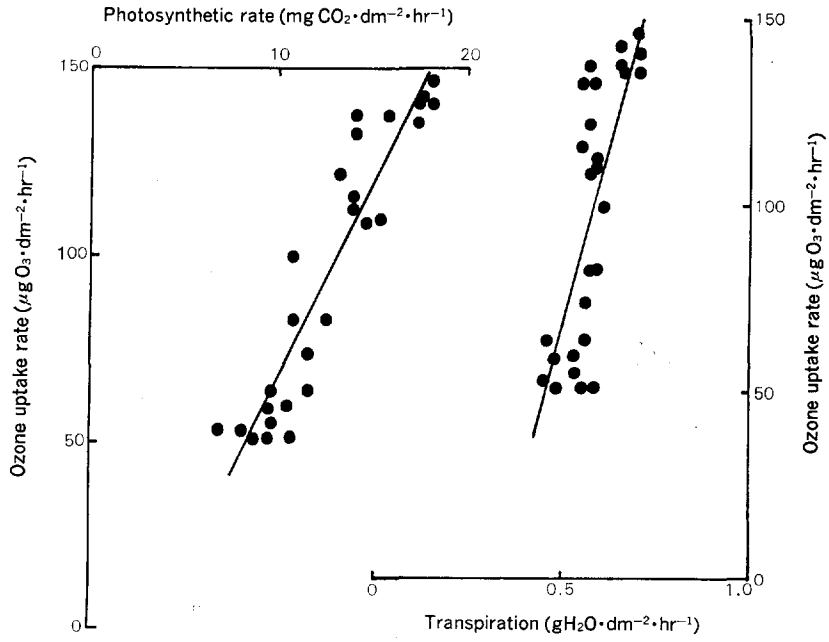

Fig. 3. Relationship between ozone uptake rate and the rate of photosynthesis or transpiration in Kamabuchi.

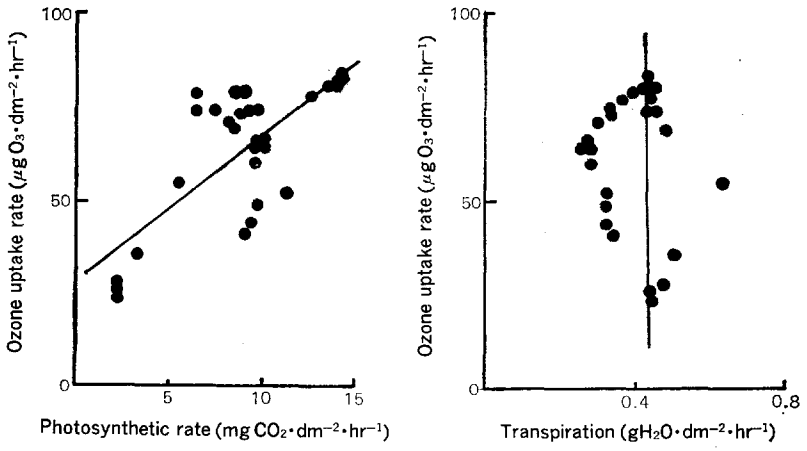

Fig. 4. Relationship between ozone uptake rate and the rate of photosynthesis or transpiration in NR 6.

in cuttings with better nutrient conditions. Figure 3 and 4 show the relationship between O_3 uptake rate and the rate of photosynthesis or transpiration, using all the data of time course shown in Fig. 2. The correlation between O_3 uptake and photosynthesis was higher than that between O_3 uptake and transpiration. The results indicate that the change in O_3 uptake rate in both clones of Kamabuchi and NR 6 may not depend on stomatal closure, though ozone is taken up mostly through stomata under light irradiation.

The reports by Hill *et al.*[18] and Furukawa *et al.*[9] showed that the decrease in photosynthetic rate induced by O_3 exposure may result from stomatal closure. On the other hand, Coyne *et al.*[3][4] observed that the loss in photosynthetic capacity exceeded the decrease in stomatal conductance, suggesting that injury to mesophyll cells or carboxylation of components of CO_2 diffusion pathway was greater than injury to stomata. The result in this study also suggests that the remarkable decrease in photosynthetic rate in Kamabuchi clone would not result from the stomatal factor (Fig. 2), and that the decrease of O_3 uptake rate also would occur in relation with the inhibition of photosynthetic system in a mesophyll cell. In general, there is no doubt that the resistance of a leaf to toxic gases depends; firstly on the gas diffusion resistance from ambient air to mesophyll cells through stomata, controlling the uptake of gas into leaves, and secondly on the tolerance of cells to the uptaken gas. In this study, it is not able to demonstrate directly whether the difference of intra-specific resistance to O_3 depended on the difference of O_3 diffusion resistance or the tolerance of cells to O_3. However, as it is found that O_3 uptake rate had high correlation with photosynthetic rate (Fig. 3, 4), the difference in O_3 resistance will be estimated from the relationship between O_3 uptake and CO_2 diffusion resistance. The diffusion resistance of CO_2 in the pathway to chloroplast of mesophyll cells through stomata is calculated from the diffusion equation[10] shown below, using the data of photosynthesis and transpiration.

$$r'_a + r'_s = \frac{W_l - W_o}{T}$$

$$r_a + r_s + r_m = \frac{C_o - C_l}{P}$$

where r'_a and r'_s are the boundary layer resistance and stomatal resistance to water vapor,

and r_a, r_s and r_m are the boundary layer resistance, stomatal resistance and mesophyll resistance to CO_2. W_l and W_o are the water vapor concentration in the leaf and air, respectively. T is the transpiration rate. C_o and C_l are the CO_2 concentration in the air and chloroplast, respectively. And P is the photosynthetic rate. Also, the relationship between $r'_a+r'_s$ and r_a+r_s is represented by the following formula,

$$r_a+r_s=\left(\frac{D_{H_2O}}{D_{CO_2}}\right)\cdot(r'_a+r'_s)$$

where D_{H_2O} and D_{CO_2} are the diffusion coefficient of water vapor and CO_2 in the air, respectively.

The calculated results are shown together with the values of O_3 uptake in Table 3. As the diffusion resistance at different times during O_3 exposure was calculated, the ranges of values in Table 3 indicate the maximum and minimum of diffusion resistance. The boundary layer resistance (r_a) was very small and was considered to be nearly constant under this experimental condition. Therefore, the stomatal resistance (r_s) is higher in NR 6 than in Kamabuchi, and nearly in the same range for every nutrient condition in each clone. In contrast, the mesophyll resistance (r_m) is lower with better nutrient conditions with which more ozone was taken up. Accordingly, it is indicated that the differences of O_3 uptake between both clones and among nutrient conditions depend on the stomatal resistance and the mesophyll resistance, respectively. Such a difference in mesophyll resistance would be related to physiological difference induced by different nutrient conditions, for example, as shown in the NOLAND's report[18] in which the loss of resistance to O_3 resulted from stomatal opening induced by osmotic action of potassium ion in guard cells induced by enriched potassium fertilizer. However, the experimental conditions used here were not enough to discuss the relationship between the resistance to O_3 and the action of nutrients. TAYLOR[19] and KIMMERER *et al.*[17] suggested that stomatal closure or continuous maintenance of lower stomatal conductance during the exposure to toxic gas is characteristic of avoidance to air pollution stress. The results of FURUKAWA *et al.*[9] with attached leaves of poplar cuttings show that ozone exposure caused stomatal closure for resistant clones, differing from clones used here. In this study, however, stomatal closure during O_3 exposure was mostly not observed in the resistant poplar clone used here, supporting the result[15] with bean cultivars that stomatal closure was not a primary factor to O_3 resistance. Therefore, it is concluded that the resistant NR 6 clone could avoid O_3 stress by the maintenance of lower stomatal conductance (higher stomatal resistance) compared with the sensitive Kamabuchi clone, and that for the difference due to nutrient conditions, mesophyll resistance would be a main factor inducing the avoidance of O_3 stress. However the above different results for stomatal behavior of different resistant poplar clones will have to be studied in the two following areas; whether it resulted from clonal difference or from the difference between detached and attached leaves.

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オゾンに対する抵抗性差異に関連したポプラクローンのガス交換反応

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摘　要

オゾンは近年発生している光化学オキシダントの主要な成分であり，樹木に可視的あるいは不可視的な被害をおこす。これらの被害は，植物の種類や生長状態によって違うことが知られており，また不可視害でも生長低下につながることもある。本報では，栄養条件を変えて育てた4種のポプラクローン（カマブチ，NR 84，NR 6，OP 29）に，0.25 ppm と 0.5 ppm のオゾン接触を環境制御室で行い，葉面可視被害の発現による抵抗性と，可視被害に先立ってみられる葉のガス交換反応への影響を調べた。

オゾン処理により葉に可視被害が生じ，その程度はクローン間および栄養条件の違いによって異なった。被害程度から抵抗性を評価すると，NR 84 とカマブチに比べて NR 6 と OP 29 は抵抗性が高く，また，どのクローンにおいても，栄養条件の良い区の個体ほど感受性が高かった。

ガス交換に対するオゾンの影響は，クローンによって違い，特に光合成速度とオゾン取り込み速度に違いがみられた。すなわち，オゾン接触が進むにつれて，カマブチの光合成速度とオゾン取り込み速度は著しく減少したが，NR 6 では特に変化はみられなかった。光合成速度とオゾン取り込み速度の減少は，蒸散速度の変化と対応しないことから，気孔閉鎖に依存するものではないと考えられた。またオゾンの取り込み総量は，クローン間では抵抗性の NR 6 に比べて感受性のカマブチの方が大きく，同一クローン内では栄養条件の良い区ほど大きかった。以上の結果およびガス拡散抵抗の解析結果から，抵抗性クローンである NR 6 は，カマブチに比べて，高い気孔抵抗を維持することによってオゾンストレスを回避しえたこと，また栄養条件による抵抗性の違いには，ガス拡散に対する葉肉抵抗の違いが主要因として関与していたことが示唆された。

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