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Ozone-Induced Expression of Stress-Related Genes in *Arabidopsis thaliana*[1]

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Ozone is a major gaseous pollutant that is known to have detrimental effects on plant growth and metabolism. We have investigated the effects of ozone on *Arabidopsis thaliana* growth and the pattern of expression of several stress-related genes. *A. thaliana* plants treated with either 150 or 300 parts per billion (ppb) ozone daily for 6 h exhibited reduced growth and leaf curling. Fresh and dry weights of ozone-treated plants were reduced 30 to 48% compared to ambient air controls. RNA blot analyses demonstrated that mRNA levels for glutathione *S*-transferase (GST), phenylalanine ammonia-lyase (PAL), a neutral peroxidase, and a cytosolic Cu/Zn superoxide dismutase (SOD) were higher in plants treated with 300 ppb ozone than in ambient air-treated controls. The mRNA levels of lipoxygenase and a catalase were not affected by ozone treatment. Of the transcripts examined, GST mRNA levels increased the most, showing a 26-fold induction 3 h after the initiation of ozone treatment. PAL mRNA was also rapidly induced, reaching 3-fold higher levels than controls within 3 h of ozone treatment. The neutral peroxidase and SOD mRNA levels rose more slowly, with both reaching maximum levels corresponding to 5-fold and 3-fold induction, respectively, approximately 12 h after ozone treatment. These studies indicate that ozone-induced expression of stress-related genes in *A. thaliana* provides an excellent model system for investigating the molecular and genetic basis of ozone-induced responses in plants.

Ozone pollution is thought to have caused more damage to vegetation in the United States than any other pollutant (Heagle, 1989). The severity of ozone damage can vary significantly, depending on the plant species or variety examined, the ozone concentration, and the duration of ozone exposure. The symptoms of ozone injury include bleaching of mesophyll cells, chlorotic mottling, changes in pigmentation, and necrosis. Acute short-term exposure to high ozone concentrations (>200 ppb) generally results in visible damage, whereas long-term, chronic exposure to lower ozone concentrations generally leads to reduced growth without visible foliar damage (reviewed by Heagle, 1989; Heck, 1989). Examples of the ozone-induced physiological changes underlying reduced growth and visible injury are reduced photosynthesis, increased respiration rate, membrane lipid peroxidation, enhanced rate of senescence, and reduced transpiration due to stomatal closing (Runeckles and Chevone, 1992). Ozone-induced decreases in crop growth and yields have generally been attributed to reduced transpiration, enhanced respiration, altered assimilate partitioning, and depressed photosynthesis. Depressed photosynthesis may be due to loss of ribulose-1,5-bisphosphate activity and reduced stomatal conductance (Pell et al., 1990).

It is thought that ozone enters the mesophyll via stomata, where it is rapidly dissolved in water and converted into reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (H_2O_2) (Grimes et al., 1983; Mehlhorn et al., 1990; Kanofsky and Sima, 1991). These active oxygen species potentially initiate oxidation reactions and generate more oxidants by reacting with various cellular macromolecules. For example, ozone can react with biogenic alkenes in isoprene-emitting plants such as spruce and fern to form phytotoxic organic peroxides and H_2O_2 (Becker et al., 1990; Hewitt et al., 1990). Active oxygen species, along with organic peroxides, are the most likely candidates for causing ozone-mediated inactivation and enhanced degradation of redox-sensitive proteins, lipid peroxidation, and other damage related to oxidative stress.

Plants employ several defense mechanisms to combat oxidative stress such as exposure to ozone. These defense systems are thought to operate either by preventing the formation of ozone-induced active oxygen species or by scavenging them once they are formed. Some of the proposed components of these oxidative defense systems are enzymes such as SOD, catalase, and peroxidase, and low molecular mass scavengers such as ascorbic acid, phenolic compounds, and the tripeptide glutathione (Scandalios, 1990; Bowler et al., 1992; Alscher and Hess, 1993). The potential role of glutathione during oxidative stress in plants has recently been reviewed (Hausladen and Alscher, 1993). Hydroxyl radicals and superoxide anions may be scavenged by GSH. In addition, glutathione acts in concert with ascorbic acid to remove H_2O_2. Ascorbic acid reacts with H_2O_2 in the presence of ascorbate peroxidase to form an intermediate monodehydroascorbate radical. The monodehydroascorbate radical can be directly reduced to ascorbic acid by monodehydroacorbate reductase. Alternatively, the monodehydroascorbate radical can dismutate to dehydroascorbate, which can be converted into ascorbic acid in a reaction catalyzed by dehydroascorbate reductase with the concomitant oxidation of GSH to GSSG. GSH is regenerated in the presence of NADPH by glutathione

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Abbreviations: GST, glutathione S-transferase; LOX, lipoxygenase; PAL, phenylalanine ammonia-lyase; ppb, parts per billion; SOD, superoxide dismutase.

reductase. Another important function of glutathione is to detoxify toxic products of lipid peroxidation and xenobiotics such as herbicides. GST catalyzes these detoxification reactions by conjugating glutathione with hydrophobic electrophiles. GST also displays peroxidase activity (Meister and Anderson, 1983; Hausladen and Alscher, 1993).

The antioxidative responses summarized above have been studied during ozone stress in several plant species with variable results (Lee and Bennett, 1982; Tanaka et al., 1985; Mehlhorn, 1990; Price et al., 1990; Sen Gupta et al., 1991; Bowler et al., 1992). In some cases increases in glutathione reductase, peroxidase, and SOD activities were due to increased enzyme synthesis. Several correlative studies have shown that increased SOD enzyme activities and mRNA levels were associated with ozone treatment of tobacco and bean (Herouart et al., 1991; Bowler et al., 1992; Pitcher et al., 1992). Other studies of the effects of ozone on plant gene expression have shown that the steady-state levels of pathogenesis-related protein 1b, β-1,3-glucanase, and chitinase increased in ozone-treated tobacco plants (Ernst et al., 1992). Ozone treatment has also been correlated with decreased mRNA levels of the large and small subunits of Rubisco in ozone-treated potato plants (Reddy et al., 1991). The mechanisms responsible for the effects of ozone on gene regulation are not known, and to date the isolation of genes that are uniquely regulated by ozone has not been reported.

The objective of this study was to determine the feasibility of using *Arabidopsis thaliana* as a model system to study the genetic and molecular basis of ozone-induced responses in plants. As a first step toward this goal, we have characterized effects of ozone treatment on *A. thaliana* growth and morphology as well as the expression patterns of several genes known to be regulated by oxidative and pathogen stress.

MATERIALS AND METHODS

Growth and Treatment of the Plants

Arabidopsis thaliana, ecotype Columbia (Col-0), plants were grown under a 12-h photoperiod in controlled-growth chambers at 22°C and 50 to 80% RH. Three- to 4-week-old plants were transferred to ozone fumigation chambers operating under similar growth conditions housed at the U.S. Department of Agriculture Forest Service Northeastern Forest Experiment Station (Delaware, OH). Plants were exposed to 150 or 300 ppb ozone for 6 h every day (9 AM to 3 PM) for 14 d. Ozone was generated by an electric discharge in dry oxygen (model 03V10–0 Orec ozone generator, Ozone Research and Equipment Corp., Phoenix, AZ). Ozone levels were continuously monitored and maintained within ±50 ppb of the set limits by a computerized control system. All experiments were completed before the onset of flowering. The ozone concentration in the ambient air control chambers was monitored and varied from 0 to 30 ppb over the course of these experiments.

Tissue Sampling

For growth studies, the aerial parts of the plants (rosettes) were harvested after 14 d of treatment and weighed to determine the fresh weight of individual plants. Dry weights of individual rosettes were determined after drying at 90°C for 2 d. Leaves to be used for RNA extractions were harvested at the indicated times, frozen in liquid nitrogen, and stored at −80°C.

RNA Extractions and Analysis

Total RNA was isolated using a phenol-SDS extraction and LiCl precipitation method (Davis et al., 1991). RNA was quantified by measuring the A_{260}, and samples containing 5 μg of total RNA were fractionated on formaldehyde agarose gels as previously described (Davis and Ausubel, 1989). Gels were blotted onto Duralon membranes (Stratagene) with 10× SSPE (1× SSPE contains 0.15 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA, pH 7.4). Damp filters were UV cross-linked and prehybridized in a solution containing 5× SSPE, 5× Denhardt's solution, 0.5% SDS, and 200 μg/mL of salmon testes DNA. DNA probes were labeled with ^{32}P using a random-primer labeling kit (BRL) and purified using a 1-mL Sephadex G-50 gel permeation spin column. Radioactive probes were heat denatured and added directly to the prehybridization solution. Hybridizations were conducted at 65°C for 16 to 24 h. Filters were washed with 0.5× SSPE containing 1% SDS at 65°C for 30 min. The air-dried filters were then exposed to Kodak XAR5 x-ray film with a Cronex intensifying screen at −80°C and/or analyzed by Molecular Dynamics (Sunnyvale, CA) PhosphorImager. To verify that equal amounts of RNA were loaded in each lane, all the filters were probed with a 28S ribosomal gene probe from pea (Wanner and Gruissem, 1991). Quantitative data from the RNA blots was obtained from PhosphorImager scans using the Molecular Dynamics ImageQuant software to calculate the total pixel values (counts) in equal-sized areas covering individual hybridizing bands. All data shown have been corrected for loading differences using the counts obtained with the control rRNA probe. All experiments were repeated three times and the data shown are from a representative experiment. The percent difference observed in these experiments ranged from 5 to 20% of the data shown.

RESULTS

Ozone-Induced Changes in Growth and Morphology

We characterized the effects of a daily 6-h ozone treatment on the growth of *A. thaliana*, ecotype Col-0, because this ecotype is one of the best genetically characterized ecotypes. Initial studies of the effects of ozone on *A. thaliana* growth and morphology demonstrated that both 150 and 300 ppb ozone caused downward curling of the leaves within the first 3 d of the experimental treatment (Fig. 1). There was no noticeable difference between the extent of leaf curling caused by the 150- and 300-ppb treatments. Besides leaf curling, a few pinhead-sized, dry lesions were observed on both the abaxial and adaxial leaf surfaces in 10 to 20% of the plants treated with 300 ppb ozone. Ambient air control plants did not show leaf curling or lesion development. Effects on growth were assessed in terms of the difference in fresh and dry weights of the plants at the end of 14 d of ozone treatment (Table I). Plants exposed to either 150 or 300 ppb ozone exhibited a reduction in fresh and dry weights of 30 to 48%

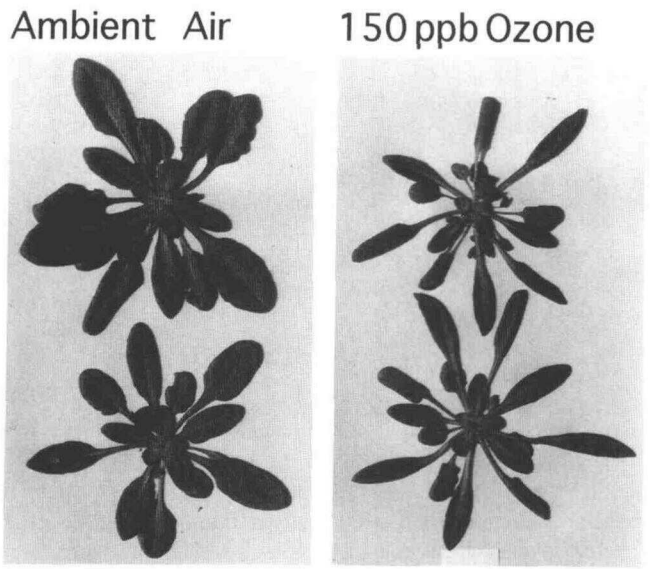

Figure 1. Ozone-induced leaf curling and reduced growth in *A. thaliana*. Plants were grown for 3 weeks in ambient air and transferred to chambers where they were maintained in ambient air or exposed to 150 ppb ozone for 6 h each day for 14 d prior to being photographed.

or 33 to 47%, respectively, compared to the ambient air controls.

Ozone-Modulated Gene Expression

RNA blot analysis was used to determine if mRNA levels for the stress-related and antioxidant proteins listed in Table II were affected in *A. thaliana* subjected to ozone fumigation. In preliminary experiments, total RNA isolated from plants treated with 150 ppb ozone for different time periods was analyzed. The 150-ppb ozone treatment did not cause consistent changes in the mRNA levels for any of the probes tested (data not shown). However, analyses of total RNA isolated from plants exposed to 300 ppb ozone demonstrated that this level of ozone treatment did consistently induce the accumulation of mRNAs of several of these stress-related genes. Therefore, detailed RNA blot experiments were conducted with plants treated with 300 ppb ozone.

The GST transcript was present at relatively low abundance in control plants and transiently accumulated to higher levels in ozone-treated plants. The highest GST mRNA level was observed at 3 h of ozone treatment, the earliest time after ozone treatment tested, after which it declined to near control levels by the end of the first 24 h of treatment (Fig. 2A). Quantitative analyses revealed that the maximal GST mRNA level observed (time = 3 h) was 26-fold higher than the corresponding control. We also monitored the GST mRNA levels on subsequent days at the end of the daily 6-h ozone treatment. GST mRNA was induced every day by ozone treatment, although in some cases the induced levels were lower than that observed after the 1st d of ozone treatment (Fig. 2B).

Since peroxidases have been suggested to have a role in detoxifying H_2O_2 in ozone-treated plants, we tested whether or not accumulation of a neutral peroxidase mRNA was affected by ozone treatment of *A. thaliana*. We observed an ozone-dependent induction of this peroxidase mRNA within 3 h. The peroxidase message level continued to increase to a maximum level of 5-fold induction by 12 h, after which it declined to near control levels by 24 h (Fig. 3A). Increased accumulation of the peroxidase mRNA was also observed on subsequent days after the daily 6-h ozone treatment (Fig. 3B).

We also tested the effects of ozone treatment of *A. thaliana* on the mRNA levels for two other putative antioxidant proteins, SOD and catalase. We found that the 300-ppb ozone treatment resulted in the induction of a Cu/Zn SOD transcript. The levels of this SOD mRNA were 2- to 3-fold higher than the levels in ambient air controls by 12 h after the onset of ozone treatment. SOD mRNA levels then decreased to control levels by 24 h (Fig. 4A). The Cu/Zn SOD transcript was detected at levels only marginally higher than, or at levels the same as, those observed in controls on subsequent days after the daily 6 h of ozone treatment (Fig. 4B). This result is consistent with the observation that the SOD mRNA level is only weakly induced after 6 h of ozone treatment (Fig. 4A). We did not observe any ozone-induced changes in catalase mRNA levels in *A. thaliana* shoots (Fig. 5).

To test the effects of ozone on another stress-related response, we examined the mRNA levels of PAL, the first enzyme in phenylpropanoid biosynthesis. As was the case with GST, the highest level of PAL mRNA observed was at 3 h after ozone treatment, the earliest time after ozone

Table I. *Effect of ozone treatment on the growth of A. thaliana*

Rosettes of plants exposed to either ambient air or ozone were harvested after 14 d of treatment prior to fresh and dry weight determinations. Values reported are the means ± SE.

Experiment (*n*)	Fresh Weight			Dry Weight		
	Ambient air	Ozone[a]	Percent control	Ambient air	Ozone	Percent control
	g			*mg*		
1 (60)	1.72 ± 0.07	0.98 ± 0.05[b]	57	145 ± 15	77 ± 9[b]	53
2 (30)	0.87 ± 0.04	0.61 ± 0.04[b]	70	65 ± 3	44 ± 3[b]	68
3 (30)	1.20 ± 0.06	0.80 ± 0.04[b]	67	85 ± 4	56 ± 3[b]	66
4 (30)	1.33 ± 0.07	0.69 ± 0.03[b]	52	93 ± 4	57 ± 2[b]	61

[a] In experiments 1 and 2, plants were treated with 150 ppb ozone; in experiments 3 and 4, plants were treated with 300 ppb ozone. [b] Significant differences between plants exposed to either ambient air or ozone at the 0.001 probability level according to a *t* test.

Table II. *Description of DNA probes used in this study*

Probe	Clone Description	Reference/Source
Peroxidase	1.9-kb *Eco*RV fragment of a neutral peroxidase genomic clone	This laboratory; Intapruk et al. (1991)
Cu/Zn SOD	0.8-kb cDNA clone	Hindges and Slusarenko (1992)
GST	0.9-kb *Bam*HI fragment of a *GST-1* cDNA clone	F. Ausubel laboratory
Catalase	1.8-kb *CAT2* cDNA clone	Chevalier et al. (1992)
LOX	1.4-kb *Eco*RI fragment of the *LOX1* gene	Melan et al. (1993)
PAL	0.5-kb *Hin*dIII fragment of *PAL1* genomic clone	This laboratory

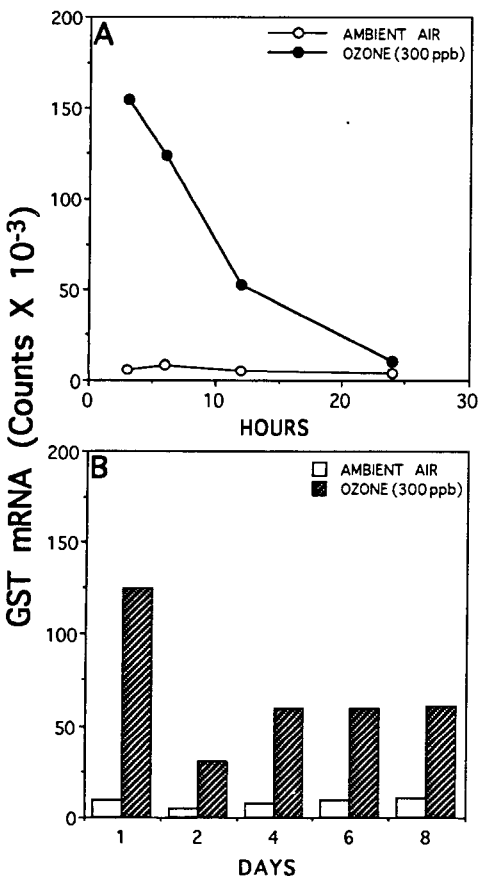

Figure 2. Ozone-dependent induction of GST mRNA accumulation in *A. thaliana*. Total RNA was isolated from leaves harvested from plants at the indicated times and subjected to RNA blot analysis using GST as a probe. The amount of hybridizing radioactivity was quantified using a PhosphorImager. Similar results were obtained in two other independent experiments. A, RNA accumulation during the 24 h after the start of the first 6-h ozone treatment. B, RNA accumulation measured on each day from tissues harvested at the end of the daily 6-h ozone treatment. Day 1 corresponds to the 6-h time point shown in A.

treatment tested. PAL mRNA levels then declined to near control levels by the end of the first 12 h of treatment (Fig. 6A). The maximum level observed was approximately 3-fold higher than that in control plants. As was observed for GST and peroxidase mRNAs, increased PAL mRNA levels were usually observed on subsequent days after the daily 6-h ozone treatment (Fig. 6B).

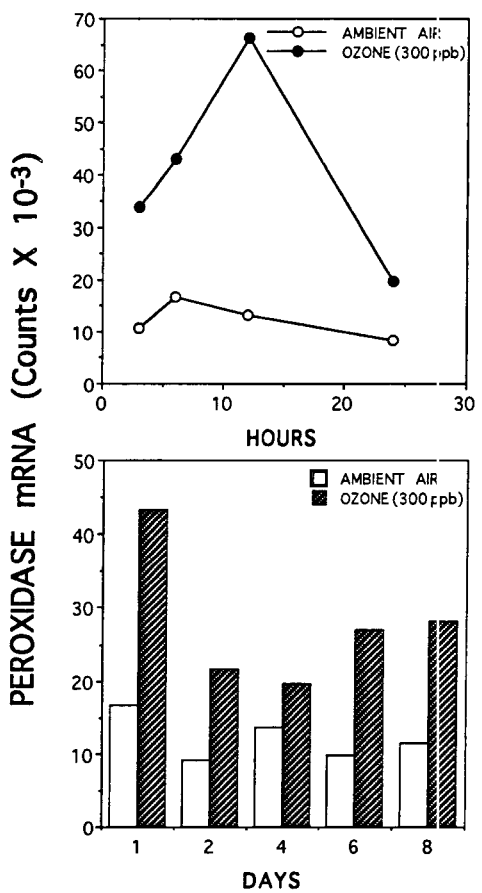

Figure 3. Ozone-dependent induction of neutral peroxidase mRNA accumulation in *A. thaliana*. Experimental details were the same as for Figure 2.

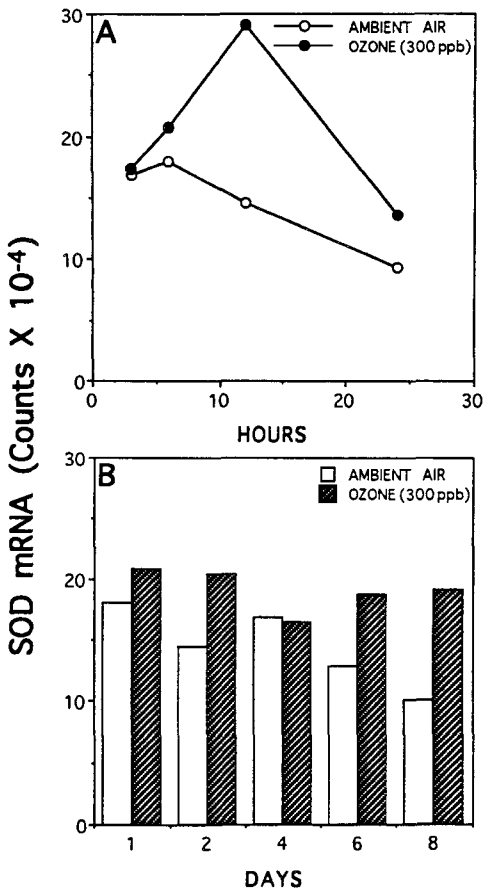

Figure 4. Ozone-dependent induction of SOD mRNA accumulation in *A. thaliana*. Experimental details were the same as for Figure 2.

We also tested whether or not mRNA levels for LOX, an enzyme involved in lipid peroxidation and the production of jasmonic acid, a potential signal molecule, was induced in *A. thaliana* by ozone exposure. *LOX1* mRNA was expressed at low levels in both control and ozone-treated plants, and no ozone-dependent changes in *LOX1* mRNA levels were observed, even after repeated daily exposures (data not shown).

DISCUSSION

In recent years the use of *A. thaliana* has led to rapid advances in our understanding of many aspects of plant growth and development. A number of mutants and genes have been isolated that affect various plant processes, including responses to environmental stresses such as pathogen attack, wounding, UV light, and oxidative stress (Davis, 1992; Hindges and Slusarenko, 1992; Zhou and Goldsbrough, 1993). Although we have learned a great deal about ozone effects on plants in other systems, the advantages that *A. thaliana* offers as an experimental system may provide a valuable opportunity to define further the molecular mechanisms responsible for ozone stress in plants.

Plant responses to ozone exposure have been a subject of ongoing investigations in several laboratories (Runeckles and Chevone, 1992). The results presented in this paper show that *A. thaliana* is sensitive to ozone treatment and that ozone effects are manifested at both the morphological level and at the level of gene expression. The general phenotypic responses to ozone stress in *A. thaliana* are similar to those observed in other ozone-sensitive plants. For example, exposure of *A. thaliana* to 150 and 300 ppb ozone for 2 weeks caused a significant reduction in biomass (Table I). In addition to reduced growth, ozone treatment also caused pronounced downward curling of rosette leaves. This leaf curling may be related to stress-induced ethylene biosynthesis, since ethylene has been proposed to be a major factor in mediating the effects of ozone in plants and is known to be associated with leaf curling symptoms (Mehlhorn and Wellburn, 1987; Reddy et al., 1991). It has been suggested that ethylene produced during ozone exposure reacts with ozone to form damaging oxygen radicals, which are primarily responsible for the foliar damage observed in pea (Mehlhorn and Wellburn, 1987). In addition, ethylene treatment of mung bean or pea before ozone exposure provided protection against damage caused by ozone, possibly by inducing defense mechanisms such as peroxidase activity (Mehlhorn, 1990). Other evidence suggesting a role for ethylene in mediating ozone effects include observations that some ozone-induced genes and enzyme

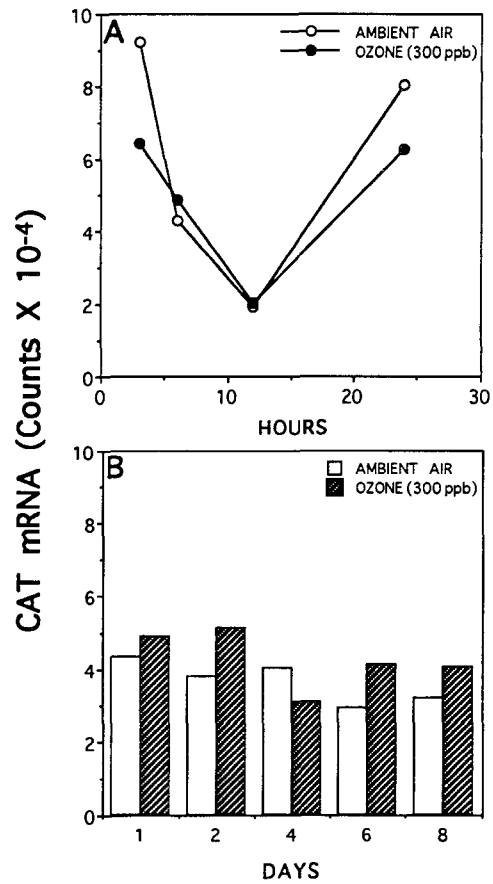

Figure 5. Lack of effect of ozone treatment on catalase (CAT) mRNA accumulation in *A. thaliana*. Experimental details were the same as for Figure 2.

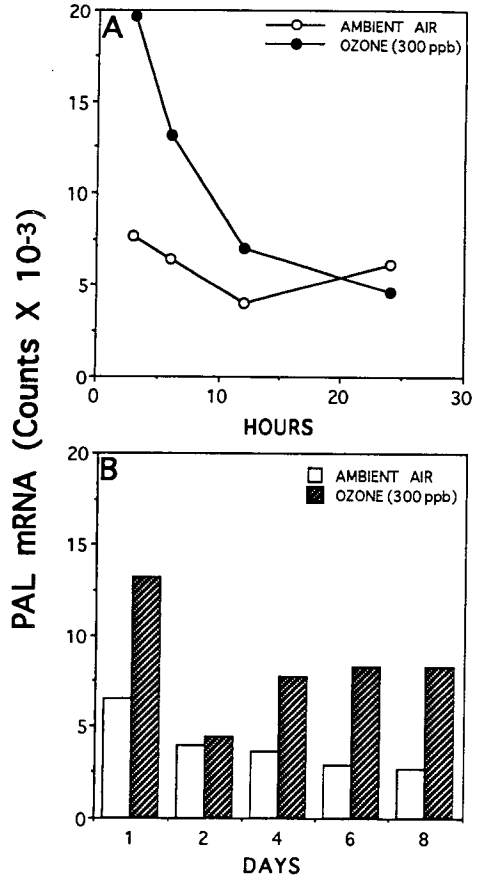

Figure 6. Ozone-dependent induction of PAL mRNA accumulation in *A. thaliana*. Experimental details were the same as for Figure 2.

activities such as peroxidase, GST, PAL, and chitinase are also induced by ethylene (Ecker and Davis, 1987; Morgens et al., 1990; Samac et al., 1990; Zhou and Goldsbrough, 1993). The availability of ethylene-insensitive and ethylene-overproducing mutants of *A. thaliana* (reviewed by Kieber and Ecker, 1993) that appear to be part of an ethylene-responsive signal transduction chain (Chang et al., 1993; Kieber et al., 1993) may prove to be valuable for further investigations of the role of ethylene during ozone stress.

In the present study, we have also shown that exposure to ozone led to increased mRNA levels of several stress-related genes. GST mRNA levels were transiently induced as much as 26-fold, reaching maximum levels within 3 h after treatment (Fig. 2). As suggested in previous studies, changes in glutathione metabolism may play a role in limiting damage to oxidative stress conditions such as exposure to ozone, sulfur dioxide, paraquat, and drought (reviewed by Hausladen and Alscher, 1993). In poplar, after an initial decline in GSH content, an overall increase in GSH, GSSG, and total glutathione content was observed in leaves in response to ozone fumigation (Sen Gupta et al., 1991). In an ozone-sensitive cultivar of *Phaseolus vulgaris*, GSH levels were found to be lower than in the ozone-tolerant cultivars (Guri, 1983). This difference was later attributed to 2-fold higher glutathione reductase activity in the tolerant cultivar. Price et al. (1990) observed a marked increase in GST activity in barley tissues as a result of ozone fumigation that was correlated with increased lipid peroxidation, suggesting that GST may play a role in the detoxification of hydrophobic electrophilic substances. Our results indicate that GST may have a similar role in protecting *A. thaliana* from ozone damage. Moreover, the relatively large increase in GST mRNA levels observed in ozone-treated plants suggests that GST may be an appropriate gene for detailed studies on ozone-mediated gene activation.

We also observed an induction of a neutral peroxidase in leaves of ozone-treated *A. thaliana*. This peroxidase was transiently induced, reaching a maximum 5-fold induction 12 h after the beginning of ozone treatment (Fig. 3). Other studies showed that ozone treatment caused a significant increase in the level of extracellular peroxidases in *Sedum* leaves, spruce needles, and bean leaves (Peters et al., 1989, and refs. therein). Similarly, a coordinated increase in glutathione reductase and ascorbate peroxidase activities was observed in response to ozone stress (Mehlhorn, 1990). In soybean, ozone-induced peroxidase activity increased more rapidly in a cultivar highly sensitive to ozone, leading to suggestions that peroxidase activity changes were important in determining differential sensitivity (Tingy et al., 1976). In bean, increased extracellular peroxidase activity was partly ascorbate dependent (Peters et al., 1989), and in another study, ascorbate peroxidase was shown to be induced by 100-ppb ozone treatment, whereas a concentration of 500 ppb caused a decrease in ascorbate peroxidase activity (Tanaka et al., 1985). The identification of a peroxidase mRNA in *A. thaliana* that is modulated by ozone treatment provides an opportunity to clarify the potential role of peroxidase in ozone stress.

Catalases have also been implicated in removing ozone-induced H_2O_2 (Lee and Bennett, 1982; Badiani et al., 1993). However, we observed no ozone-induced changes in catalase mRNA levels in *A. thaliana* leaves (Fig. 5). Although it is possible that circadian induction of this catalase mRNA may have minimized detection of an ozone effect, our results suggest that at least this catalase is not part of the ozone-induced defense systems in *A. thaliana*. However, it remains possible that ozone regulation of catalase activity at the translational or posttranslational level may be important. Our results are consistent with those observed in spinach, where no differences were found between the catalase activity in ozone-sensitive and ozone-tolerant spinach cultivars (Tanaka et al., 1985), and in maize, where ozone treatment did not cause any increase in catalase activity (Matters and Scandalios, 1987).

The current understanding of the role of another antioxidative enzyme, SOD, in providing protection against ozone is not very clear (Bowler et al., 1992). We observed a 2- to 3-fold increase in cytosolic Cu/Zn SOD mRNA level after ozone exposure (Fig. 4), suggesting that this enzyme may have some role in oxidative stress responses in *A. thaliana*. Previous studies have shown that ozone treatment caused increased SOD enzyme activities and mRNA levels in other plants (Herouart et al., 1991; Bowler et al., 1992; Pitcher et al., 1992). Studies of SOD overproduction in transgenic plants have yielded conflicting results. Overproduction of a petunia

chloroplast Cu/Zn SOD in genetically engineered tobacco did not confer an increased tolerance to 300 ppb ozone as determined by foliar damage (Pitcher et al., 1991). In contrast, Van Camp et al. (1994) found that overproduction of SOD in tobacco chloroplasts resulted in a 3- to 4-fold reduction in the visible damage caused by 80 to 150 ppb ozone. These contrasting results may be due to differences in the levels of SOD expression in the transgenic plants studied or the ozone concentrations used. Taken together, the available data indicate that SOD may be a component of plant defenses against ozone, and further studies of SOD and ozone in *A. thaliana* should prove informative.

Plant phenolics and other phenylpropanoid derivatives may function as antioxidants because of their free radical-trapping properties (Lewis, 1993). These compounds are also thought to be an important part of induced defense systems in response to environmental stresses such as pathogen infection, wounding, and UV exposure (Hahlbrock and Scheel, 1989). Ozone treatment induced isoflavonoid and flavonoid biosynthesis in soybean and pine, respectively (Keen and Taylor, 1975; Rosemann et al., 1991). Activities of PAL and other biosynthetic enzymes of the central phenylpropanoid and flavonoid pathways were shown to be increased by 8 h after ozone exposure in pine (Rosemann et al., 1991). We observed that ozone treatment rapidly and transiently increased PAL mRNA levels in *A. thaliana* leaves within 3 h of ozone treatment (Fig. 6), indicating that increased synthesis of phenylpropanoid derivatives may play a protective role in *A. thaliana* during ozone stress.

The mechanisms responsible for ozone-induced changes in gene expression have not been elucidated. It has been suggested that active oxygen species generated during pathogen attack and oxidative stress conditions such as elevated ozone levels act as signal molecules leading to induction of gene expression (Apostol et al., 1989; Ernst et al., 1992). This so-called "oxidative burst" results in the formation of oxygen radicals identical to those thought to be produced during ozone exposure. These oxygen radicals may in some way interact directly with a signal-transduction pathway that ultimately controls plant defense gene expression. For example, LOX can generate jasmonic acid, a product of plasma membrane lipid peroxidation that has been implicated as a signaling molecule in inducing defense proteins such as PAL and proteinase inhibitors (Gundlach et al., 1992). The observation that some of the same genes are induced by pathogen infection and ozone suggests that ozone may activate a signaling pathway that has components in common with the pathways induced during disease resistance (Apostol et al., 1989; Ernst et al., 1992). In soybean, LOX activity and mRNA levels were shown to be increased during ozone stress (Maccarone et al., 1992). However, we were not able to detect any changes in mRNA levels corresponding to a *LOX1* gene in *A. thaliana*. It is possible that another *A. thaliana* LOX gene recently described by Bell and Mullet (1993) is induced by ozone.

In conclusion, we have established that *A. thaliana* responds to ozone treatment in a manner similar to that observed in other plants. These initial data provide the basic framework for further investigations into the mechanisms of ozone action and the activation of protective systems. In addition, we can now evaluate the role of ethylene and active oxygen species as the links between the overlapping patterns of gene expression that are induced by ozone, pathogen infection, and wounding. A clearer understanding of the signal transduction pathways involved in the induction of protective antioxidative molecules by these stresses may directly relate to how plants respond to other oxidative stresses including drought, radiation, sulfur dioxide pollution, and other xenobiotic stresses.

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