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Effects of ozone fumigation on photosynthesis and membrane permeability in leaves of spring barley, meadow fescue, and winter rape

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Abstract

Seedlings of spring barley, meadow fescue, and winter rape were fumigated with 180 μg kg^{-1} of ozone for 12 d, and effect of O_3 on photosynthesis and cell membrane permeability of fumigated plants was determined. Electrolyte leakage and chlorophyll fluorescence were measured after 6, 9, and 12 d of fumigation, while net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured 9 d after the start of ozone exposure. O_3 treatment did not change membrane permeability in fescue and barley leaves, while in rape a significant decrease in ion leakage was noted within the whole experiment. O_3 did not change the photochemical efficiency of photosystem 2 (PS2), *i.e.*, F_v/F_m, and the initial fluorescence (F_0). The values of half-rise time ($t_{1/2}$) from F_0 to maximal fluorescence (F_m) decreased in fescue and barley after 6 and 9 d of fumigation. P_N decreased significantly in ozonated plants, in the three species. The greatest decrease in P_N was observed in ozonated barley plants (17 % of the control). The ozone-induced decrease in P_N was due to the closure of stomata. Rape was more resistant to ozone than fescue or barley. Apparently, the rape plants show a large adaptation to ozone and prevent loss of membrane integrity leading to ion leakage.

Additional key words: *Brassica napus* var. *oleifera*; chlorophyll fluorescence; electrolyte leakage; *Festuca pratensis*; *Hordeum vulgare*; net photosynthetic rate; ozone; stomatal conductance.

Introduction

Ozone alters basic metabolic processes in plants, including P_N (Soja *et al.* 1998, Soldatini *et al.* 1998a, Guidi *et al.* 1999), ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Reid *et al.* 1998), and accelerates leaf senescence (Koch *et al.* 1998, Reichenauer *et al.* 1998). Ozone acts as strong oxidant destroying the structure and function of biological membranes, leading to electrolyte leakage (Soldatini *et al.* 1998, Guidi *et al.* 1999). O_3 may also alter the properties of thylakoids, thereby changing the yield of chlorophyll (Chl) *a* fluorescence. Measurement of Chl fluorescence after dark adaptation provides information on photoinhibition of PS2. Guidi *et al.* (1999) observed an increase in ion leakage that occurred simultaneously with a decrease in F_v/F_m ratio in ozone-treated *Vicia faba* plants. Reichenauer *et al.* (1998) found a decrease in F_v/F_m ratio in ozone-fumigated wheat leaves, while Soldatini *et al.* (1998a) noted damage to PS2 reaction centres in poplar under ozone fumigation. A depression in P_N is often the first detectable symptom observed in ozone-treated plants (Reichenauer *et al.* 1998).

The aim of the present work was to determine whether elevated ozone concentration causes changes in P_N and membrane permeability in leaves of spring barley, meadow fescue, and winter rape. The ozone concentration used in this experiment (180 μg kg^{-1}) caused leaf yellowing and led to premature senescence.

Materials and methods

Plants: Seeds of *Festuca pratensis* (Hud.) cv. Skra, *Hordeum vulgare* (L.) cv. Mobek, and *Brassica napus* (L.) var. *oleifera* cv. Górczański were sown in plastic pots, containing a mixture of soil : peat : sand (2 : 2 : 1,

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Abbreviations: Chl – chlorophyll; F_0 – initial level of chlorophyll fluorescence; F_m – maximal fluorescence; F_v/F_m – photochemical efficiency of PS2, where $F_v = F_m - F_0$; g_s – stomatal conductance; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; RH – relative humidity; $t_{1/2}$ – half rise time from initial (F_0) to maximal fluorescence (F_m).

v/v/v) at pH 5.8. The barley and fescue seedlings were grown in glasshouse in daylight at temperatures ranging between 17/25 °C (night/day) and at a relative humidity (RH) between 55 and 65 %. The rape plants were grown at PPFD of 150 μmol m^{-2} s^{-1} with 12 h photoperiod at a constant temperature of 12 °C and at 80 % RH. The plants were fertilised weekly with Hoagland's liquid medium.

Ozone treatment: Seven-week-old seedlings of fescue and five-week-old seedlings of rape and barley were transferred into two growth chambers and were grown at constant temperature of 20 °C, 60 % RH, and PPFD of 300 μmol m^{-2} s^{-1} with 16 h photoperiod. In one chamber ozone was produced with a Fischer ozone generator (model *500 M*) during the light period. The O_3 concentrations were monitored with photometric *49C* analyser (*Thermo Environmental Instruments*, Franklin, MA, USA) (for details see Skoczowski *et al.* 2000). In the control chamber the ozone concentration did not reach more than 10 μg kg^{-1}, while in the fumigation chamber it averaged 180 μg kg^{-1}. At night the ozone concentration in all chambers dropped to 5 μg kg^{-1}. The plants were ozonated for 12 d. The measurements of Chl fluorescence and electrolyte leakage were taken 6, 9, and 12 d after the start of fumigation, while the gas exchange was measured after 9 d.

Electrolyte leakage: Five leaf disks cut to a size of 5 mm in diameter were placed in a vial containing 13 cm^3 ultrapure water. They were shaken (1.7 s^{-1}) at 20 °C. Conductivity was measured after 2 h, and the vials were stored at –50 °C overnight. After thawing, the vials were shaken for 2 h and the conductivity was measured again. The measurements on frozen and thawed tissues represent the conductivity of the total ion content in the tissue. Membrane permeability is expressed as a percentage of total electrolyte leakage.

Chl fluorescence was measured on the 3rd leaf using a plant stress meter *Mark II* (*Biomonitor S.C.I.*, Umea, Sweden) after dark adaptation for 20 min. The saturating PPFD was 600 μmol m^{-2} s^{-1}, and the induction time was 2 s. Photochemical efficiency of PS2 (F_v/F_m), the initial fluorescence value (F_0), and the half-rise time ($t_{1/2}$) from initial (F_0) to maximal (F_m) fluorescence were measured.

Gas exchange: The rates of gas exchange were measured 4 h after the end of the O_3 exposure on the youngest fully expanded leaf, at 20 °C using an infrared gas analyser (*LCA-2*; *Analytical Development Co.*, Hoddeson, UK), operated within an open system with a fixation chamber *PLC (N)*. Gas exchange parameters were determined at the PPFD of 800 μmol m^{-2} s^{-1}, in air with CO_2 concentration of 345 μmol mol^{-1}, and at 60 % RH. P_N, and g_s were measured.

Statistical analysis: The results, obtained from 7 (electrolyte leakage and Chl fluorescence) or 6-9 replicates (gas exchange) were statistically analysed using Duncan's multiple range test at $p<0.05$. All effects of ozone on the studied parameters of fumigated plants were tested with *F*-test (multi-factorial analysis of variance ANOVA/MANOVA, *Statistica 5.0*).

Results

Exposure to ozone caused accelerated leaf senescence. About 75 % of fescue and barley leaves became yellow after 5 d of fumigation. In rape only older leaves yellowed. In some leaves, exposure to ozone resulted in anthocyanin accumulation (values not shown).

Ozone did not change the membrane permeability of fescue leaves, while in barley a significant increase in ion leakage was observed after 12 d of fumigation. In rape leaves a significant decrease in membrane permeability was observed over the whole period of the experiment (Table 1).

Ozone treatment generally did not change the photochemical efficiency of PS2 (F_v/F_m) and initial fluorescence (F_0), however, the changes in half-rise time from F_0 to F_m were observed (Table 2). The $t_{1/2}$ values decreased in fescue leaves after 6 and 9 d. In barley leaves the $t_{1/2}$ values fell only after 6 d of fumigation. In contrast, O_3 fumigation in rape seedlings caused an

Table 1. Electrolyte leakage [% of total ion content] from leaves of meadow fescue, spring barley, and winter rape ozonated at 180 μg kg^{-1} for 12 d. The values are means of seven replicates. Critical ranges were calculated according to the Duncan's test ($p<0.05$). The significance of the *F*-ratio following the ANOVA/MANOVA test is shown. *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS, $p>0.05$.

Plant	Control			Ozonated			Critical range	*F*		
	6	9	12	6	9	12		Time	O_3	Time×O_3
Fescue	22.2	21.2	21.7	15.2	21.2	21.3	9.2	NS	NS	NS
Barley	22.2	29.6	21.7	26.3	32.3	41.4	9.9	*	***	**
Rape	29.1	22.5	30.2	15.8	15.3	15.9	6.5	NS	***	NS

increase in $t_{1/2}$ after 6 d. P_N decreased significantly in ozonated plants similarly to g_s. Statistical analysis proved that the ozone-induced depression in P_N was dependent on the closure of stomata. Analysis of covariance indicated that changes in g_s, caused by ozone, were the main source of variance in P_N (Table 3). The correlation coefficients between g_s and P_N were: 0.885, 0.983, and 0.987 for barley, fescue, and rape, respectively (all coefficients significant for $p<0.00001$). In fescue and rape the closure of stomata caused by ozone may be responsible for the observed decrease in P_N (Table 3). The greatest decrease in P_N was observed in ozonated barley leaves (17 % of the control). In fumigated rape P_N decreased to 60 %, while in fescue plants to 45 % of the control. The largest decrease in g_s caused by ozone was found in fescue, where values fell to 2.5 times those of the controls.

Table 2. The effect of ozone on photosynthesis. The photochemical efficiency of PS2 (F_v/F_m), the initial fluorescence (F_0), and the half time ($t_{1/2}$) of increase from initial (F_0) to maximal (F_m) fluorescence were recorded on leaves of meadow fescue, spring barley, and winter rape ozonated at 180 μg kg^{-1} (16 h d^{-1}) for 12 d. Net photosynthetic rate, P_N [μmol(CO$_2$) m^{-2}s^{-1}] and stomatal conductance, g_s [mmol(CO$_2$) m^{-2}s^{-1}) were measured on mature leaves after 9 d of fumigation. Each value is the mean of seven replicates. The differences between control and ozonated plants are marked with asterisks following the Duncan's test. $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; NS, $p>0.05$. The fluorescence values are the means of seven replicates. Critical ranges were calculated according to the Duncan's test ($p<0.05$). The significance of the *F*-ratio following the ANOVA/MANOVA test is shown.

Plant	Character	Control			Ozonated			Critical range	*F*		
		6	9	12	6	9	12		O_3	Time	Time×O_3
Fescue	F_v/F_m	0.768	0.760	0.742	0.714	0.699	0.773	0.09	**	NS	NS
	F_0	0.28	0.25	0.27	0.29	0.23	0.28	0.04	NS	***	NS
	$t_{1/2}$	89	97	98	69	79	85	18	**	NS	NS
	P_N		2.67			1.20**					
	g_s		82.86			32.86**					
Barley	F_v/F_m	0.757	0.760	0.761	0.787	0.781	0.738	0.05	NS	NS	NS
	F_0	0.20	0.20	0.22	0.19	0.20	0.24	0.04	NS	***	NS
	$t_{1/2}$	126	117	110	107	119	110	18	NS	NS	***
	P_N		5.78			0.99***					
	g_s		32.99			19.99**					
Rape	F_v/F_m	0.713	0.769	0.761	0.774	0.775	0.786	0.04	**	*	NS
	F_0	0.22	0.22	0.20	0.19	0.25	0.20	0.04	NS	**	NS
	$t_{1/2}$	121	121	127	148	107	130	23	NS	**	**
	P_N		4.23			2.54*					
	g_s		66.15			39.22*					

Table 3. Covariance analysis of the effect of stomatal conductance (g_s) on ozone-induced decreases in photosynthesis.

Plant	Source	df effect	MS effect	df error	MS error	*F*	*p*
Fescue	g_s	1	5.0493	10	0.0393	128.600	0.0000
	others	1	0.0301	10	0.0393	0.766	0.4021
Barley	g_s	1	65.0595	14	0.6179	105.300	0.0000
	others	1	9.3700	14	0.6179	15.160	0.0016
Rape	g_s	1	31.1075	17	0.0728	427.300	0.0000
	others	1	0.0104	17	0.0728	0.142	0.7111

Discussion

Ozone-induced acceleration of leaf senescence has been described in many plant species (Koch *et al.* 1998, Reichenauer *et al.* 1998, Miller *et al.* 1999). This phenomenon is connected with protein and Chl degradation and lipid peroxidation (Miller *et al.* 1999). Ozone acts as a powerful oxidant modifying the structure and function of biological membranes, leading to membrane leakage (Soldatini *et al.* 1998a). In the present experiment many leaves on the barley and meadow fescue plants became yellow after 5 d of ozone fumigation. The accumulation of anthocyanins observed in some of the leaves on the fumigated rape plants may be due to the induction of phenolic metabolism (Lo and Nicholson 1998, Solecka *et al.* 1999). Anthocyanins appear in plants after exposure to abiotic stresses (Krupa *et al.* 1996, McKown *et al.* 1996), and may protect against photoinhibition (Solecka *et al.* 1999). Activation of plant defence systems is a key process in the adaptation of plants to the stresses such as elevated ozone concentration.

The primary site of ozone damage is the plasmalemma. Ozone damages the plasma membrane and thereby changes the ionic and solute relations of the cell. In plants exposed to O_3 the leakage of cellular electrolytes is an early symptom of membrane disruption; it results from the oxidation of the plasma-membrane protein sulfhydryl groups and lipids (Guidi *et al.* 1999). In the present experiments an increase in membrane permeability was observed only in barley plants ozonated for 12 d. Rape seedlings responded to ozone treatment by a decrease in ion leakage which may increase repair of the plasma membrane. Membrane permeability depends on many factors, but the structure of membrane particularly on the degree of non-saturation of fatty acid (Yoshida and Uemura 1990). The ability of the plant to modify its plasma-membrane structure under stress is the most important feature, and may be manifested as decreased solute leakage. This process is probably an adaptation to the stress.

Many types of stress cause the damage to the Chl-protein complexes located in thylakoids, especially in the PS2 reaction centre. Changes in Chl fluorescence are frequently used to measure damage to the PS2 reaction centre (Lichtenthaler 1996). A decrease in the dark-addapted F_v/F_m ratio indicates photoinhibition (Krause and Weis 1984, Björkman and Demmig 1987). Soja *et al.* (1998) found that ozone could modify the induction of Chl fluorescence. These authors showed a significant decrease in F_v/F_m values in ozonated leaves of apple plants. A decrease in F_v/F_m ratio and a significant increase in F_0 values were found in ozone-treated bean leaves (Guidi *et al.* 1999). A change in F_0 indicates effects on the energy in PS2 centre. In the present study no changes in either the photochemical efficiency of PS2 or in F_0 values were observed. However, a decrease in P_N was found. Similarly, Farage *et al.* (1991) found a decrease in P_N, but no decrease in F_v/F_m, after ozone fumigation of wheat plants.

A decrease in $t_{1/2}$ value can indicate a decrease in the rate of reduction of plastoquinone pool or in the dark reaction rate. Baszyński *et al.* (1980) observed changes in $t_{1/2}$ in tomato plants following cadmium treatment. They interpreted this as a limitation on the plastoquinone pool. In the present experiment, significant decreases in values of $t_{1/2}$ were found for ozonated fescue and barley seedlings. The interpretation of such data is complex, particularly when there is a general decrease in Chl content. It is therefore not possible to further interpret the results in terms of modified electron transport within PS2.

Soldatini *et al.* (1998a) reported a decrease in P_N and small changes in g_s in ozonated poplar plants. In the present work g_s influenced the depression in P_N. In green parts of *Hedera canariensis* variegated leaves P_N was depressed *via* a stomatal mechanism, but in white parts of the leaf such effect was not observed (Soldatini *et al.* 1998b). Farage *et al.* (1991) showed that exposure of wheat to ozone resulted in a significant reduction of P_N and in an increase in the g_s. Reichenauer *et al.* (1998) reported that O_3 fumigated wheat plants had lower g_s and decreased P_N; in this case stomatal closure was excluded as the main cause of the decrease in P_N. In the present study ozone caused closure of stomata together with a decrease in P_N values. Among the species studied, barley was the most ozone-sensitive; in this case stomatal closure may not be the exclusive cause of the decrease in P_N. Other important factors include ozone damage to the enzymes of CO_2 assimilation, particularly ribulose-1,5-bisphosphate carboxylase/oxygenase, and to electron transport components.

Among the species studied, only rape demonstrated an adaptation to high (2.5 times higher than that of air) ozone concentration. Its higher resistance to O_3 was demonstrated by (*1*) antioxidant pigment (anthocyanin) production, (*2*) decreased solute leakage, and (*3*) greater retention of photosynthetic capacity. Similarly, in a previous study on rape seedlings, fumigated at 180 μg kg^{-1} of ozone no changes in dark respiration were observed (values not shown), but at the same O_3 concentration fescue and barley plants had much lower respiration rates than controls. Farage and Long (1999) found that cereals were more resistant to ozone treatment than the other studied plant species.

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