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Response of Photosynthesis and Cellular Antioxidants to Ozone in *Populus* Leaves[1]

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ABSTRACT

Atmospheric ozone causes formation of various highly reactive intermediates (*e.g.* peroxyl and superoxide radicals, H_2O_2, etc.) in plant tissues. A plant's productivity in environments with ozone may be related to its ability to scavenge the free radicals formed. The effects of ozone on photosynthesis and some free radical scavengers were measured in the fifth emergent leaf of poplars. Clonal poplars (*Populus deltoides* × *Populus* cv *caudina*) were fumigated with 180 parts per billion ozone for 3 hours. Photosynthesis was measured before, during, and after fumigation. During the first 90 minutes of ozone exposure, photosynthetic rates were unaffected but glutathione levels and superoxide dismutase activity increased. After 90 minutes of ozone exposure, photosynthetic rates began to decline while glutathione and superoxide dismutase continued to increase. Total glutathione (reduced plus oxidized) increased in fumigated leaves throughout the exposure period. The ratio of GSH/GSSG also decreased from 12.8 to 1.2 in ozone exposed trees. Superoxide dismutase levels increased twofold in fumigated plants. After 4 hours of ozone exposure, the photosynthetic rate was approximately half that of controls while glutathione levels and superoxide dismutase activity remained above that of the controls. The elevated antioxidant levels were maintained 21 hours after ozone exposure while photosynthetic rates recovered to about 75% of that of controls. Electron transport and NADPH levels remained unaffected by the treatment. Hence, elevated antioxidant metabolism may protect the photosynthetic apparatus during exposure to ozone.

All organisms that have evolved in aerobic environments have a variety of enzymatic and nonenzymatic mechanisms to prevent oxidation of cellular components. It is quite probable that existing mechanisms for detoxifying oxyradicals are invoked in response to ozone since it causes the formation of some highly reactive oxyradicals in aqueous solutions, a major product being the superoxide anion (11). The superoxide anion can be metabolized by several isozymes of superoxide dismutase found in plants (14). The hydrogen peroxide formed by this reaction is toxic. It can inactivate —SH containing enzymes (5, 13) or react with superoxide to form the hydroxyl radical, which can attack many macromolecular species. It is to the cell's advantage, therefore, to remove the hydrogen peroxide quickly. This occurs through a series of oxidations and reductions of glutathione, ascorbic acid, and NADPH (9, 22). We have observed that ozone causes an increase in the activity of components of the superoxide dismutase-ascorbate-glutathione pathway in clonal poplars. In our experimental system, photosynthesis was inhibited even under conditions when levels of NADPH were not detectably affected. In another experimental system, reductant was found to accumulate as a result of ozone exposure, while the reductive pentose phosphate pathway was inhibited (17, 18). Since NADPH did not accumulate in our system on exposure to ozone, it may have been consumed in other metabolic processes; for example, the increased synthesis and rereduction of oxidized glutathione.

In an ozone enriched environment, a successful plant would be one that effectively removes the increased free radicals formed by ozone. The formation of free radicals by ozone is very fast; thus, the antioxidant scavenging system should respond quickly before extensive ozone damage can occur. To our knowledge, there have been no published reports of the initial effects of ozone on the scavenging system of plants. Antioxidant increases have indeed been shown to occur in plants (21) after prolonged ozone exposure but no information is available on the pattern of antioxidant accumulation. In this report we document the initial effects of ozone on some aspects of the antioxidant scavenging system of plants. A preliminary report of our findings has appeared elsewhere (27).

MATERIALS AND METHODS

Growth Conditions

Poplar trees (*Populus deltoides* × *Populus* cv *caudina*, NE clone 353) were used in our studies. Trees were planted in "Cornell Mix" (peat moss:vermiculite 1:2) in 1-gallon pots and watered every other day. Trees were grown in charcoal-filtered greenhouses and periodically trimmed such that only one terminal shoot was present per tree, the last pruning occurring at least 7 d before fumigation. Trees were grown in a 12 h photoperiod. The temperature within the fumigation chamber was maintained at 22°C (days) and 18°C (night), and light intensities of 300 to 400 μmol photons m^{-2} s^{-1}, RH 60%, 16-h photoperiod. A 5000 W metal halide lamp was used as a supplementary light source. On the fourth day, fumigation was initiated 4 h after the beginning of the light

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period in the chamber. Ozone was generated by a Griffith Technics Corporation ozone generator and monitored by a Monitorlabs ozone analyser model 8410. Flow rates of ozone were adjusted as necessary by a micrometer valve such that the trees in the chamber were always exposed to 180 ppb[2] ozone. Ozone was delivered to the chamber in Teflon tubing. Trees were fumigated for 3 h. Eight trees were used in each experiment. Of these, three trees were used as controls (charcoal-filtered air, ozone about 1 ppb) and five were used as a source for ozone exposed tissue. The fifth emergent leaf or the first fully expanded leaf was used for all measurements. Biochemical measurements were made on extracts from leaves harvested at periodic intervals during the fumigation and 4 and 21 h after the end of fumigation. Leaves that were used for biochemical measurements were frozen in liquid nitrogen immediately following harvesting. These samples were stored at −40°C until they were used.

Photosynthesis measurements were made on control trees just before, and during the period of fumigation. Photosynthesis readings were taken on fumigated trees before, during, and up to 21 h after fumigation had ceased. The controls for 21 h after ozone exposure were untreated plants that were maintained in the charcoal-filtered chamber, for the same length of time.

Photosynthesis

Stomatal resistance and photosynthesis measurements were made using a Li-Cor 6200 gas exchange system (LI-COR, Lincoln, NE). A 4 L leaf cuvette was used and the entire leaf was inserted in the cuvette for gas exchange measurements.

Glutathione

Glutathione was measured by a method described by Smith (28). A known amount of GSH was added as an internal standard to at least two randomly picked replicate samples of control and ozone exposed tissue in each experiment. In all cases about 95 to 99% of the added GSH could be recovered.

Superoxide Dismutase

Eight hundred milligrams of leaf tissue were homogenized by a "Polytron" homogenizer (setting at No. 7) in a 0.5 M potassium phosphate buffer (pH 7.8), which contained 0.1% BSA and 0.1 mM EDTA. The homogenate was filtered through cheesecloth and centrifuged at 5856*g* using a fixed angle SS-34 rotor, in a Sorvall superspeed centrifuge for 60 s. The pellet was washed once with the isolation buffer and centrifuged. The supernatants were combined and the resulting solution dialyzed overnight against the isolation buffer without the BSA. The dialyzing solution was changed three times. Proteins were separated by PAGE according to Beauchamp and Fridovich (4). The running and stacking gel was made of 10 and 7% polyacrylamide, respectively. The stacking gel was light polymerized with riboflavin as catalyst. A Tris-glycine running buffer (pH 8.3) was used as the running buffer. The gels were run at 4°C, using 125 V until the tracking dye (bromophenol blue) reached the lower end of the gel.

The presence of the enzyme was determined by soaking the gels for 1 h in a staining buffer (0.05 M potassium phosphate, 0.1 mM EDTA [pH 7.8]) which contained 1 mg riboflavin, 16 mg NBT, and 0.2 mL Temed. After 1 h the solution was poured off and the gels rinsed with double distilled deionized water and exposed to fluorescent light for 10 min. The presence of the enzyme was inferred from the presence of colorless regions in the gel. The Mn isozyme was identified by soaking the gels in 5 mM KCN before staining with the NBT/Temed solution described above. The activity of the enzymes was determined by scanning get densitometry at 560 nm. A unit of enzyme activity was defined as the quantity of superoxide dismutase required to produce a 50% inhibition of reduction of NBT. The colorless regions were compared with the background (where NBT reduction occurred) and the percentage inhibition of reduction of the dye was calculated from the difference in absorption of the two regions. Known quantities of superoxide dismutase from bovine heart were used as standards and the range in which a linear relationship existed between units of enzyme activity and inhibition of reduction of the dye within the gel established. Standards were run on every occasion on the same gels as the poplar samples.

Superoxide dismutase was also assayed independently by a spectrophotometric method described in Dhindsa *et al.* (8).

Pyridine Nucleotide

Reduced and oxidized pyridine nucleotide were assayed according to a procedure published by Matsumura and Miyachi (20). Tissues were extracted with hot NaOH or perchloric acid (0.1 N) final concentration for reduced and oxidized coenzyme, respectively. Known amounts of the oxidized and reduced coenzymes were added to appropriate control and fumigated samples which were picked at random. Recovery of the added pyridine nucleotide was always between 95 and 98%.

Chl

Tissues were extracted by 80% acetone and Chl determined by Arnon's (3) method.

Thylakoid Isolation

Thylakoids were isolated by the method of Alscher *et al.* (2). One gram of leaf tissue was ground in 50 mM $Na_2H_2P_2O_7$ (pH 7.0), 5 mM $MgCl_2$, 4 mM D-isoascorbic acid, 4 mM $K_2S_2O_3$, 5 mM DTE, 330 mM sorbitol, 20% PEG, 3000 units catalase mL^{-1}, for 3 to 5 s. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 90 s at 3000*g*. The resultant pellet was reusupended in 10 mL of 50 mM Hepes (pH 6.7), 2 mM $NaNO_3$, 2 mM EDTA, 2 mM isoascorbic acid, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 0.5 mM K_2HPO_4, 20 mM NaCl, 330 mM sorbitol, and 3000 units catalase mL^{-1} and centrifuged for 1 min at 30*g*. The resultant superantant was decanted, centrifuged for 3 min at 6000*g*, and the pellet resuspended in 1 mL 5 mM Tricine (pH 8.0), 10 mM $MgCl_2$, 1 mM $MnCl_2$. Electron transport capacity of the thylakoid

[2] Abbreviations: ppb, parts per billion; NBT, nitro blue tetrazolium; Temed, (*N,N,N′N′*-tetramethylethylenediamine).

preparation was measured using a Clark-type oxygen electrode, (YSI, Yellow Springs, OH), 25°C, light intensity 400 micromol m^{-2} s^{-1}, with ferricyanide (1.3 mM) acting as the electron acceptor, ammonium chloride (0.02 M) as the uncoupler and Chl concentration adjusted to 150 μg mL^{-1} with resuspension buffer. Solutions were deoxygenated before use in the oxygen electrode.

RESULTS

Photosynthesis ultimately declined in trees exposed to ozone (Fig. 1). Rates were unaffected, however, during the first 90 min of the fumigation. The decline in photosynthesis began to occur 90 to 120 m after the onset of fumigation and it continued for at least 4 h after the ozone was turned off. Twenty-one hours after the termnation of ozone exposure, photosynthesis had recovered partially to about 75% of that of the controls (14.2 ± 1.4 μmol CO_2 fixed m^{-2} s^{-1}, fumigated plants 21 h after the end of fumigation *versus* 18.9 ± 0.8 for control plants, means of four experiments with four replicates in each experiment).

Stomatal resistances were unaffected by ozone both during and after the exposure (Fig. 2 and 0.98 ± 0.06 control *versus* 0.98 ± 0.05 m^2 s mol^{-1} fumigated both 4 and 21 h after fumigation). Thus, the photosynthetic decline measured was

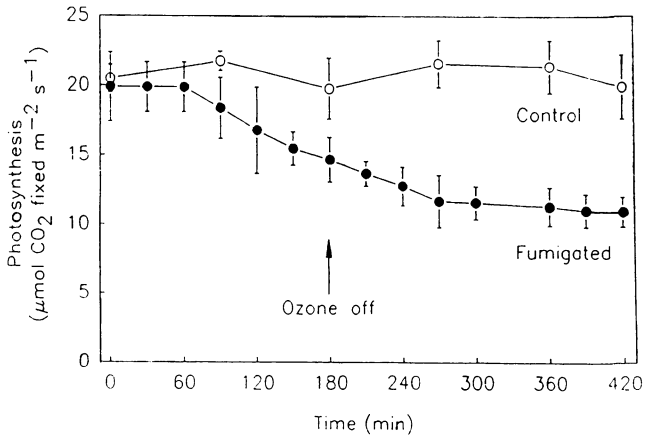

Figure 1. Effect of exposure to ozone (180 ppb) for 4 h on apparent photosynthesis in hybrid poplar. Trees were grown, maintained, and acclimated in the exposure chambers as described in "Materials and Methods." On the fourth day, fumigation was initiated 4 h after the beginning of the light period as described in "Materials and Methods." Trees were fumigated for 3 h. Eight trees were used in each experiment. Of these, three trees were used as controls and five were used as a source for ozone exposed tissue. The fifth emergent leaf or the first fully expanded leaf was used for all measurements. Leaves that were used for control measurements were harvested before fumigation and the leaves were removed from the trees harvested 4 h after the end of fumigation. Photosynthesis measurements were made on control trees just before fumigation. Photosynthesis readings were taken on fumigated trees at regular intervals before, during, and 4 h after fumigation had ceased. Stomatal resistance and photosynthesis measurements were made using a Li-Cor 6200 gas exchange system. A 4 L leaf cuvette was used and the entire leaf was inserted in the cuvette for gas exchange measurements.

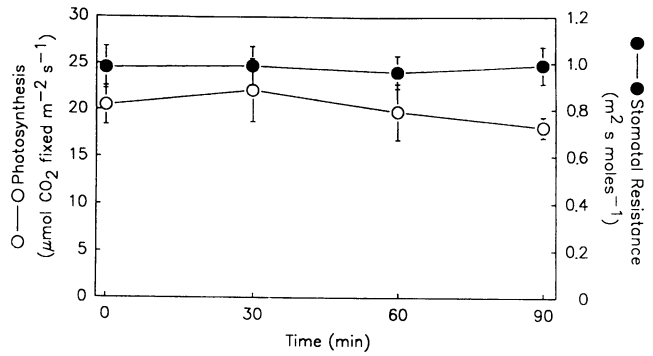

Figure 2. Effect of exposure to 180 ppb ozone on apparent photosynthesis and stomatal resistance in hybrid poplar during the first 90 min of exposure. Exposures were carried out as described in Figure 1. Stomatal resistance and photosynthesis measurements were made using a Li-Cor 6200 gas exchange system. A 4 L leaf cuvette was used and the entire leaf was inserted in the cuvette for gas exchange measurements.

not a result of lowered effective ozone doses or lowered carbon dioxide.

There were no detectable differences in NADPH and NADP levels in control and fumigated trees (Table I). The ratio of NADPH/NADP was also unchanged in both sets of trees. The same pattern was seen for NADH, NAD, and NADH/NAD ratios in control and fumigated trees. Electron transport measurements made with thylakoids isolated from control and fumigated leaves showed that the electron transport capacity of the chloroplasts was not changed with ozone exposure. Thylakoids from leaves of control trees had electron transport rates of 250 ± 35 μmol O_2 mg^{-1} Chl h^{-1}, while thylakoids from fumigated leaves (3 h of ozone exposure) had electron transport rates of 230 ± 41 O_2 μmol mg^{-1} Chl h^{-1}. These measurements were made on thylakoids isolated from leaves from one experiment only, with three replicates used for the controls and five trees for the ozone exposed measurements.

Total glutathione levels increased during ozone fumigation. The increase was first detected after 30 min of fumigation and continued until the 90 min sampling time (Fig. 3). Four hours after fumigation the total glutathione levels were similar to those of leaves sampled at the 90 min time point during fumigation (Table II; Fig. 3). This elevated level was still observed 21 h later. The ratio of reduced glutathione to oxidized glutathione changed during the fumigation. Fifteen minutes after ozone exposure had begun total glutathione was unchanged from that of unexposed tissue. However, the ratio of GSH:GSSG had shifted from 29 to 0.67 with more than half of the glutathione being present in the oxidized form (Fig. 3). Thirty minutes after the initiation of the exposure, at the time when an increase in total glutathione was first observed, the GSH:GSSG ratio was approximately the same as that seen at 15 min. As more glutathione continued to be synthesized, the GSH:GSSG ratio changed from 1.08 at 60 min to 1.25 at 90 min. This ratio was maintained 4 h after the ozone was turned off even though the total glutathione content was slightly higher than that at 90 min. The total

Table I. *Effect of Ozone Exposure on Pyridine Nucleotide Levels*

Plants were equilibrated in the growth chamber to be used for exposures for 2 d at saturating light and about 65 to 70% RH. All measurements were conducted on the fifth emergent leaf 4 h after the end of the fumigation period. Values shown are the means of six separate exposures. Five replicate leaves were sampled on each occasion for the fumigated plants and three for the controls. Pyridine nucleotides were measured by the method of Matsumara and Miyachi (20). Results are expressed as $\times 10^{-9}$ mol mg^{-1} Chl. Standard errors are indicated in parentheses.

Pyridine Nucleotide	Treatment	
	Control	Fumigated
NADPH	4.5 (2.5)	6.1 (1.9)
NADP	3.7 (1.8)	4.6 (2.0)
NADPH/NADP	1.22	1.31
NADH	14.4 (3.0)	14.5 (0.8)
NAD	9.7 (1.2)	10.4 (1.3)
NADH/NAD	1.47	1.39

glutathione content was maintained 21 h later but the GSH:GSSG ratio had now shifted to 10 with most of the glutathione now present in the reduced form. Poplars which were not exposed to ozone but sampled for glutathione at the same time as the trees at 4 h after fumigation had total glutathione levels of 122 nmol mg^{-1} Chl. Almost all (95%) of this glutathione was present in the reduced form.

Total superoxide dismutase activity was initially unaffected by ozone (0–60 min of ozone) (Fig. 3). The first increase in superoxide dismutase activity (about 10% increase relative to control) was observed in leaves of trees that had been exposed to ozone for 90 min. Four hours after the end of ozone exposure, superoxide dismutase activity had increased by about 34% and this increased level of activity was maintained 21 h later (Table III). The data shown in Table III were obtained using the gel scanning method, while those shown in Figure 3 were obtained independently using the spectrophotometric method. It should be noted that the values obtained by the two methods were quite similar.

DISCUSSION

Varying effects of ozone on photosynthesis have been reported (7). Among the parameters that were measured in the poplar system, photosynthesis was the least sensitive to ozone. Furthermore, the inhibition and recovery of photosynthesis observed after fumigation were apparently not regulated by the stomata. Both glutathione levels and superoxide dismutase activity increased before there was any observable ozone effect on photosynthesis. The decline in photosynthesis observed after 90 min of fumigation may, thus, be a secondary effect of ozone. Both glutathione synthesis and the reduction of oxidized glutathione require NADPH. Thus, the photosynthetic decline observed may be a result of less reductant available for carbon reduction. In wheat, ribulose bisphosphate carboxylase levels and activity have been shown to decline by about a third after fumigation with ozone for the entire growing season (17, 18). Changes in ribulose bisphosphate carboxylase might be responsible for the photosynthesis

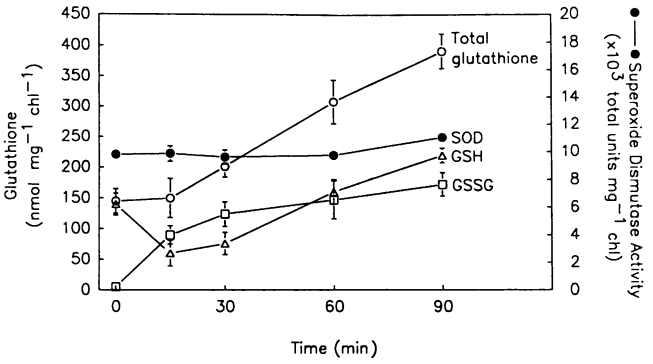

Figure 3. Effect of exposure to 180 ppb ozone on total, reduced (GSH), and oxidized (GSSG) and on superoxide dismutase (SOD) in leaves of hybrid during the first 90 min of exposure. Trees were cultivated and exposed to zone as described in "Materials and Methods" and in Figure 1. Leaves that were used for biochemical measurements were frozen in liquid nitrogen immediately following harvesting. These samples were stored at −40°C until they were used. GSH, GSSG, and SOD were assayed as described in "Materials and Methods."

decline observed in poplars, but this is probably not the first site of ozone damage in these plants. The trees used in our study were equilibrated and fumigated in growth chambers under saturating light and high humidity and this may have modified any effect of ozone on stomatal closure. Field poplars may behave differently when exposed to ozone.

NADPH and NADP levels were stable and not significantly altered by fumigation. The electron transport capacity in chloroplast thylakoids was also unaffected; thus reductant availability did not change in ozone-exposed tissues. NADH and NAD levels were not detectably altered either. Coulson and Heath (6) have shown that ozone does not uncouple ATP synthesis even under conditions where electron transport is inhibited. Schreiber *et al.* (26) report that electron transport between photosystems is eventually inhibited after ozone exposure and that the extent of injury depends on concentration of pollutant, exposure time, and leaf age. It is improbable, therefore, that the reduction of carbon uptake observed in our system was due to a lack of ATP and/or reductant. In fact, our results suggest that there was adequate reductant for

Table II. *Postfumigation Glutathione Levels in Poplar*

Experimental treatment is described in Table I.

Control	Postfumigation	
	4 h	2 h
	nmol mg^{-1} Chl	
Total glutathione (GSH + GSSG)		
136 ± 30	462 ± 27	440 ± 41
Reduced glutathione (GSH)		
133 ± 10	257 ± 32	400 ± 20
Oxidized glutathione (GSSG)		
3 ± 3	205 ± 19	40 ± 38
GSH : GSSG		
44.33	1.25	10.00

Table III. *Postfumigation Superoxide Dismutase Activity in Poplar*

Plants were fumigated with 180 ppb ozone for 3 h at saturating light intensities as described in "Materials and Methods." Superoxide dismutase activity was measured by the spectrophotometric method described (8) before fumigation as well as 4 and 21 h after fumigation. Measurements were made on the fifth emergent leaf or on the first fully expanded leaf. The results reported are the means of four experiments with four replicates in each experiment.

Control	SOD Activity Postfumigation	
	4 h	21 h
	total units mg^{-1} Chl	
9088 ± 501	14,098 ± 321	14,211 ± 432

photosynthesis. The decline in photosynthesis observed could be due to an inhibition of the ribulose bisphospate carboxylase enzyme (either directly or indirectly) by ozone or to some other process as yet unknown. Either a diversion of reductant away from carbon fixation, or an inhibition of Rubisco, or both may explain our results.

Both initial GSH levels and the increases observed as a result of fumigation are quite similar to those reported previously (1). Total glutathione did not change in the first 15 min of ozone fumigation. During this period, GSH levels were reduced by more than half while GSSG levels increased dramatically from 5 to 90 nmol mg Chl_{-1}. The first detectable increase in total glutathione occured 30 min after the onset of ozone fumigation. At this time the glutathione was still present primarily in the oxidized form. After 60 min reduced glutathione levels began to recover, and at 90 min the GSH:GSSH ratio was 1.25. This ratio was maintained 4 h after the ozone fumigation had been terminated while the total glutathione levels were greater at that time than at 60 min of ozone exposure. The results suggest that initially GSSG reduction was not keeping pace with GSH oxidation. At the 30 min time point of ozone fumigation GSSG reduction appeared not to be keeping pace with either GSH oxidation or synthesis. GSH levels began to recover between 60 and 90 min of fumigation. Between 60 and 90 min of ozone fumigation the reduction of GSSG began to equal GSH synthesis and oxidation. Four hours after the ozone was turned off the GSH:GSSG ratio was 1.25 while 21 h later it was 10. Apparently the synthesis and oxidation of glutathione was stimulated to a greater degree by ozone than was the reduction of GSSG. The GSH:GSSG ratios in these plants only approximated that of nonfumigated poplars at the 21 h sampling time.

The initial decline in GSH and accumulation of GSSG observed are consistent with the proposal that GSH inhibits γ-glutamyl cysteine synthetase and thus glutathione synthesis (25). Thus, the low GSH:GSSG ratios observed initially may stimulate glutathione biosynthesis. As GSH began to accumulate, glutathione biosynthesis may have been shut off (4 and 21 h after fumigation). The increased activity and/or induction of glutathione reductase presumably both renewed GSH for further scavenging of free radicals and also prevented GSSG from accumulating in toxic amounts. GSSG is thought to prevent protein synthesis (15). Reduced glutathione has been shown to increase under conditions of oxidative stress (1, 10). In *Phaseolus vulgaris* reduced glutathione levels were significantly lower in two sensitive cultivars, while in two ozone tolerant cultivars the reduction in GSH was slight (10). These divergent results probably reflect varying conditions of pollutant exposure and/or dose.

Glutathione synthesis and its reduction from the oxidized state occurs in the chloroplast and perhaps also in the cytosol and both of these processes require substrates and, ultimately, reductant. Lernherr *et al.* (17, 18) have shown that, in the case of wheat grown in elevated ozone levels, both the ATP:ADP ratio and the triose phosphate:3-phosphoglycerate ratio increase when apparent photosynthesis is inhibited. In their system, ribulose bisphosphate continued to be regenerated and the inhibition of apparent photosynthesis observed was a result of decreased ribulose bisphosphate carboxylation (23). They conclude that photochemical products can accumulate in an ozone environment when the reductive pentose phosphate pathway is inhibited. In our case, under very different conditions (short-term *versus* prolonged exposure), NADPH (one product of photochemistry) did not accumulate, even though carbon fixation was inhibited. Substrates and reductant required for antioxidant biosynthesis may originate from photochemical processes and this may result in lowered carbon fixation. The decreased photosynthesis observed may also be partly the result of decreased Rubisco activity brought about by exposure to ozone.

Superoxide dismutase activity increased by about 15% after 90 min of fumigation and then increased by another 15 to 20% 4 h after the ozone was turned off. In our system, glutathione responded before superoxide dismutase. It may be that if the stress imposed were milder (lower concentrations of ozone and/or shorter duration of fumigation) the glutathione response might have been adequate for indirectly scavenging free radicals, and the dismutase may not have been invoked.

Like glutathione, superoxide dismutase levels have been shown to change in foliar tissue exposed to oxidative stress (16). In an ozone sensitive snap bean cultivar exposure to (*N*-[2-(2-oxo-1-imidazolidinyl ethyl]*N*-phenylurea (an antiozonide), tolerance to ozone injury was always correlated with increases in superoxide dismutase levels and leaf age. Young leaves had greater dismutase levels and were more tolerant to ozone (16). Superoxide dismutase in poplar leaves increases after exposure to sulfur dioxide and these leaves become more tolerant to sulfhur dioxide exposures (30). Paraquat, benzyl viologen, methyl viologen (agents which cause increases in the superoxide radical in cells) cause increases of superoxide dismutase (12, 19, 24). In our study, superoxide dismutase activity increased with fumigated trees having one and a half more enzyme activity than control trees after the 4 h fumigation (9.1×10^3 *versus* 14.1×10^3 units mg^{-1} Chl, control and fumigated, respectively). There was no change in total Chl (data not shown); so the elevated enzyme and glutathione levels reported in fumigated leaves represent true increases. The Cu-Zn and Mn isozymes isozymes of superozide dismutase all increased with ozone exposure in the poplar. There was no enhanced increase of one isozyme over another.

If the glutathione and superoxide dismutase do provide an adaptive function by scavenging free radicals, as has been

suggested (29), one would expect that the plants at 21 h after ozone fumigation, with their elevated glutathione and superoxide dismutase, would be less susceptible to another ozone exposure. This raises the intriguing possibility that plants can be acclimated to ozone exposure and perhaps much of the contradictory field evidence gathered may be the result of data gathered from acclimated and nonacclimated plants.

LITERATURE CITED

1. **Alscher R, Bower J, Zipfel W** (1987) The basis for different sentitivities of photosynthesis to SO_2 in two cultivars of pea. J Exp Bot **38:** 99–108
2. **Alscher RG, Amundson RG, Cumming JR, Fellows S, Fincher J, Rubin G, Van Leuken P, Weinstein LH** (1989) Seasonal changes in the pigments, carbohydrates and growth of red spruce as affected by ozone. New Phytol **113:** 211–223
3. **Arnon DI** (1949) Copper enzymes in chloroplasts. Polyphenoloxidases in Beta vulgaris. Plant Physiol **24:** 1–14
4. **Beauchamp CO, Fridovich I** (1973) Isozymes of superoxide dismutase from wheat germ. Biochim Biophys Acta **317:** 50–64
5. **Charles SA, Halliwell B** (1980) Effect of hydrogen peroxide on spinach (*Spinacea oleraceae*) chloroplast fructose bisphosphatase. Biochem J **189:** 373–376
6. **Coulson C, Heath RL** (1974) Inhibition of the photsynthetic capacity of isolated chloroplasts by ozone. Plant Physiol **53:** 32–38
7. **Darrell NM** (1989) The effect of air pollutants on physiological processes in plants. Plant Cell Environ **12:** 1–30
8. **Dhindsa RS, Plumb-Dhindsa P, Thorpe TA** (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dosmutase and catalase. J Exp Bot **32:** 93–101
9. **Gordon D, Beck E** (1979) H_2O_2 destruction by ascorbate-dependant systems from chloroplasts. Biochim Biophys Acta **546:** 426–435
10. **Guri A** (1983) Variation in glutathione and ascorbate acid content among selected cultivars of Phaseolus vulgaris prior to and after exposure to ozone. Can J Plant Sci **63:** 733–737
11. **Halliwell B, Gutteridge JMC** (1984) Free Radicals in Biology and Medicine. Clarendon Press, Oxford
12. **Harper DB, Harvey BMR** (1978) Mechanism of parquat tolerance in perennial ryegrass. II. Role of superoxide dismutase, catalase and peroxidase. Plant Cell Environ **1:** 211–215
13. **Hassan MA, Asada K** (1984) Inactivation of ascorbate peroxidase in spinach chloroplasts on dark addition of hydrogen peroxide: its protection by ascorbate. Plant Cell Physiol **25:** 1285–1295
14. **Jackson C, Dench J, Moore AL, Halliwell B** (1978) Subcellular localisation and identification of superoxide dismutase in the leaves of higher plants. Eur J Biochem **91:** 339–344
15. **Kan B, London LM, Levins DH** (1988) Role of reversing factor in the inhibition of protein synthesis initiation by oxidized glutathione. J Biol Chem **263:** 15652–15656
16. **Lee EH, Bennett JH** (1982) Superoxide dismutase. A possible protective enzyme against ozone injury in snap beans (*Phaseolus vulgaris* L.) Plant Physiol **69:** 1444–1449
17. **Lernherr B, Grandjean A, Machler F, Fuhrer J** (1987) The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. J Plant Physiol **130:** 189–200
18. **Lernherr B, Machler F, Grandjean A, Fuhrer J** (1988) The regulation of photosynthesis in leaves of field-grown spring wheat (*Triticum aestivum* L., cv Albis) at different levels of ozone in ambient air. Plant Physiol **88:** 1115–1119
19. **Lewinson E, Gressel J** (1984) Benzyl viologen mediated counteraction of diquat and paraquat. Plant Physiol **76:** 125–130
20. **Matsumura H, Miyachi S** (1980) Cycling assay for nicotinamide adnine dinucleotides. Methods Enzymol **69:** 465–470
21. **Mehlorn H, Seufert G, Schimdt A, Kunert KJ** (1986) Effect of SO_2 and O_3 on production of antioxidants in conifers. Plant Physiol **82:** 336–338
22. **Nakano Y, Asada K** (1980) Spinach chloroplasts scavenge hydrogen peroxide on illumination. Plant Cell Phyiol **21:** 1295–1307
23. **Pell EJ, Pearson NS** (1983) Ozone-induced reduction in quantity of ribulose-1, 5-bisphosphate carboxylase in alfalfa foliage. Plant Physiol **73:** 185–187
24. **Rabinowitch HD, Fridovich I** (1985) Growth of Chlorella sorokiniana in the presence of sulfite elevates cell content of superoxide dismutase and imparts reistance towards paraquat. Planta **141:** 524–528
25. **Rennenberg H** (1982) Glutathione metabolism and possible biological roles in higher plants. Phytochemistry **21:** 2778–2781
26. **Schreiber U, Vidaver W, Runeckles V, Rosen P** (1978) Chlorophyll fluorescence assay for ozone injury in intact plants. Plant Physiol **61:** 80–84
27. **Sen Gupta A, Alscher RG, McCune DC** (1990) Ozone exposure, glutathioe levels and photosynthesis in hybrid poplar. *In* H Rennberg, C Brunold, L De Kok, I Stulen, eds, Sulfur Nutrition and Sulfur Assimilation in Higher Plants. SPB Academic Publishing, The Hague, pp 195–197
28. **Smith IK** (1985) Stimulation of glutathione synthesis in photorespiring plants by catalase inhibitors. Plant Physiol **79:** 1044–1047
29. **Tanaka K, Suda Y, Kondo N, Sugahara K** (1985) O_3 tolerance and the ascorbate dependent H_2O_2 decomposing system in chloroplasts. Plant Cell Physiol **26:** 1425–1431
30. **Tanaka K, Sugahara K** (1980) Role of superoxide dismutase in the defense against SO_2 toxicity and induction of superoxide dismutase with SO_2 fumigation. Res Rep Natl Inst Environ Stud **11:** 155–164