Effects of Ozone and UV-B Radiation on Pigments, Biomass and Peroxidase Activity in Soybean

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ABSTRACT

Tropospheric ozone (O_3) in the eastern United States is estimated to reduce the production of some crops by as much as 15% or more. However, another environmental stress, ultraviolet-B (UV-B) radiation, may be increasing due to stratospheric O_3 depletion. Studies have shown that UV-B radiation can alter growth and development in some plants. Because little is known about the combined effects of these stresses, plant responses to O_3 in combination with UV-B radiation were examined. Soybeans were treated in open-top field chambers equipped with UV-B lamp banks or with similar facilities in a greenhouse. Results showed that O_3 treatments (80 – 100 ppb) accelerated chlorophyll loss, suppressed biomass accumulation and increased peroxidase activity compared with controls. None of these responses consistently occurred in plants treated in the field with UV-B radiation simulating up to a 37% loss of stratospheric O_3. Extracts of leaves treated with supplemental UV-B radiation showed increased absorbance in the UV-B waveband. This suggested that screening of UV-B radiation by foliar pigments provided protection from UV-B injury. Overall, our results suggested that increased tropospheric O_3 resulting from increased solar UV-B radiation may be an important factor when assessing the impact of stratospheric O_3 loss on soybean production.

INTRODUCTION

Little is known about plant responses to the combined effects of increased ultraviolet-B (UV-B) radiation and gaseous pollutants [1]. Solar UV-B radiation from 290 - 320 nm may be increasing at the earth's surface in temperate latitudes due to stratospheric ozone (O_3) depletion. Studies have shown that UV-B radiation can reduce growth and yield in some crops [2]. Tropospheric O_3, the most phytotoxic regional air pollutant, reduces the production of some crops by as much as 15% or more [1, 3]. In addition, the photochemical production of O_3 is driven by solar UV-B radiation, which suggests that levels of both stresses could increase with stratospheric O_3 depletion [4]. Therefore, it is important to understand how increased UV-B radiation in combination with O_3 might affect the physiology, growth and yield of a major agricultural crop such as soybean.

Chronic exposure of soybean to O_3 typically suppresses biomass accumulation, including yield, and induces chlorosis [5]. The effect of increased UV-B radiation on soybean growth and yield, however, has been variable in the few studies conducted under field conditions [2, 6]. Little change in chlorophyll content occurred when plants were treated with UV-B radiation in combination with high visible light levels. In contrast, the foliar concentration of UV-absorbing pigments, particularly flavonoids, typically increased in plants treated with UV-B radiation [2]. The level of phenylpropanoid monomers and polymers also increased in plants treated with O_3 [7]. In addition, changes in the activity of antioxygenic enzymes such as peroxidase and catalase sometimes have been observed in plants treated with either O_3 or UV radiation [8-11]. Increased antioxygenic enzyme activity appears to be a protective response for reducing the level of activated oxygen species in the plant [12]. Altogether, measurements of pigments, biomass and antioxygenic enzyme activity should indicate the level of injury, growth suppression and oxidative stress sustained by plants in response to O_3 and increased UV-B radiation.

To address these research objectives, experiments were conducted in two field studies and in a greenhouse. Chronic treatment effects on plant responses, mainly growth and yield, were examined in the 1990 field experiment. In 1991, experiments to test whether UV-B radiation promoted or protected plants from O_3 injury were conducted in the greenhouse and the field. In these experiments, plant growth and treatment methods were designed to elicit responses to O_3 quickly, and short-term pigment and enzymatic responses were closely monitored. Using these two approaches, the magnitude of the potentially deleterious interactions between O_3 and increased UV-B radiation could be examined.

METHODS AND MATERIALS

Growth Conditions, O_3 and UV-B Radiation Treatments

Soybeans *(Glycine max* (L.) Merr. cv. Essex) were grown in a medium of sandy loam soil and Metromix (3:1) in pots. Ozone treatments were administered in open-top field chambers or in continuous-flow stirred tank reactors (CSTRs) in the greenhouse [3]. Supplemental UV-B radiation was provided using banks of 40 W fluorescent lamps (model UVB-313, Q-Panel Co.) filtered with cellulose diacetate film (0.13 mm thickness) to remove radiation less than 290 nm or with polyester film to remove radiation less than 315 nm. Polyester-filtered radiation served as a control for the UV-A (320 – 400 nm) and visible radiation emitted by the lamps in the supplemental UV-B radiation treatments. Output from a lamp bank was controlled with a fluorescent lamp dimmer control, and height of the lamp bank was

adjusted to maintain a fixed distance above the plant canopy (0.4 m). The lamp bank and chamber assembly shaded about 24% of the solar UV and visible radiation in the open-top chambers [13]. Similar lamp banks were used for greenhouse applications.

Irradiance was measured with a UV-visible spectroradiometer (model 742, Optronic Laboratories, Inc.) fitted with a 3.7 m long quartz fiber-optic cable and diffuser head. The spectroradiometer was calibrated using a NIST-traceable 200-W tungsten-halogen lamp standard of spectral irradiance (model 220A, Optronic Laboratories) and regulated power supply. Wavelength calibration of the spectroradiometer was checked by comparison with Hg spectral emission lines from a UVB-313 lamp. Broad-band erythemal meters (Robertson-Berger meter and model 2D, Solar Light Co.) were used to monitor solar UV radiation continuously and to adjust lamp bank irradiance levels, respectively. Broad-band erythemal meters were calibrated against the spectroradiometer. Biologically-effective UV-B ($UV\text{-}B_{BE}$) irradiance was calculated by applying Caldwell's [14] generalized plant action spectrum, normalized to 300 nm, to the spectroradiometer scans.

Simulations of stratospheric O_3 loss and corresponding supplemental UV-B radiation treatments were calculated using a radiative transfer model requiring inputs for location, time of year, environment, barometric pressure, relative humidity and aerosol level [15]. Values for column O_3 thickness can be specified by the user or generated by the model. The column O_3 thickness value generated by the model for 21 June was 0.350 cm, which we used. Barometric pressure (1002 mb) and relative humidity (54%) were long-term averages of summer data collected at the Raleigh-Durham International Airport Weather Station. The aerosol level was set to a value of 1. The daily solar $UV\text{-}B_{BE}$ irradiance calculated by the model for our location (35.8° N, 78.7° W) on 21 June was 5.45 kJ m^{-2}, which was in good agreement with ground-based measurements. The average (± s.d.) daily solar $UV\text{-}B_{BE}$ irradiance measured by our Robertson-Berger meter was 4.89 ± 0.51 kJ m^{-2} for the 30 d surrounding 21 June in 1990 and 1991.

Experimental Design

Chronic treatment effects on plant responses, mainly growth and yield, were examined in the 1990 field experiment. Plants were treated from germination for 120 d with either charcoal-filtered air (CF) or 1.5x ambient O_3 in combination with three supplemental UV-B radiation treatments. Seasonal 12 h mean O_3 concentrations in the CF and O_3 treatments were 24 and 83 ppb, respectively. On 21 June, the daily supplemental $UV\text{-}B_{BE}$ irradiance in the three UV-B treatments was 0.00 (control), 4.43 (medium) and 8.13 (high) kJ m^{-2}. The UV-B radiation treatments corresponded to an increase in column O_3 thickness in the control of 15% (due to shading of ambient UV-B radiation) and decreases in the medium and high treatments of 22 and 37%, respectively. Supplemental UV-B treatments were administered as a constant addition daily with biweekly adjustments for seasonal changes in photoperiod and solar UV-B irradiance [13]. Four open-top chambers equipped with lamp banks were used for each treatment combination (n = 24).

Experiments to test if pretreatment with UV-B radiation promoted or reduced O_3 injury were conducted in the greenhouse and the field in 1991. From February to April, plants were grown for 35 d in the greenhouse and then treated with a daily supplemental $UV\text{-}B_{BE}$ irradiance of either 0 or 14 kJ m^{-2} for 7 d (one lamp bank per treatment). Plants were then transferred to CSTRs and treated 8 d with either CF air or 100 ppb O_3 6 h daily (two CSTRs per treatment). The experiment was conducted twice. During this period, average (± s.d.) daytime and nighttime temperatures were 23° ± 2° and 18° ± 2° C, relative humidity was 46% ± 13% and photosynthetic photon flux was 366 ± 158 μmole quanta m^{-2}

sec^{-1}. From May to August, plants were grown for 34 d in open-top field chambers in CF air. Plants were then treated 7 d with a daily supplemental UV-B_{BE} irradiance of either 0.0 (control) or 5.67 kJ m^{-2}. The UV-B radiation treatment corresponded to a column O_3 loss of 27%. Afterward, plants from both the control and supplemental UV-B treatments were treated for 17 d with either CF air, O_3 or O_3 plus supplemental UV-B radiation. Ozone was administered as a constant addition 7 h daily, and average concentrations in the CF and O_3 treatments were 29 and 97 ppb, respectively. There were three replicates of each treatment combination and the experiment was conducted twice.

Pigments and Biomass

During the 1990 field study, one plant was harvested biweekly from each treatment replicate. Tissue samples from main stem leaves were extracted with 95% ethanol for 2 d in the dark. Extracts were assayed spectrophotometrically for chlorophyll and UV-absorbing pigments [16, 17]. Plant height, oven-dried biomass and leaf area were also measured.

Peroxidase and Catalase Assays

Leaf tissue samples were frozen in liquid N_2 and homogenized with a Tekmar Tissumizer in cold 100 mM phosphate buffer solution (pH 6.5), 2 mM ethylenediaminetetraacetic acid and 1% (w/v) polyvinylpyrrolidone, 15 ml to 0.5 g tissue (fresh wt). The homogenate was filtered through one layer of Miracloth and centrifuged 15 min at 15,000x g and 4° C. Enzyme activities in the supernatant were assayed in 100 mM phosphate buffer solution (pH 6.0) at 30° C in a total volume of 2 ml. Pyrocatechol-*p*-phenylenediamine peroxidase (PC-PPD), a nonspecific peroxidase, was assayed in buffer solution containing 10 µmole H_2O_2, 6 µmole PC, 3 µmole PPD and 25 µl of leaf extract [18]. Activity was determined by the change in absorbance at 544 nm. Syringaldazine peroxidase (SYR), a peroxidase associated with lignification, was assayed in buffer solution containing 1 µmole H_2O_2, 100 µl of leaf extract and 0.25 µmole syringaldazine [19]. Syringaldazine (10 mM) was dissolved in dimethylsulfoxide. Activity was determined by the change in absorbance at 530 nm. Catalase was assayed in buffer solution (pH 7.0) containing 10 µmole H_2O_2 and 100 µl of leaf extract [20]. Activity was determined by the change in absorbance at 240 nm. Protein was measured using Bradford's reagent [21] (Bio-Rad Protein Assay) with bovine serum albumin as the standard.

RESULTS

Pigments

In all our experiments, treatment with O_3 accelerated the loss of chlorophyll from leaves compared with CF air treatment, but supplemental UV-B radiation had no significant effect. In the 1990 field experiment, average chlorophyll concentration in the tenth main stem leaf on O_3 -treated plants was significantly lower than that in control plants by 75 d after planting and thereafter ($p < 0.001$) (Figure 1). In the 1991 field experiment, chlorophyll loss in plants exposed to O_3 occurred more rapidly. In this experiment, average chlorophyll concentration in the fifth main stem leaf of plants treated with O_3 for 4 d was 8% less than that in plants treated with CF air ($p < 0.03$). This difference increased to 25% after 8 d of O_3 treatment. However, no significant UV-B treatment effect or UV-B x O_3 interaction was observed.

Extracts of leaf tissue from plants treated with medium or high supplemental UV-B radiation in the 1990 field experiment showed increased absorbance in the UV-B waveband compared with leaf

tissue extracts from the control treatment ($p < 0.05$) (Figure 2). Greenhouse experiments indicated that increased UV absorbance of leaf tissue extracts could be observed after treatment with supplemental UV-B radiation for 3 d. Scans of leaf tissue extracts from plants treated with either O_3 or O_3 in combination with supplemental UV-B radiation also showed increased UV absorbance (Figure 2). Absorbance was highest in extracts from the O_3 plus high supplemental UV-B radiation treatment. Increased UV absorbance of leaf tissue extracts from O_3-treated plants coincided with chlorophyll loss and visible foliar injury.

Biomass

Total biomass accumulation was suppressed by O_3 in the 1990 field experiment starting at approximately 60 days after planting. By 104 d after planting, average biomass of O_3 -treated plants was 28% less than that of CF air-treated plants ($p < 0.001$) (Figure 3). No significant difference in biomass among plants in the control, medium or high supplemental UV-B radiation treatments was observed (Figure 3). Average plant height, leaf area and pod weight were similarly affected (data not shown). Chlorophyll concentration and biomass at 104 d after planting were significantly correlated ($p < 0.001$, $r = 0.79$).

Peroxidase and Catalase Assays

At 19 d after planting in the 1990 field experiment, average activity of PC-PPD and SYR in the first trifoliolate leaf was 1.4 and 3.3 times higher, respectively, in plants treated with O_3 than in plants treated with CF air ($p < 0.05$). By 75 d after planting, average PC-PPD and SYR activities in the tenth trifoliolate leaf from O_3 -treated plants were 2.0 and 12.9 times higher, respectively, than in CF air-treated plants ($p < 0.001$). After just 4 d of treatment with O_3 in the 1991 field experiment, PC-PPD and SYR activities in the fifth trifoliolate leaf were higher than in CF air-treated plants ($p < 0.001$) (Figures 4 and 5). Peroxidase activities, particularly SYR activity, in O_3 -treated plants continued to increase over the course of this experiment.

The activities of PC-PPD and SYR were not significantly affected by supplemental UV-B radiation treatments in either field or greenhouse experiments (Figures 4 and 5). In contrast, greenhouse experiments in which plants were first treated with supplemental UV-B radiation and then treated with O_3 suggested that the O_3 -induced increase in SYR activity was reduced in plants previously treated with supplemental UV-B radiation. However, results from the 1991 field experiment did not indicate that supplemental UV-B radiation affected the O_3 -induced increase in SYR activity (Figure 5).

Catalase activity was not significantly affected by either O_3 or supplemental UV-B radiation treatments in either field or greenhouse experiments.

DISCUSSION

The results of our experiments showed that O_3 accelerated chlorophyll loss, suppressed biomass accumulation and increased peroxidase activity in soybean. None of these responses consistently occurred in plants treated with supplemental UV-B radiation, nor were any significant interactions observed. Under field conditions, increased UV-B radiation neither promoted nor protected plants from O_3 injury.

Chlorosis and visible foliar injury have been correlated with decreased net photosynthesis and

yield in soybean treated chronically with O_3 [22, 23]. In our 1990 field experiment, chlorophyll concentration was closely correlated with biomass at 104 d after planting. The 28% average difference in biomass between the O_3-treated and CF air-treated soybeans in this experiment was typical of previous studies [5]. In the 1991 field experiment, a significant loss of chlorophyll was detected in plants treated 4 d with O_3. Rapidly occurring chlorosis and necrosis in plants first grown in CF air and then treated with O_3 has been related to an interaction between O_3 and the plant hormone ethylene [24]. It was suggested that O_3 stimulated a sudden production of ethylene, which was followed by reactions between O_3 and ethylene that produced free radicals, H_2O_2 and subsequent injury [24]. Much less ethylene was produced by plants grown from seed in elevated O_3, and foliar injury was slower to develop.

Chlorophyll concentration decreased in several species sensitive to UV-B radiation in a growth chamber study [2], but generally it has not been affected by UV-B radiation in plants grown under visible light levels greater than 600 μmole quanta m^{-2} sec^{-1} [25]. In our greenhouse experiments, no effect of UV-B radiation on average chlorophyll content was observed in plants grown under an average visible light level of 343 μmole quanta m^{-2} sec^{-1} even though leaf bronzing was occasionally noticed.

The reported effects of increased UV-B radiation on soybean growth and yield in field studies have been variable. Under optimal environmental conditions, supplemental UV-B radiation significantly suppressed growth and yield of field-grown Essex soybean [26, 27]. However, increased UV-B radiation had no effect on growth and yield reductions caused by drought, mineral nutrient deficiency or high summer temperatures [26-28]. A field experiment in which soybeans were treated with supplemental UV-B radiation comparable to our high UV-B radiation treatment found no significant UV-B radiation effect on accumulated dry wt or seed yield [29]. No significant UV-B radiation effect on biomass or yield occurred in our 1990 field experiment even though plants were well-watered and fertilized (Figure 3). These results contrasted somewhat with those we observed in a short-term field experiment conducted in 1989. In that study, significant interactions between O_3 and supplemental UV-B radiation during early vegetative growth reflected less than an additive response of biomass to the two stresses in combination [30]. Results from the 1990 field study strongly suggested, however, that O_3-induced stress was not significantly affected by supplemental UV-B radiation treatments.

Numerous studies indicate that screening of UV-B radiation by flavonoids and other pigments provides effective protection from injury [2]. It is noteworthy that the production of UV-B absorbing compounds in response to supplemental UV-B radiation occurred in plants already exposed to the sun (Figure 2). The addition of a small supplement of UV-B radiation to the solar spectrum elicited the synthesis of additional pigments, indicating the effectiveness of UV-B radiation for inducing flavonoid synthesis [17]. However, changes in phenolic composition from exposure to increased UV-B radiation could affect many processes at the community and ecosystem levels [6].

Studies have shown that acute treatment of soybean and bean with O_3 led to the production of phenolics such as caffeic acid and isoflavonoids [31]. The increased absorbance in the UV region of extracts from O_3-treated plants (Figure 2) suggested that phenolic levels increased [32]. Abnormal pigmentation in O_3-injured leaves probably results from the production and polymerization of phenolics [31]. The formation of foliar lesions in O_3-treated plants has been correlated with increased peroxidase activity [7].

In our experiments, increased peroxidase activity, particularly SYR, was observed in plants treated with O_3 (Figures 4 and 5). A number of studies have found increased peroxidase activity in O_3-treated plants [9]. Increased peroxidase activity may be a protective mechanism for reducing peroxide levels [9]. It has been suggested that SYR peroxidase also participates in lignification reactions [19]. Peroxidase activity has been associated with suberization and the polymerization of phenolic acids in cell walls [33]. Increased peroxidase activity and phenolic biosynthesis may thus affect cell wall composition. Ultraviolet-fluorescence microscopy revealed that mesophyll cell walls autofluoresced blue in plants visibly injured by O_3, which suggested that cell walls were impregnated with phenolic monomers and polymers [34]. However, supplemental UV-B radiation did not affect cell wall autofluorescence (Booker, unpublished data).

Increased peroxidase activity in response to supplemental UV-B radiation has been observed in detached *Hibiscus* leaves [10]. Murali and Teramura [8] reported that UV-B radiation altered peroxidase isozyme expression in greenhouse-grown soybean, but total peroxidase activity was unchanged. In our 1991 field experiment, we observed no significant effect of supplemental UV-B radiation alone or in combination with O_3 on the activity of either PC-PPD or SYR peroxidase (Figures 4 and 5).

Catalase, which would also prevent accumulation of H_2O_2, was unaffected by treatments with O_3 and UV-B radiation in our experiments. Neither O_3 nor SO_2 significantly increased the level of catalase in maize leaves [35]. Soluble catalase activity was unchanged in needle extracts from O_3-treated Norway spruce, but activity increased significantly in the particulate fraction of the extracts [11]. Peroxisomes, which proliferated in response to O_3, are a major site of particulate catalase in plant tissues [11]. We assayed catalase in the soluble fraction of leaf extracts in our experiments, and thus changes in catalase activity in the particulate fraction would not have been detected.

Increased peroxidase activity can be induced by a variety of stresses such as air pollutants, metals, chilling, wounding or pathogenic infection [36]. Stress conditions such as these can cause oxygen activation in plants [12]. Peroxidase induction is likely related to oxidative reactions at the membrane [36]. On this basis, results of the peroxidase assays suggested that O_3 caused significant oxidative stress in soybean, whereas UV-B radiation did not. Results of the chlorophyll and biomass assays further support the conclusion that the potentially detrimental effects of increased UV-B radiation on soybean crop production may be of less concern than the demonstrated phytotoxic effects of tropospheric O_3. Researchers have suggested that plants possess effective protective mechanisms probably sufficient to cope with the forecasted increase in UV-B radiation [6]. However, recent calculations from an atmospheric transport model indicate that a stratospheric O_3 depletion of 10% and a surface temperature increase of 1.6°C could increase average tropospheric O_3 levels by 3% as well as the number of exceedences of threshold values [4]. The results of our research suggest that changes in tropospheric O_3 levels due to increased solar UV-B radiation may be an important factor in assessing the impact of stratospheric O_3 loss on soybean crop production.

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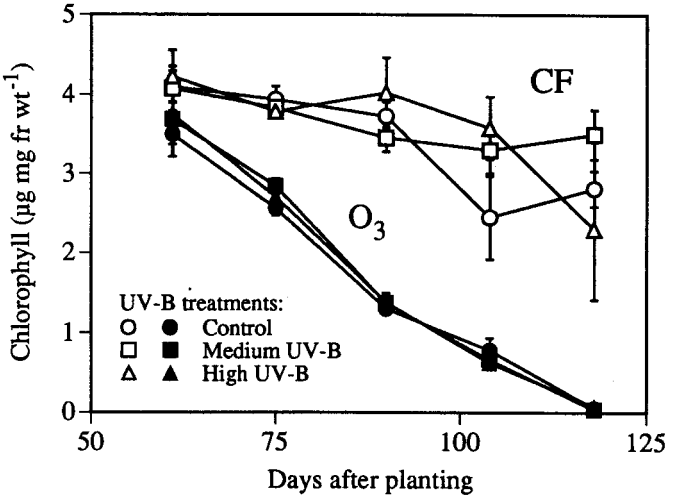

Figure 1. Mean (± s.e.) chlorophyll concentration in the tenth main stem leaf on plants treated with either CF air (O, □, △) or 1.5x ambient O_3 (●, ■, ▲) in combination with either control, medium or high supplemental UV-B radiation in the 1990 field experiment.

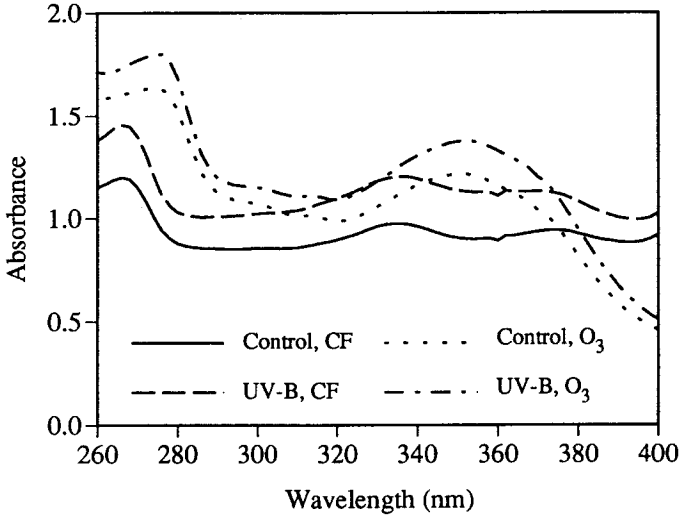

Figure 2. Mean absorbance of ethanolic extracts of leaf tissue (7 cm^2) from the twelfth main stem leaf on plants treated 90 d with either CF air or 1.5x ambient O_3 in combination with either control or high supplemental UV-B radiation in the 1990 field experiment (n = 16).

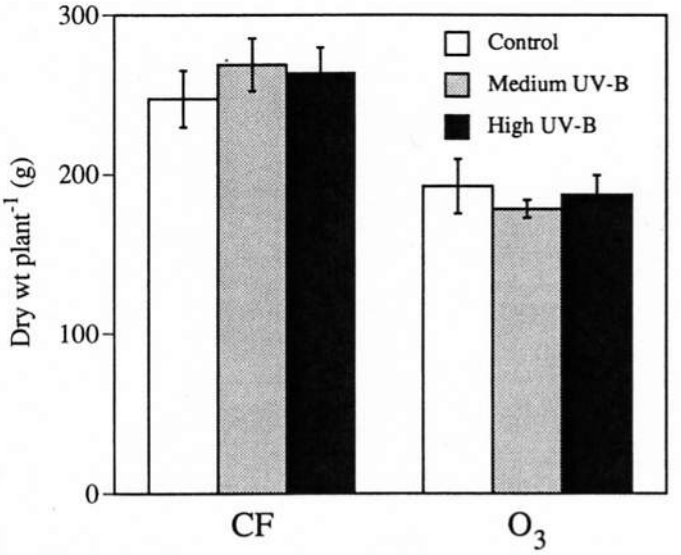

Figure 3. Mean (± s.e.) plant biomass after treatment for 104 d with either CF air or 1.5x ambient O_3 in combination with either control, medium or high supplemental UV-B radiation during the 1990 field season (n = 24).

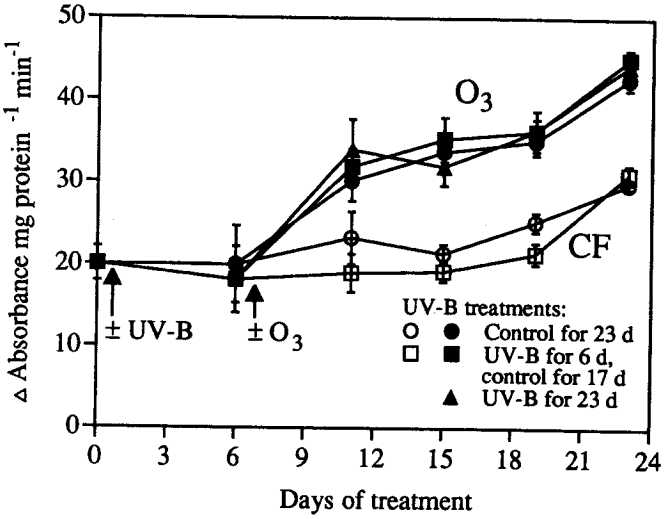

Figure 4. Mean (± s.e.) pyrocatechol-*p*-phenylenediamine peroxidase activity in the fifth main stem leaf on plants grown 34 d in CF air and then treated with control or supplemental UV-B radiation for 6 d. The UV-B treatment was followed by treatment with either CF air (○, □), O_3 (●, ■) or O_3 plus supplemental UV-B radiation (▲) for 17 d. Data are from the 1991 field experiment (n = 30).

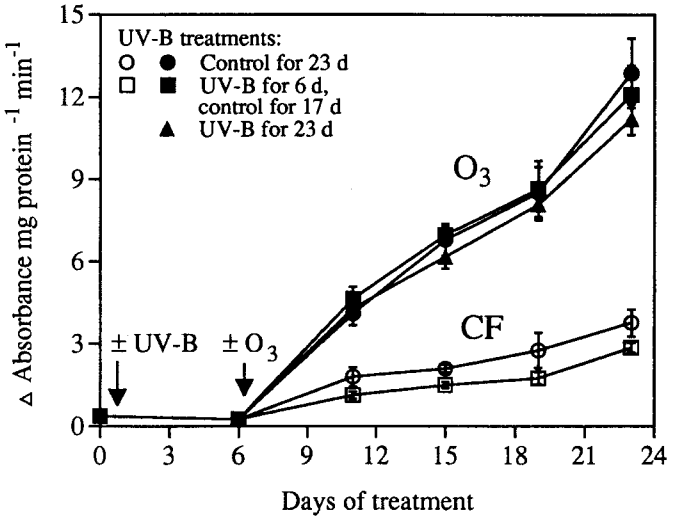

Figure 5. Mean (± s.e.) syringaldazine peroxidase activity in the fifth mean stem leaf on plants grown 34 d in CF air and then treated with control or supplemental UV-B radiation for 6 d. The UV-B treatment was followed by treatment with either CF air (O, □), O_3 (●, ■) or O_3 plus supplemental UV-B radiation (▲) for 17 d. Data are from the 1991 field experiment (n = 30).

Transactions

Tropospheric Ozone and the Environment II

Effects, Modeling and Control

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