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A preliminary study of the response of red spruce to O_3 and SO_2

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ABSTRACT

A laboratory branch chamber experiment determined the response of a young red spruce (*Picea rubens*) sapling to ozone (O_3) and sulfur dioxide (SO_2). A treatment branch was exposed to concentrations of O_3 ($\sim$90 ppbv) and SO_2 ($\sim$15 ppbv) representative of the summertime maxima observed in the high elevation spruce-fir forests of the eastern United States. A control branch of the same age and comparable biomass received clean air only. Pollution exposure, lasting 78 days, consisted of 4 sequential stages designed to compare the tree's response to each individual pollutant with its response to the combination of $SO_2 + O_3$. The treatment branch exhibited distinctly different physiological responses to O_3 alone, SO_2 alone, and the $SO_2 + O_3$ combination. Ozone exposure in the 1st stage induced an increase in CO_2 assimilation (20% over 26 days, relative to the control branch) while transpiration and nighttime respiration were unaffected. Ozone uptake increased markedly during this stage. In the 16-day 2nd stage of exposure, the combination of $SO_2 + O_3$ induced a factor of $\sim$2 decrease in CO_2 assimilation, transpiration and O_3 uptake within 1 week. In the 3rd stage, the removal of SO_2 resulted in the partial recovery of photosynthetic uptake and other gas fluxes. A final stage of exposure to SO_2 alone produced large day-to-day variations in CO_2 assimilation. The isoprene emission rate of the treatment branch declined relative to the control branch over the course of the entire experiment. The results of this preliminary experiment suggest that, singly and in combination, O_3 and SO_2 may disrupt physiological functions of red spruce. Further experiments are needed to determine whether these results pertain to larger populations of red spruce and the occurrence of red spruce decline in the northeastern United States.

1. Introduction

Explanations of forest decline are complex, often involving the interaction of multiple stresses. Experimental studies have shown that the influence of combined stresses on vegetation is unpredictable and frequently nonadditive. This paper presents the results of a laboratory branch chamber study aimed at getting a first look at how 2 chemical stresses, ozone and sulfur dioxide, interact in the case of red spruce. 4 sequential exposure stages tested the combined and individual effects of $\sim$90 ppbv (parts per billion by volume) O_3 and $\sim$15 ppbv SO_2 on a red spruce sapling.

Stands of red spruce in the eastern United States are experiencing growth reductions, foliar damage and dieback (Johnson and Siccama, 1983; Johnson and McLaughlin, 1986; Siccama et al., 1982; Hornbeck and Smith, 1985). Atmospheric pollution has been suggested as one factor which could account for the timing of the decline and its greater severity at high-elevation sites. Most past and current research has focused on ozone, acid rain and acid mist as possible atmospheric factors which may act together with winter injury. Gas-phase exposure studies of red spruce seedlings have focused almost entirely on ozone. Initial work revealed that ozone alone does not influence growth (Laurence et al., 1989; Taylor et al., 1986; Kohut et al., 1990; Alscher et al., 1989) or induce foliar symptoms (Skelly et al., 1983). Lee et al. (1990) observed decreased seedling growth as a result of ozone exposure, although photosynthesis

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and transpiration were unaffected. Only one study has investigated the effects of $\geqslant 2$ trace gases on red spruce (Ennis et al., 1990b). That study demonstrated an increase in nighttime respiration in a 3-week exposure of a red spruce sapling to the combination of gaseous O_3, SO_2 and H_2O_2.

The experiment described here applies branch chambers in an intensive study of a sapling-sized red spruce tree approximately 20 years in age. Each of 2 entire branches on the same whorl of 1 tree is enclosed in a branch chamber. Fluxes of CO_2, water and pollutants are measured in situ once per hour for the treatment branch and the control branch. In addition, isoprene emission rates are determined on selected days. Investigations have shown that isoprene (2-methyl-1,3 butadiene) makes a major contribution to tropospheric ozone in the rural eastern US (Trainer et al., 1987) and that exposure of isoprene emitters to ozone produces organic hydroperoxides which may damage the plant (Hewitt et al., 1990).

Branch chambers have been used by other researchers to conduct exposure experiments (Miller et al., 1963; Botkin et al., 1972; Thompson and Kats, 1975; Skärby et al., 1987) or to measure the dry deposition of pollutants to foliage (Bengtson et al., 1980; Johansson et al., 1983a and 1983b; Johansson, 1987; Granat and Johansson, 1983). We have chosen to use branch chambers for several reasons. A primary consideration is that the approach allows us to study trees rather than seedlings in our exposure experiments. It has been noted that seedling physiology is distinct from that of mature trees and therefore may be difficult to use as a basis for making extrapolations to the real-world forest (Reich, 1987; Taylor et al., 1986). We have used a native red spruce sapling from the Adirondack forest, rather than a nursery-grown sapling, again in an effort to obtain results that pertain well to the forest. By conducting the experiment on 2 approximately equivalent (same-whorl) branches of a single tree, environmental history and genetics are eliminated as variables. Intensive measurement of several gaseous fluxes allows us to follow the complete evolution of a response throughout a long exposure period. Despite these advantages, the recognized trade-off of our branch chamber approach is that our sample size is limited to one tree. Accordingly, our experiment should be regarded as an exploratory study that suggests hypotheses for further testing on larger populations of red spruce. It is also possible that untreated branches can compensate for the effects of stress in a treated branch. In this respect, the results of our branch chamber experiment might be considered to be conservative.

2. Methods

The experiment was conducted on a young red spruce sapling, 15–20 years old and healthy in appearance, obtained in early September of 1988 from ~ 1000 m elevation in the forest of Essex County, New York. The tree was potted in a 13-inch diameter plastic pot using soil from the mountain site. The tree remained near the site for a few weeks to determine that it was healthy with no signs of transplantation shock or other stresses. Transportation to Boulder, Colorado was via overnight express airfreight in an insulated and reinforced container. The tree was acclimated to the environmental conditions of the growth laboratory ($\sim 50\%$ relative humidity and $\sim 20°C$) for several weeks prior to the exposure experiment. The sapling was watered regularly and showed no signs of stress prior to the exposure experiment. Elongation of the current-year shoots and needles was complete when the experiment began.

The exposure facility was used as previously described (Ennis et al., 1990a) with some modifications. Briefly, the system consists of a humidified and cooled tree enclosure; two stirred Teflon whole-branch exposure chambers; a 1000-Watt metal halide lamp with polycarbonate UV filter and water tray infrared filter; a dynamic-dilution gas mixing system comprised of a commercial clean air source, compressed gas cylinders, humidifiers, teflon tubing and electronic mass flow controllers; and an array of commercial detectors for dewpoint, CO_2, SO_2, O_3 and temperature. Each branch chamber contains a K-type thermocouple to monitor air temperature in a shaded area ~ 0.5 cm under the foliage. A computer-based data acquisition system has replaced the datalogger used in earlier work. A 286AT computer with a commercial software package (Laboratory Technologies, Inc.) samples each of the detectors once every 10 s and moves a gas sampling valve every 15 min. 15-min averages at each of 4 sampling positions (inlets and outlets of the 2 branch chambers) are used to compute hourly values of

fluxes for CO_2, H_2O, SO_2 and O_3 for the two branches. Analysis was done using daily flux averages, computed from the hourly values for steady-state daytime and nighttime periods. Fluxes are corrected for chamber wall effects (Ennis et al., 1990a). Isoprene emissions were determined near the end of the photoperiod on 6 days during the experiment, noted in Fig. 1, using methods that have been described previously (Ennis et al., 1990b).

The exposure was conducted continuously, day and night, and consisted of 1 preliminary stage and 4 treatment stages. In the preliminary stage, both branches received clean, humidified air for 3 days. For the next 78 days, 4 sequential pollutant stages were applied to the treatment branch (Fig. 1). These stages tested the effects of the individual pollutants and the combination of $SO_2 + O_3$. Transitions between stages were made based on analysis of trends in the physiological response of the branch. Chamber concentrations of O_3 and SO_2 were similar to maximum summertime values observed in high elevation spruce-fir forests of the eastern US (Lefohn et al., 1990; Mueller and Weatherford, 1988; Kelly, 1985; Pinkerton and Lefohn, 1987). Our approach of using a constant day-and-night exposure mimics the nearly flat diurnal pattern of pollutant concentrations that is observed in high-elevation forests (Saxena et al., 1989; Mohnen and Kadlecek, 1989; Lovett and Kinsman, 1990). Chamber concentrations of CO_2 were maintained near ambient values ($\sim$350 ppmv) for both branches throughout the experiment. Average day/night temperature and relative humidity conditions in the branch chambers were 22/17°C and 70/90% RH, and light exposure (photosynthetically active radiation, PAR) was $\sim$600 μmoles m^{-2} s^{-1} for 8 h per day. The pollutant exposure was interrupted for about five hours each week to determine correction factors for empty-chamber uptake of gases. The tree was watered approximately weekly.

Immediately after the experiment, an estimate of branch biomass and surface area was made by a bead-coating technique (Ennis et al., 1990a). Fluxes reported here are based on the total needle surface area estimated by this technique, which is larger than the projected area by about a factor of two. The branch chambers each enclosed an entire branch, with treatment and control branches

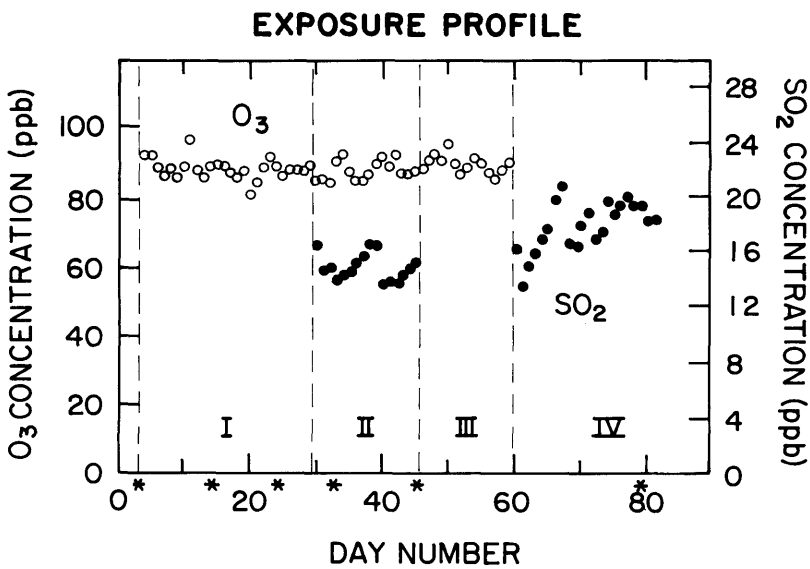

Fig. 1. Exposure profile of the treatment branch, with separation of exposure stages denoted by dashed lines. Each point represents the average daytime concentration as measured at the outlet of the branch chamber. Isoprene emission rates were determined on 6 days marked with asterisk symbols. Day-to-day variability in the exposure concentrations is determined primarily by variations in plant uptake of the pollutants; inlet concentrations were much more stable than outlet concentrations (Ennis et al., 1990a).

located on the same whorl near the midsection of the tree. Thus, the observed fluxes give the cumulative response of current-year growth plus several years of old growth on an 8- to 10-year-old branch of the sapling.

After this exposure experiment, a separate experiment was conducted on a different but comparable red spruce sapling to determine the normal physiological relationships between two branches which are treated identically with clean air. In this 37-day experiment, CO_2 and H_2O fluxes were determined exactly as described above. Isoprene measurements were made on 9 of the last 10 days of the experiment by injecting 20-ml samples of branch chamber air into a gas chromatograph equipped with a HgO/Hg reductive atomic absorption analyzer (Trace Analytical, model RGD2). This clean-air experiment explores the expected behavior and relationships of two branches which are unstressed and treated identically. The data provide a standard to use in detecting responses to pollutant treatments in the O_3/SO_2 experiment.

3. Results

Figs. 2, 3 give average daily values of the daytime and nighttime fluxes of CO_2, water and pollutants for the treatment and control branches. The dominant qualitative impression of the daytime data is that the physiological responses of the treatment and control branches are quite distinct. In particular, the net photosynthetic uptake of the treatment branch undergoes changes in each stage of the experiment which are remarkable in comparison to the uniform behavior of the control branch. The behavior of the treatment branch CO_2 flux resembles the H_2O and O_3 fluxes of that branch, while the SO_2 flux behavior is unique. Further, the treatment branch behavior is different in each of the 4 stages of the experiment.

In contrast to the daytime data, the nighttime fluxes are more similar for the 2 branches (Fig. 3). Nighttime transpiration is small compared to daytime values but is frequently measurable in this experiment, as in our previous study (Ennis et al., 1990b). Occasionally, deposition of water to branch surfaces exceeds transpiration, leading to values below zero in Figs. 3b and 3f. Because of this effect, we will not attempt to attach physiological significance to the nighttime transpiration measurements. The nighttime ozone fluxes also are problematical because they are small or sometimes negative (Fig. 3c). This suggests that either our corrections for ozone loss to the empty chamber surfaces are too large, or that perhaps ozone desorbs from plant surfaces at night. Because of this ambiguity, we will not discuss the nighttime ozone any further. We present the nighttime ozone and transpiration fluxes for the sake of completeness and to make other investigators aware of the challenges posed by their measurement.

For each stage of the exposure, we apply a regression analysis to determine whether the data exhibit statistically significant temporal trends over the entirety of the stage (Table 1). Trends are reported when the regression of flux versus day number yields a slope which differs from zero at the $P < 0.05$ level of significance. In Table 2, relationships among fluxes are explored by computing contemporaneous cross correlations. Fluxes which exhibited trends in Table 1 were detrended using a linear model prior to this correlation analysis. Cross correlations are reported as significant if the probability is less than 5% that they are due to chance ($P < 0.05$). For both the regression and correlation analyses, standard errors were inflated to account for autocorrelation in the original time series of flux data. Autocorrelation, which occurs when data taken in one time interval are related to data taken in one or more of the preceding time intervals, reduces the number of truly independent observations in the time series. Our data showed that an order 1 autoregressive model was usually appropriate (lag of 1 day). Consequently, first-order autocorrelation coefficients were used to correct the standard errors of trends and cross correlation coefficients (see Katz and Brown, 1991; Tiao et al., 1990).

The correlation results of the 37-day clean-air experiment (Table 2) provide a measure of which inter-branch and intra-branch correlations can be expected to occur normally. This experiment revealed that for a given molecule (CO_2 or H_2O), correlations between two branches are normally positive (column 5, lines 1 and 2 of Tables 2A, B). Thus, the transpiration, assimilation and respiration are coupled for 2 branches which are unstressed and treated identically. Within a branch, the fluxes show no consistent pattern of

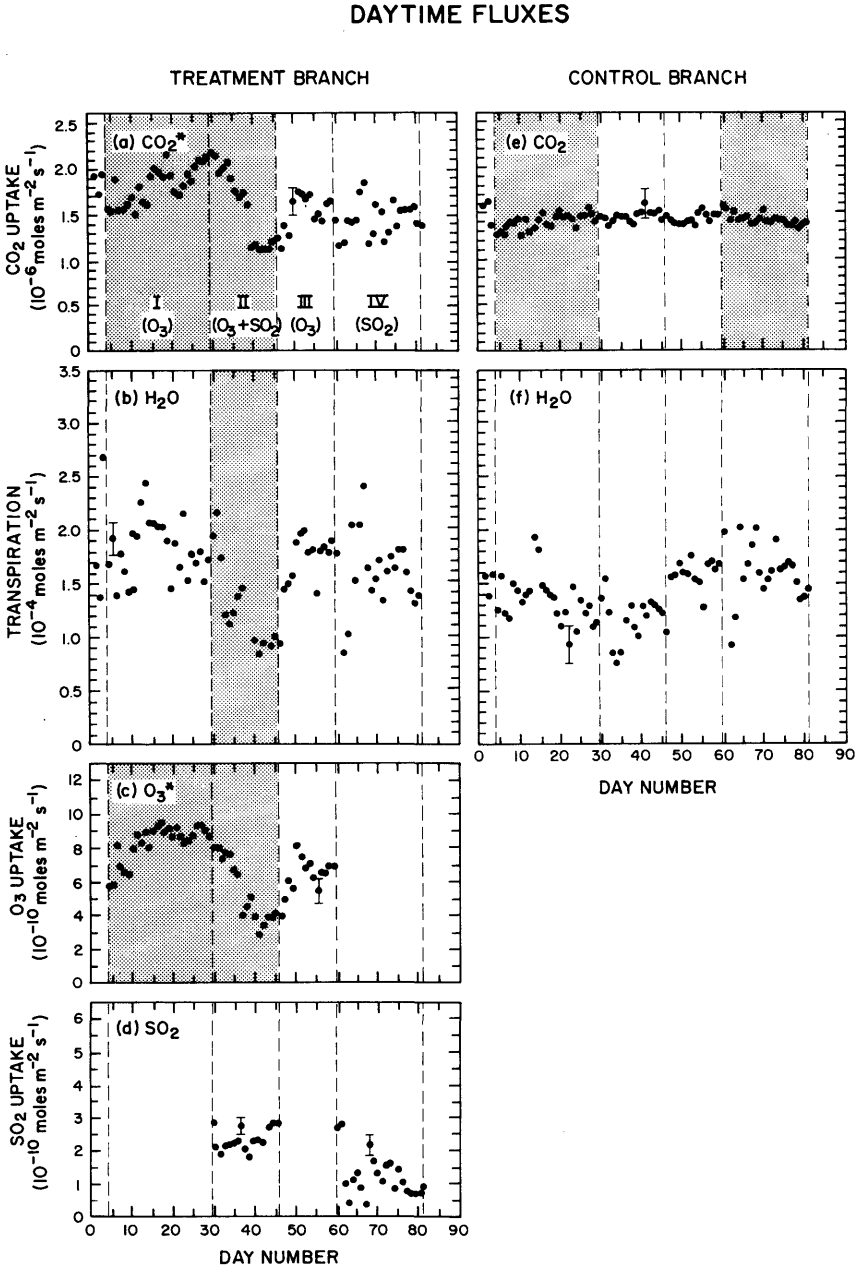

Fig. 2. Daytime net photosynthesis (P_n), transpiration (T_S) and pollutant fluxes for the treatment and the control branch as a function of day of the experiment. Each point is an average of generally 5 hourly measurements. A stage is shaded if the flux exhibited a significant temporal trend over that interval of time ($P \leqslant 0.05$). If a significant trend occurred for a flux over the entire 81-day experiment, an asterisk appears by the upper left hand label. The daily standard deviation of the mean of the hourly fluxes was calculated for each day's point ($\sigma_{\bar{x}}$). Error bars represent the average of all the daily $\sigma_{\bar{x}}$ values during the experiment ($\pm 2\bar{\sigma}_{\bar{x}}$), and are shown on only one point in the interest of clarity.

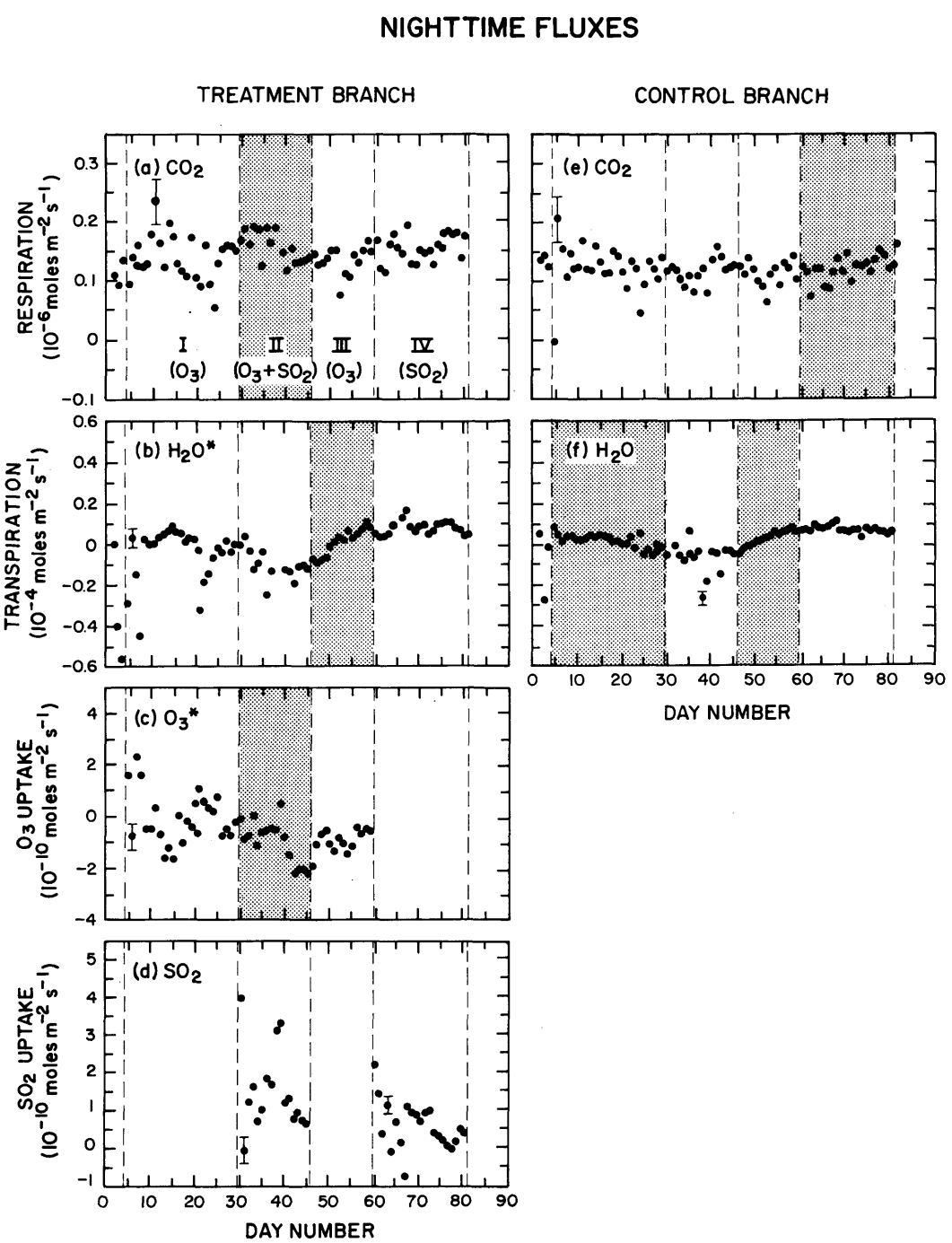

Fig. 3. Nighttime respiration (R_D), transpiration (T_S) and pollutant fluxes for the treatment and the control branch as a function of day of the experiment. Each point is an average of generally 12 hourly measurements. Other features are as described for Fig. 2.

Table 1. *Regression results* [+]

(a) Daytime trends

Days (stage)	Treatment branch P_N	Treatment branch T_S	Treatment branch O_3	Treatment branch SO_2	Control branch P_N	Control branch T_S
4–29 (I)	2.8** (2.0×10^{-8})	−0.01	2.4* (1.0×10^{-11})	—	3.0** (7.8×10^{-9})	−1.5
30–45 (II)	−3.6** (-8.1×10^{-8})	−2.2* (-6.8×10^{-6})	−2.4* (-3.5×10^{-11})	1.3	1.2	0.3
46–59 (III)	1.2	1.6	1.2	—	1.2	1.4
60–81 (IV)	0.8	0.8	—	−1.6	−3.0** (-7.1×10^{-9})	0.7
1–81 (All)	−1.8	−1.1	−0.9	—	0.4	1.5

(b) Nighttime trends

Days (stage)	Treatment branch R_D	Treatment branch T_S	Treatment branch O_3	Treatment branch SO_2	Control branch R_D	Control branch T_S
4–29 (I)	−0.4	0.5	0.7	—	−0.5	−3.6** (-3.3×10^{-7})
30–45 (II)	−2.7* (-3.6×10^{-9})	−1.6	−2.7* (-1.3×10^{-11})	−1.2	1.0	−0.9
46–59 (III)	0.5	3.7** (1.5×10^{-6})	1.7	—	0.1	4.5** (8.6×10^{-7})
60–81 (IV)	1.5	0.1	—	−1.7	2.7* (1.9×10^{-9})	−1.5
1–81 (All)	1.3	2.1* (2.9×10^{-7})	−2.3* (-2.7×10^{-12})	—	−0.1	1.4

[+] The t-values for the regression of average daily plant fluxes versus day number for various stages of the experiment (I–IV) and the whole experiment. Fluxes were expressed as uptakes by the plant, except for transpiration (T_S) and dark respiration (R_D). P_N = net photosynthetic uptake. The t-values give the ratio of [slope of regression]/[standard error of slope]. Statistically significant trends are noted by a * ($P<0.05$ level) or a ** ($P<0.01$ level). The slope for significant trends is given in units of moles $m^{-2}\,s^{-1}\,d^{-1}$ in parentheses below the t-value. Significance levels were adjusted to account for autocorrelation in the original data (see text).

correlation. These results provide us with one measure of detecting a response in our pollutant exposure experiment. If the usual strong inter-branch correlations are broken, we conclude that pollutant treatment has elicited a physiological response. Furthermore, such a break would be evidence that branches are able to respond independently when unequal stresses are applied. Major findings of the experiment are outlined below.

3.1. Stage I. During the ozone-only exposure of Stage I, the net photosynthesis of both branches increased, with the treatment branch increasing by 20% relative to the control

Stage I of the experiment, days 4–29, tested the response of the treatment branch to ozone at a concentration of about 90 ppbv (Fig. 1). For each branch, CO_2 assimilation declined during the first few days of the stage, and then gradually increased.

Table 2. *Flux cross correlations*[a)]

(A) Daytime

	I (O_3)	II (O_3+SO_2)	III (O_3)	IV (SO_2)	Clean air exper.
Inter-branch					
T'_S–T_S	+0.70**	+0.77*	+0.84**	+0.90**	+0.80**
P'_N–P_N	+0.20	−0.45	+0.06	−0.03	+0.74**
T'_S–P_N	−0.18	−0.39	−0.19	−0.33	+0.24
P'_N–T_S	−0.15	−0.48	+0.37	+0.33	+0.42*
Intra-branch					
P_N–T_S	−0.10	+0.05	−0.39	−0.26	+0.32
P'_N–T'_S	−0.14	+0.14	+0.79*	+0.67**	+0.46*
P'_N–O'_3	+0.39	+0.69*	+0.82*		
P'_N–SO'_2		+0.21		−0.61**	
T'_S–O'_3	+0.35	+0.01	+0.90**		
T'_S–SO'_2		+0.36		−0.40	
O'_3–SO'_2		+0.01			

(B) Nighttime

	I (O_3)	II (O_3+SO_2)	III (O_3)	IV (SO_2)	Clean air exper.
Inter-branch					
T'_S–T_S	−0.04	+0.45	+0.49	+0.35	+0.89**
R'_D–R_D	+0.59**	−0.46	+0.44	+0.29	+0.55**
T'_S–R_D	+0.48*	+0.10	−0.07	−0.07	−0.10
R'_D–T_S	−0.11	+0.25	−0.43	+0.07	+0.04
Intra-branch					
R_D–T_S	−0.12	−0.37	−0.15	+0.21	+0.05
R'_D–T'_S	+0.40	−0.37	−0.23	+0.49*	−0.20
R'_D–O'_3	−0.35	+0.05	+0.09		
R'_D–SO'_2		−0.09		−0.52*	
T'_S–O'_3	−0.78**	−0.39	−0.47		
T'_S–SO'_2		+0.02		−0.62**	
O'_3–SO'_2		+0.41			

[a)] Contemporaneous cross correlation coefficients for fluxes in the SO_2/O_3 and clean-air experiments. P_N = net photosynthetic uptake; R_D = dark respiration; T_S = transpiration; primes denote treatment branch. Significant cross correlations are denoted by a * ($P<0.05$ level) or a ** ($P<0.01$ level). Significance levels were adjusted to account for autocorrelation in the original data and variables with significant trends (Table 1) were detrended before the correlation analysis (see text).

In the case of the treatment branch, assimilation eventually surpassed the clean-air values of days 1–3. Overall for the stage, both branches showed a statistically significant positive trend in photosynthetic uptake (Table 1A). Despite similarities in trends, the cross correlation was not significant for the two CO_2 fluxes (Table 2A). This is a departure from the usual positive correlation which is expected based on the clean-air experiment. To further test for any differential response between

the two branches, we computed the ratio of their daily CO_2 fluxes and tested for a trend. For Stage I, the treatment branch exhibited an increase in CO_2 assimilation of about 20% relative to the control branch. This physiological response was not repeated in Stage III (also an ozone-only exposure) or in any other stage. Similar to the CO_2 flux, the ozone uptake of the treatment branch showed an overall upward trend and a local minimum near the end of the stage (Table 1A, Fig. 2c).

The absence of an upward trend in the transpiration data of either branch (Figs. 2b and 2f) suggests that the gross CO_2 and O_3 flux trends probably cannot be attributed to a decrease in stomatal resistance. Intra-branch cross correlations of the treatment branch support this hypothesis, because transpiration does not correlate with either O_3 or CO_2 uptake (Table 2A). Transpiration fluxes of the two branches are correlated positively, as expected from the clean-air experiment, further suggesting that stomatal behavior probably does not underlie the observed CO_2 and O_3 flux changes.

Deviation from normal behavior was not detected in the case of nighttime respiration for Stage I. There were no trends in the respiration of either branch (Figs. 3a, e). Cross correlation (Table 2B) gave a positive inter-branch correlation for respiration, just as expected from the clean-air experiment.

3.2. *Stages II and III. During exposure to $O_3 + SO_2$ in Stage II, daytime treatment branch fluxes for CO_2, O_3 and H_2O declined by a factor of 2; removal of SO_2 in Stage III led to a partial recovery of previous flux levels*

From days 30–45, the ozone exposure of Stage I continued and SO_2 at ~ 15 ppbv was added to the mixture. Whereas the control branch exhibited no Stage II trends for either daytime or nighttime fluxes, the treatment branch had a remarkable physiological response. The daytime data (Fig. 2, Table 1A) show that transpiration, net photosynthesis and O_3 uptake all declined markedly. The appearance of the decline is similar in all 3 cases: after a short 3–5 day delay, the fluxes decrease for ~ 5 days and then stabilize at new values for the final ~ 5 days of this stage. The total decrease from start to finish in Stage II is about a factor of 2 for each flux. These flux data are consistent with the idea that stomatal closure may have occurred in the treatment branch during Stage II. If true, the transpiration data suggest that the closure occurred gradually over the course of ~ 5 days in the middle of Stage II, before a new level was achieved and sustained. The treatment branch response during this stage is clearly distinct from the observed ozone-only response of Stage I. Whether it is due to SO_2 or the $SO_2 + O_3$ combination is unclear; a subsequent stage of the experiment addresses this question. It is interesting that while the addition of SO_2 in this stage was seemingly critical to the observed effects, its own flux to the leaf was not altered during the stage. In fact, the SO_2 flux behavior is unique and is set apart from all other fluxes in the cross correlations.

In Stages III (days 46–59), SO_2 was removed from the treatment branch mixture, leaving O_3 as the only pollutant. Stage III therefore returns to the exposure conditions of Stage I and tests for the reversibility of the physiological responses observed in Stage II. Daytime fluxes of Fig. 2 show that the treatment branch fluxes recovered only partially. All three treatment fluxes show a ~ 4-day increase and then stabilize at new levels. For both CO_2 and O_3 uptake, this new level is about 20% lower than the level measured at the end of Stage I. CO_2 assimilation, which climbed upward with no signs of stabilizing in the Stage I ozone exposure, appears to stabilize in the Stage III ozone treatment. Transpiration returns to previous Stage I levels. None of the treatment fluxes shows temporal trends over the entirety of this stage (Table 1), but this is due to the fact that recovery is so rapid. Over the shorter interval of days 46–52, significant upward trends are obtained for all daytime treatment fluxes. Control fluxes give no daytime trends for Stage III.

3.3. *Stage IV. The response to SO_2-only exposure was distinct from the responses of each previous stage*

Stage IV exposed the treatment branch to SO_2 at a concentration of ~ 18 ppbv. One purpose of this stage was to determine if SO_2 alone is sufficient to produce the flux decreases observed during the $O_3 + SO_2$ exposure of Stage II. Fig. 2 shows that the fluxes of the treatment branch did not exhibit trends in response to SO_2 exposure. Table 2 daytime correlations reveal that the normal inter-branch correlation for CO_2 assimilation

is not present during Stage IV, and that treatment branch assimilation correlates negatively with SO_2 uptake. At night, inter-branch correlations are broken for CO_2 fluxes, and the SO_2 flux correlates negatively with respiration. These results suggest that while SO_2-only exposure does not induce sustained flux decreases, it may influence daytime and nighttime physiological processes within the branch. An interesting effect is the extremely high variability in the treatment branch data for CO_2 assimilation (Figs. 2a,.e). Compared to the control branch, the treated branch variability (± 2 standard deviations of the daily means) is three times greater. This effect occurs only in the daytime CO_2 data; the variability of the other fluxes is similar for the two branches.

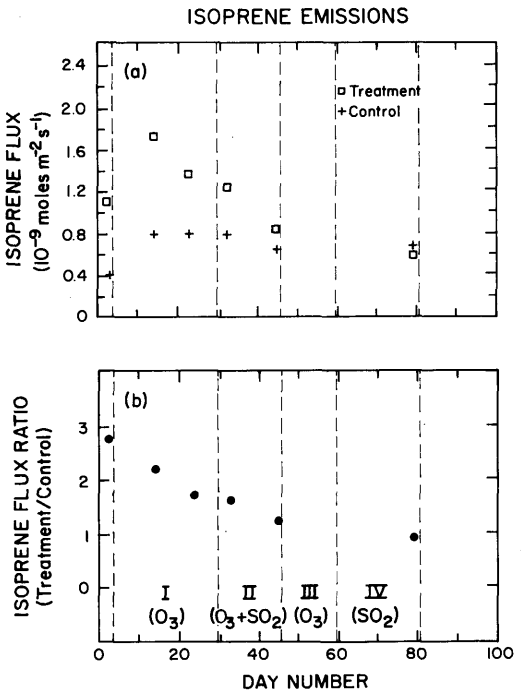

Fig. 4. Isoprene fluxes (a) for the treatment and control branch, as measured on 6 days of the 81-day experiment. Fluxes are normalized to 298 K using the isoprene-temperature algorithm of Guenther et al. (1991). The ratio of the two fluxes is plotted in (b), to show changes in the treatment branch emissions relative to the control branch. The ratio shows a significant negative trend ($t = -4.9$, $P \ll 0.01$) over the course of the entire experiment.

3.4. Isoprene emissions of the treatment branch declined relative to the control over the course of the entire experiment

Fig. 4a gives the isoprene emission rates determined on six days of the experiment. Isoprene fluxes were in the range of $(0.4\text{–}1.8) \times 10^{-9}$ moles $m^{-2} s^{-1}$, which is within about a factor of two of our previous values for a similar red spruce sapling (Ennis et al., 1990b). Since significant temporal variation is observed in both branches, the ratio of the two emission rates is plotted in Fig. 4b. All of the ratios in Fig. 4b lie below the baseline point taken on day 3 during the preliminary clean air phase, indicating a relative decrease in treatment branch isoprene emissions. From the first to the last isoprene measurements, a relative decline of about $68 \pm 4\%$ occurred. Limits are derived from our estimates of the uncertainty in measuring temperature, which is used to normalize our measured emission rates to 298 K (Fig. 4; Guenther et al., 1991). In the clean-air experiment, no trend was seen in 9 isoprene measurements taken over a 10-day period (data not shown).

4. Discussion

The 4 stages of this experiment identified distinct responses of red spruce to O_3, SO_2 and $O_3 + SO_2$. Sustained decreases in transpiration and assimilation occurred only when both pollutants were present; exposure to ozone alone led to an increase in photosynthetic uptake; and exposure to SO_2 alone led to large fluctuations in net photosynthesis. Our use of sequential pollutant treatments leaves open the possibility that earlier treatment stages influenced the responses observed in later stages of the experiment. It is also possible that the response we observed in the treatment branch has been buffered due to a compensatory response from other untreated branches of the tree. In this respect, the results of branch chamber experiments are probably conservative. The sample size in our exploratory study was limited to one tree. Considering these limitations, we describe our results as suggestive but not conclusive. Additional tests of the observed SO_2/O_3 effects, using other experimental approaches and larger populations of red spruce, are needed to complement our study. In particular, our preliminary evidence of a possible synergism involving a stomatal response

of red spruce to $SO_2 + O_3$ warrants further investigation.

The effect of a 26-day ozone-only exposure, Stage I, was a relative increase ($\sim 20\%$) in the CO_2 assimilation of the treatment branch. The more typical vegetation response to ozone has been a reduction of net photosynthesis (Reich and Amundson, 1987), but the increase we observed is not without precedent. Increased growth and/or photosynthesis have been seen in ozone exposures of loblolly pine (Kress et al., 1989), Norway spruce (Eamus et al., 1990), and red maple (D. Gates, personal communication of data from University of Michigan summer field study). Studies of the effects of ozone on red spruce seedlings have generally shown that there is little effect on growth or photosynthesis (Taylor et al., 1986; Alscher et al., 1989; Laurence et al., 1989; Lee et al., 1987; Kohut et al., 1990). Our study, which is conducted on a native and more mature *P. rubens*, indicates that depression of photosynthesis occurs initially but that assimilation recovers and then surpasses its original level. Increased growth is one possible consequence of this response, but under actual field conditions, nutrients and/or water may be limiting factors and growth stimulation would not necessarily occur. Real-world ozone episodes are usually a few days in duration, so that the initial decrease we observed in net photosynthesis may be more pertinent.

The SO_2 exposures in Stages II (with ozone) and IV (alone) gave peculiar results. In Stage II, it was clearly demonstrated that SO_2 uptake was unaffected by the apparent stomatal closure that reduced all other fluxes by a factor of two. The implication is that the SO_2 flux is entirely to plant surfaces. At the same time, it seems from Stages I–IV that the presence of SO_2 was an essential factor in the apparent stomatal closure seen during this stage. One possible explanation is that surface reactions between deposited O_3 and SO_2, perhaps to form sulfuric acid, may disrupt stomatal function. Another peculiar effect of SO_2 occurred during Stage IV, when exposure to SO_2 induced very high variability in the CO_2 assimilation of the treatment branch. While the SO_2 treatment appears to have simply induced "biological noise" in the assimilation, the effect is more systematic because net photosynthesis and the SO_2 flux correlated negatively during Stage IV. This correlation suggests a cyclic process related to the concentration of SO_2 inside the leaf, but we are unable to offer a full explanation for the behavior at present.

Although the isoprene measurements were not frequent enough to allow us to draw conclusions about the effect of each stage of the experiment, the overall trend we observed was a relative decline in the treatment branch emissions. If the results of this exploratory experiment can be generalized, they suggest that a stabilizing biosphere-atmosphere feedback may occur, whereby ozone exposure reduces the emission rate of biogenic hydrocarbons which are key players in the tropospheric photochemistry which produces ozone. 2 other investigations have had similar findings. Our earlier study showed that compared to a control branch, the emission rates of isoprene and monoterpenes declined in a red spruce branch treated with O_3, SO_2 and H_2O_2 (Ennis et al., 1990b). Hewitt et al. (1990) have shown that ozone exposure reduced the isoprene emissions of California poppies. Clearly, more studies are needed before firm conclusions can be made.

Finally, we note that the responses observed in the four stages of this experiment were different than the increased respiration we obtained in our earlier study of red spruce exposure to $O_3 + SO_2 + H_2O_2$ (Ennis et al., 1990b). One possible interpretation is that each of the four pollutant exposures tested in the two studies (O_3 alone, SO_2 alone, $O_3 + SO_2$, and $O_3 + SO_2 + H_2O_2$) elicits a unique physiological response. If true, the results imply that the presence of H_2O_2 was critical to the respiration increase observed in our previous study. Further, the absence of H_2O_2 may have been important to the apparent stomatal closure seen in the $SO_2 + O_3$ exposure stage reported in this paper. However, we cannot be certain that later pollutant stages were unaffected by the earlier stages in the experiment described in this paper. Alternatively, the difference in the responses may be explained by individual differences between the two native red spruce trees used in the two studies. It has been noted that genetic differences within a given species can lead to differing responses to stress (Kress et al., 1982) and that individual trees may exhibit variability in their responses (Lefohn and Ormrod, 1984). Clearly, these possibilities underscore the need for additional studies to determine whether the responses we observed can be generalized. Such studies will advance our

understanding of how multiple chemical stresses affect red spruce.

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