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The Regulation of Photosynthesis in Leaves of Field-Grown Spring Wheat (*Triticum aestivum* L., cv Albis) at Different Levels of Ozone in Ambient Air

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BERCHTOLD LEHNHERR*, FELIX MÄCHLER, ARIANE GRANDJEAN, AND JÜRG FUHRER
Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene, CH-3097 Liebefeld-Bern, Switzerland (B.L., A.G., J.F.); *and Institut für Pflanzenwissenschaften, Eidgenössische Technische Hochschule, Universitätstrasse 2, CH-8092 Zürich, Switzerland* (F.M.)

ABSTRACT

Wheat (*Triticum aestivum* L. cv Albis) was grown in open-top chambers in the field and fumigated daily with charcoal-filtered air (0.015 microliters per liter O_3), nonfiltered air (0.03 microliters per liter O_3), and air enriched with either 0.07 or 0.10 microliters per liter ozone (seasonal 8 hour/day [9 AM–5 PM] mean ozone concentration from June 1 until July 10, 1987). Photosynthetic $^{14}CO_2$ uptake was measured *in situ*. Net photosynthesis, dark respiration, and CO_2 compensation concentration at 2 and 21% O_2 were measured in the laboratory. Leaf segments were freeze-clamped *in situ* for the determination of the steady state levels of ribulose 1,5-bisphosphate, 3-phosphoglycerate, triose-phosphate, ATP, ADP, AMP, and activity of ribulose, 1,5-bisphosphate carboxylase/oxygenase. Photosynthesis of flag leaves was highest in filtered air and decreased in response to increasing mean ozone concentration. CO_2 compensation concentration and the ratio of dark respiration to net photosynthesis increased with ozone concentration. The decrease in photosynthesis was associated with a decrease in chlorophyll, soluble protein, ribulose bisphosphate carboxylase/oxygenase activity, ribulose bisphosphate, and adenylates. No decrease was found for triose-phosphate and 3-phosphoglycerate. The ratio of ATP to ADP and of triose-phosphate to 3-phosphoglycerate were increased suggesting that photosynthesis was limited by pentose phosphate reductive cycle activity. No limitation occurred due to decreased access of CO_2 to photosynthetic cells since the decrease in stomatal conductance with increasing ozone concentration did not account for the decrease in photosynthesis. Ozone-stressed leaves showed an increased degree of activation of ribulose bisphosphate carboxylase/oxygenase and a decreased ratio of ribulose bisphosphate to initial activity of ribulose bisphosphate carboxylase/oxygenase. Nevertheless, it is suggested that photosynthesis in ozone stressed leaves is limited by ribulose bisphosphate carboxylation possibly due to an effect of ozone on the catalysis by ribulose bisphosphate carboxylase/oxygenase.

Air pollution due to ozone decreases photosynthesis and yield in field-grown plants (2, 8, 14, 17). The decrease in photosynthesis can be associated with decreased Chl (6, 16) and RuBPCO[1] contents (8, 15) and with increased stomatal resistance (1, 13). CO_2 response curves of net photosynthesis show that photosynthesis of ozone-stressed leaves is decreased to similar extents at low and high CO_2 concentrations suggesting that RuBPCO activity is limiting photosynthesis (8). However, the decrease in the amount of activatable RuBPCO in ozone-stressed leaves is partially compensated for by an increase in the degree of activation of this enzyme (8). The results of the present study relate this effect of ozone on RuBPCO regulation to ozone-induced changes in other components of the photosynthetic metabolism. It is shown that the increase in the degree of activation of RuBPCO in ozone-stressed leaves is associated with increased contents of products from photochemical reactions and a decreased ratio of RuBP to initial activity of RuBPCO.

MATERIALS AND METHODS

Growing conditions. Spring wheat (*Triticum aestivum* L. cv Albis) was sown on April 3, 1987 (16.7 cm row-spacing) at a site located 25 km northeast of Bern, near Oeschberg-Koppigen on the Swiss Plateau at 480 m above sea level. The general growing conditions were as described earlier (8). The soil (weakly humic loam) was fertilized according to standard agricultural practice and additional nitrogen was given by hand during anthesis. Availability of water in the soil was sufficient or, episodically, even excessive.

Open-top chambers (1.5 m diameter; 1.8 m height) similar to those described by Elphinstone *et al.* (5) were placed over 16 experimental plots on April 27. The air volume of each chamber was exchanged 2.5 times per minute. Ventilation was turned off from 10 PM to 7 AM. Chambers were supplied either with charcoal-filtered, nonfiltered, or ozone-enriched air. Four replicate chambers for each treatment were used. Ozone additions started on May 13. Ozone was generated by electrical discharge in dry air and was added daily from 9 AM to 5 PM. During this period, O_3 concentrations in ambient air are usually highest and plants are most active metabolically. Mean O_3 concentrations for daily 8-h periods (9 AM–5 PM) from June 1 until July 10 were 0.015, 0.03, 0.07, and 0.1 μL/L in charcoal-filtered, nonfiltered, slightly O_3-enriched and heavily O_3-enriched air, respectively. Stomatal conductance during anthesis at noon varied between 0.87 and 1.08 cm s^{-1} depending on the treatment and allowed for easy access of ozone to photosynthetic cells. Seasonal 24 h/d mean air temperature was 17.1°C inside open-top chambers and 15.7°C in the open field. Concentrations of O_3 and NO_2 were recorded in one chamber of each treatment and in ambient air (Dasibi 1003 AH for O_3 and Tecan CLD 502 NO/NO_x for NO_2). Seasonal 24 h/d mean concentration of NO_2 was between 12

[1] Abbreviations: RuBPCO, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; TP, triose phosphate; PGA, 3-phosphoglycerate: P_A, assimilatory power; Pi, inorganic phosphate; Γ, CO_2 compensation concentration; P_N, net photosynthesis; D_R, dark respiration.

Table I. *Effect of Ozone Concentration During Growth in Open-Top Chambers on Stomatal Conductance of Flag Leaves During Anthesis (as determined on July 1, 1987)*
Means of four measurements and SE are indicated.

Time of Measurement	Ozone Concentration (μL/L)			
	0.015	0.03	0.07	0.10
	stomatal conductance (cm s^{-1})			
8 AM	0.93 ± 0.08	0.88 ± 0.06	0.80 ± 0.04	0.77 ± 0.06
10 AM	1.01 ± 0.09	1.00 ± 0.09	0.94 ± 0.10	0.84 ± 0.12
12 AM	1.08 ± 0.08	1.04 ± 0.12	1.05 ± 0.13	0.87 ± 0.06

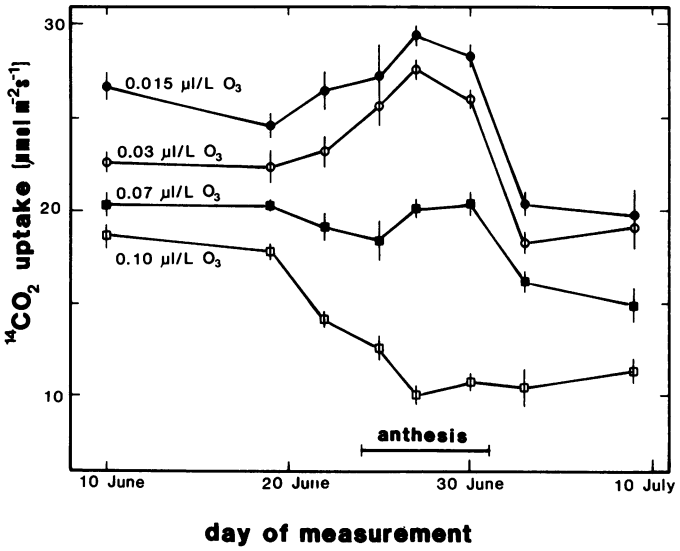

FIG. 1. Time course of photosynthetic $^{14}CO_2$ uptake per leaf area of flag leaves of wheat plants grown at various ozone concentrations in open-top chambers. Data points are means of four replicates and SE are indicated.

and 14 μL/L in the various ozone-treatments and in ambient air.

Measurement of Photosynthetic $^{14}CO_2$ Uptake and Stomatal Conductance. Flag leaves from the main shoots were equally exposed to light during the measurement of photosynthesis and of stomatal conductance. Measurements were taken in the morning when photosynthetic irradiance on the surfaces of the flag leaves was 1600 to 2000 μE m^{-2} s^{-1} and stomatal conductance was near its daily maximum (9–11 AM, depending on the weather conditions). Leaf temperature was about 25°C. Photosynthetic $^{14}CO_2$ uptake of attached flag leaves was measured *in situ* using equipment similar to that described by Shimishi (18). Leaves were exposed to $^{14}CO_2$ for 20 s. Fixed ^{14}C was determined as described earlier (8). Stomatal conductance was determined with the LiCor steady state porometer (Lambda).

Measurement of Net Photosynthesis, Dark Respiration, and of Γ. Wheat shoots were cut at the base during anthesis in the morning and transferred to the laboratory. Net photosynthesis (at 1400 μE m^{-2} s^{-1}), dark respiration and Γ were analyzed by infrared gas analysis in a system as described earlier (8). Segments from the middle part of flag leaves were used. Temperature was 15°C.

Chl and Soluble Protein. Chl content was determined according to Knudson *et al.* (6). Soluble protein was determined using the Bio Rad reagent according to Bradford (3).

RuBPCO Activity and Total Foliar Steady State Metabolite Levels. Flag leaves were sampled during anthesis between 9 and 11 AM using freeze stop tongs. Light and temperature conditions before sampling were similar to those during $^{14}CO_2$ uptake measurement. Frozen leaf segments were homogenized in liquid nitrogen in a mortar and extracted either for RuBPCO assays or for metabolite determinations. RuBPCO was extracted and initial activity determined at 15°C as described earlier (9). An aliquot of the extract was incubated for 10 min in the presence of 10 mM $NaHCO_3$ and 20 mM $MgCl_2$ at 30°C prior to the

Table II. *Effect of Ozone Concentration During Growth in Open-Top Chambers on Photosynthetic $^{14}CO_2$ Uptake and on Chl, Soluble Protein, and Adenylates (ATP + ADP + AMP) in Flag Leaves during Anthesis*
Means of six or more replicates and SE are indicated.

Measurement	Ozone Concentration (μL/L)			
	0.015	0.03	0.07	0.10
Photosynthetic $^{14}CO_2$ uptake per leaf area (μmol m^{-2} s^{-1})	28.5 ± 1.05	26.4 ± 1.51	19.6 ± 1.50	11.2 ± 0.98
Chl per leaf area (g m^{-2})	0.54 ± 0.041	0.50 ± 0.048	0.37 ± 0.022	0.22 ± 0.023
Adenylates per leaf area (μmol m^{-2})	31.3 ± 2.08	27.0 ± 2.11	20.8 ± 2.01	12.5 ± 1.36
Soluble protein per leaf area (g m^{-2})	3.10 ±0.143	2.99 ± 0.130	2.35 ± 0.131	1.63 ± 0.109
Photosynthetic $^{14}CO_2$ uptake per Chl (μmol s^{-1} g^{-1})	52.8	52.8	53.1	51.1
Soluble protein per Chl (mg mg^{-1})	5.7	6.0	6.4	7.4
Adenylates per Chl (μmol g^{-1})	58.0	54.0	56.2	56.8

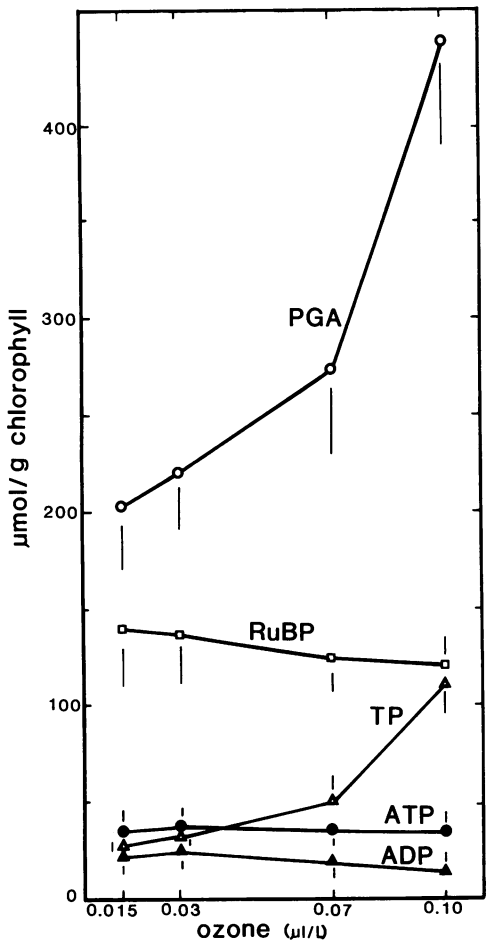

FIG. 2. Effect of ozone concentration during growth in open-top chambers on molar steady state levels of ATP, ADP, TP, PGA, and RuBP per Chl content in flag leaves during anthesis. Data points are means of five replicates and SE are indicated.

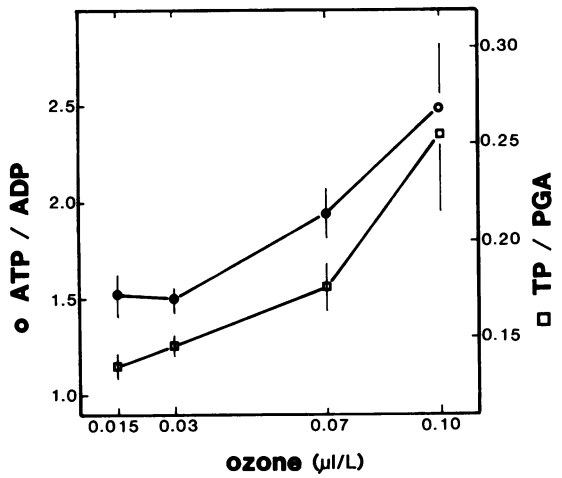

FIG. 3. Effect of ozone concentration during growth in open-top chambers on molar steady state ratios of ATP/ADP and TP/PGA in flag leaves during anthesis. Data points are means of five to six replicates and SE are indicated.

determination of RuBPCO activity. Metabolites were extracted as described by Leegood and Furbank (7). TP and PGA were analyzed spectrophotometrically, RuBPCO was analyzed by *in vitro* assay using $H^{14}CO_3^-$ and purified RuBPCO from wheat, and adenylates were analyzed in the luminometer as described earlier (12).

RESULTS AND DISCUSSION

Photosynthetic $^{14}CO_2$ Uptake and Stomatal Conductance. $^{14}CO_2$ uptake of flag leaves was measured when photosynthetic irradiance on the surfaces of the leaves was 1600 to 2000 $\mu E\ m^{-2}\ s^{-1}$ and stomatal conductance was near its daily maximum (Table I). This occurred between 9 and 11 AM depending on the weather conditions. Photosynthetic $^{14}CO_2$ uptake by flag leaves was highest in filtered air and decreased as ozone concentration was increased (Fig. 1). The inhibition by ozone was most pronounced during anthesis when photosynthesis in filtered air was increased. Stomatal conductance was high during this period (Table I), and photosynthetic cells were easily accessible by ozone. A lesser effect of ozone was found before anthesis when flag leaves were not yet fully developed and after anthesis when photosynthesis in filtered air was decreased due to unfavorable weather conditions. During anthesis, stomatal conductance was less affected by ozone than $^{14}CO_2$ uptake (Table I; Fig. 1). Effects on stomatal conductance did not account for effects on photosynthesis indicating that access of CO_2 to photosynthetic cells was not impaired in the presence of ozone. Intercellular CO_2 concentrations during anthesis as calculated from stomatal conductance (Table I), $^{14}CO_2$ uptake (Fig. 1), and an ambient CO_2 concentration of 320 $\mu L/L$ increased clearly with ozone concentration (259, 263, 273, and 292 $\mu L/L$ CO_2 at 0.015, 0.03, 0.07, and 0.10 $\mu L/L$ ozone, respectively).

Chl, Adenylates, and Soluble Protein. The content of Chl, adenylates, and soluble protein per leaf area decreased as ozone concentration was increased (Table II). The decrease in Chl and adenylates was parallel to the decrease in photosynthesis and the ratios of Chl to adenylates and of Chl to photosynthesis were constant. Soluble protein decreased less than Chl and the ratio of protein to Chl increased with increasing ozone concentration.

The results suggest that Chl content is more suitable than leaf area as a basis for the comparison of photosynthetic parameters in leaves grown at various ozone concentrations. Metabolite pools and RuBPCO activity were therefore related to Chl content (Figs. 2 and 4).

Total Foliar Steady State Levels of ATP, ADP, PGA, TP, and RuBP. On a Chl basis, TP and PGA increased strongly as ozone concentration increased whereas RuBP, ATP, and ADP were less affected (Fig. 2). The content of TP per Chl increased more than PGA resulting in an increase in the ratio of TP/PGA at high ozone levels (Fig. 3). A similar increase was found for the ratio of ATP to ADP.

ATP, ADP, TP, and PGA measurements include components from inside and outside the chloroplasts; however, the concentrations in both compartments are in equilibrium due to the TP/PGA shuttle. It is therefore suggested that changes in ATP/ADP ratios observed in the leaf extracts reflect effects on actual ratios in the chloroplast.

The ATP/ADP ratio in chloroplasts is part of the assimilatory power, P_A as expressed by Eq. 1.

$$P_A = [ATP][NADPH]/[ADP][Pi][NADP^+]. \quad (1)$$

The TP/PGA ratio in chloroplasts is directly related to P_A since the formation of TP from PGA in the pentose phosphate reductive cycle consumes ATP and NADPH in a reaction which is not far from thermodynamic equilibrium (4). The relationship between P_A and TP/PGA is expressed by Eq. 2.

$$P_A = [TP]9.8\times10^{-6}/[PGA][H^+]. \quad (2)$$

The increase in ATP/ADP and TP/PGA ratios with increasing ozone concentration, as found in leaf extracts in this study,

Table III. *CO_2 Compensation Concentration (Γ) at 2 and 21% O_2 and the Ratio of Net Photosynthesis (P_N) to Dark Respiration (D_R) of Plants Grown at Different Ozone Concentration in Open-Top Chambers*

Flag leaves were measured in the laboratory by IRGA at 15°C. Light intensity was 1400 μE m^{-2} s^{-1} for P_N and 500 μE m^{-2} s^{-1} for Γ. Means of four determinations and SE are shown.

Measurement	Ozone Concentration (μL/L)			
	0.015	0.03	0.07	0.10
Γ at 2% O_2 (μL L^{-1})	2.1 ± 0.1	2.4 ± 0.1	3.3 ± 0.3	5.6 ± 0.2
Γ at 21% O_2 (μL L^{-1})	28.8 ± 0.7	30.4 ± 1.6	32.1 ± 0.8	34.6 ± 0.6
Ratio P_N/D_R	7.0 ± 0.39	6.0 ± 0.56	5.6 ± 0.24	3.8 ± 0.43

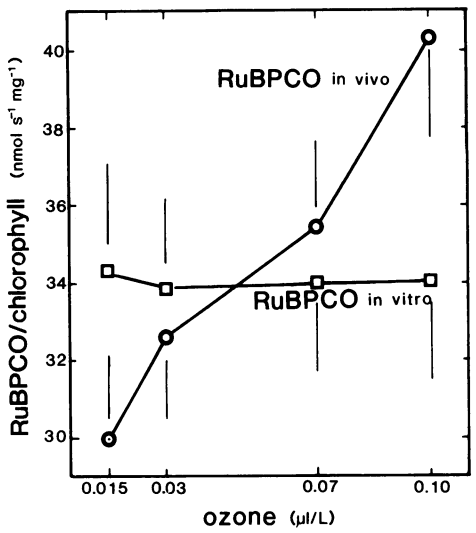

FIG. 4. Effect of ozone concentration during growth in open-top chambers on the activity of RuBPCO per Chl content in flag leaves during anthesis. RuBPCO *in vivo* is initial activity and RuBPCO *in vitro* activity after incubation in the presence of CO_2 and Mg^{2+}. Temperature during assays was 15°C. Data points are means of six replicates and SE are indicated.

suggests increasing P_A. The increase in P_A appears to be the result of a decreased consumption of ATP and NADPH due to reduced pentose phosphate reductive cycle activity and possibly due to increased Pi. On the other hand, it seems likely that contribution of ATP synthesis from 'dark' respiration in the light is increased in ozone-stressed leaves. Γ measured at 2 and 21% O_2 increased as ozone concentration was increased (Table III) suggesting an increase in the ratio of respiration in the light to photosynthesis. This is supported by the observation that the ratio of net photosynthesis at 1400 μE m^{-2} s^{-1} to CO_2 evolution in the dark decreases with increasing ozone concentration (Table III). ATP synthesis associated with respiration may therefore contribute to the increase in P_A in ozone-stressed leaves.

RuBPCO Activity and RuBP Content. The content of activatable RuBPCO (RuBPCO activity after incubation in the presence of CO_2 and Mg^{2+}) relative to Chl content was not affected by ozone (Fig. 4). This shows that RuBPCO content followed the general decrease of components of the photosynthetic apparatus relative to leaf area (Table II). A decrease in quantity of RuBPCO in alfalfa foliage was found earlier by Pell and Pearson (15). Effects on activatable RuBPCO (Fig. 4) and RuBP (Fig. 2) suggest that the ratio of RuBP to RuBPCO was not decreased by ozone and that CO_2 fixation was not limited by the regeneration of RuBP in ozone stressed leaves. On the other hand, initial activity of RuBPCO expressed on Chl content increased with ozone concentration suggesting that the ratio of RuBP to number of active sites was decreased in ozone stressed leaves and that a limitation by RuBP was possible.

The degree of activation of RuBPCO (ratio of initial activity to activity after incubation in the presence of CO_2 and Mg^{2+}) increased with ozone concentration. This increase was associated with an increase in the ATP/ADP ratio (Fig. 3). A similar relationship between ATP and RuBPCO activation has been found in a study on the effect of Pi on chloroplast photosynthesis (10, 11) and in a study of the effect of nitrogen nutrition on leaf photosynthesis in wheat (12). These findings support the notion that ATP is important for the regulation of RuBPCO. ATP is needed for the activation of RuBPCO by the enzyme RuBPCO activase (19). On the other hand, changes in stromal ATP concentration can be associated with changes in Pi concentration and pH, both of which are known to affect RuBPCO activity.

CONCLUSIONS

Ozone at ambient or at elevated concentrations decreased photosynthetic C-fixation in flag leaves of wheat during anthesis. The reduction in photosynthesis was associated with decreases in various components of the photosynthetic apparatus such as the contents of Chl, soluble protein, adenylates, RuBP, and RuBPCO. No decrease was found for the contents of PGA and TP.

The ratios of ATP/ADP and TP/PGA were increased at elevated ozone concentrations, suggesting that the products of the photochemical reactions were accumulated and that limitation of photosynthesis occurred in the pentose phosphate reductive cycle. No limitation occurred due to decreased access of CO_2 to photosynthetic cells since the decrease in stomatal conductance did not account for the decrease in photosynthesis.

The results show that limitation of photosynthesis in ozone-stressed leaves is either due to decreased RuBP regeneration from TP or due to decreased RuBP carboxylation. The data cannot exclude one of the two processes. A limitation due to decreased regeneration of RuBP from TP cannot be excluded since the ratio of RuBP to initial activity of RuBPCO was decreased, although the ratio of RuBP to activatable RuBPCO was not affected. Neither can a limitation by RuBP carboxylation be excluded since catalysis of this reaction could be inhibited by ozone, although the degree of activation of RuBPCO was increased. However, the CO_2 response curves of photosynthesis published in an earlier paper (8) show that photosynthesis in ozone-stressed leaves is decreased to similar extents at low and high CO_2 concentration. A limitation due to decreased RuBP regeneration would result in a decrease at high CO_2 concentrations only. It is therefore suggested that limitation is due to decreased RuBP carboxylation and not due to decreased RuBP regeneration. A study of the effect of ozone on catalysis of RuBP carboxylation by purified RuBPCO should be considered for the future.

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