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Flux determinations and physiological response in the exposure of red spruce to gaseous hydrogen peroxide, ozone, and sulfur dioxide

By CHRISTINE A. ENNIS, ALLAN L. LAZRUS and PATRICK R. ZIMMERMAN,
National Center for Atmospheric Research, P.O. Box 3000, Boulder, Colorado 80307, USA*
and RUSSELL K. MONSON, *Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado 80309, USA*

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ABSTRACT

We report on the 3-week exposure of a branch of a forest-grown red spruce (*Picea rubens*) sapling to the combination of gaseous hydrogen peroxide, ozone, and sulfur dioxide. The exposure was conducted continuously using concentrations of H_2O_2, O_3, and SO_2 that have been observed during the summertime on the summit of Whiteface Mountain, New York. Fluxes of H_2O, CO_2, and the three pollutants were determined throughout the exposure. At weekly intervals, measurements of chlorophyll fluorescence, stomatal conductance, and hydrocarbon emissions were made. The response of the branch was compared to an equivalent branch of the same tree which received no pollutants but was otherwise treated identically. The exposure produced no visible injury symptoms but did produce an increase in dark respiration; the respiration rate more than doubled during the 21-day exposure period. Net photosynthesis was unaffected for both the experimental and the control branches. Nighttime fluxes of SO_2 and H_2O_2 to external plant surfaces were significant. The stomatal component of O_3 uptake by the branch displayed a linear increase during the experiment, and showed no evidence of saturating. Daytime and nighttime fluxes of H_2O_2 were increasing at the end of the experiment. We observed that isoprene is emitted from red spruce, but saw no clear-cut change in emission rate in response to the exposure experiment. Possible relationships between our experimental results and observations of red spruce decline in the forests of the northeastern United States are suggested for further investigation.

1. Introduction

The decline of red spruce (*Picea rubens*) populations has been observed in the high-elevation forests of the northeastern US since the mid-1960s (Siccama, 1974; Siccama et al., 1982; Johnson and Siccama, 1983; Foster and Reiners, 1983; Scott et al., 1984). Red spruce is very important ecologically and is the species which appears to have the greatest sensitivity to damage. Surveys have shown that at high elevations, more than half of the red spruce are dead or in the advanced stages of decline (Johnson and Siccama, 1983; Johnson and McLaughlin, 1986). The observed positive correlation of damage with elevation suggests atmospheric wet and/or dry deposition as a possible factor, since deposition tends to be greater at higher elevations (Lovett et al., 1982). Recent enhancement of atmospheric pollutant levels, induced by anthropogenic activity, may be related to the onset of the current forest decline.

A few experimental studies of the exposure of red spruce to various components of atmospheric wet and dry deposition have been conducted, but none has conclusively built a case for a particular pollutant as the cause of damage. Exposure of red

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spruce seedlings to ozone (O_3) did not influence growth (Taylor et al., 1986; Lee et al., 1987) or induce foliar symptoms (Skelly et al., 1983). Exposure to simulated acid precipitation caused decreased seedling growth and altered development in one study (Percy, 1983) but did not influence biomass in others (Taylor et al., 1986; Lee et al., 1987; Seiler and Paganelli, 1987). The combinations of O_3 + acid precipitation and O_3 + acid mist did not interact to inhibit growth or CO_2 assimilation (Taylor et al., 1986; Lee et al., 1987). Investigations of the effects of other gaseous pollutants (SO_2, NO_x, oxidants) on red spruce have not yet been reported.

In this paper, we report on the exposure of a forest-grown red spruce sapling to the combination of gaseous hydrogen peroxide (H_2O_2), sulfur dioxide (SO_2), and ozone. The subject of the experiment was a red spruce tree transplanted from the forest of the Adirondack Mountains in New York. The experiment was conducted on two branches of the tree, with one branch receiving pollutants continuously for three weeks and the second branch receiving clean air. The experiment made use of our branch chamber exposure system and techniques, described previously (Ennis et al., 1990). Physiological response was compared for the two branches throughout the exposure period, and fluxes of CO_2, H_2O, and pollutants to or from the branches were determined hourly. Ultraviolet light and misting episodes, which have been suggested as possible factors in the occurrence of forest decline, were absent in this experiment.

To date, experimental exposure studies of red spruce have made no tests of gases other than ozone. In this investigation we look at a combination of several gases, in particular H_2O_2, SO_2, and O_3. These three gases occur simultaneously at Whiteface Mountain, at concentrations that are highest during the summer growing season (S. McLaren, 1987. Personal communication of data from Whiteface Mountain field station, Wilmington, New York, USA). Although NO_x and many other gases occur in the ambient atmosphere of the forest, we chose to study the three-pollutant mixture of H_2O_2, SO_2, and O_3 because we postulate that these particular gases interact synergistically to cause injury to red spruce. Our model for this interaction includes the chemical processes which produce acid rain in the atmosphere. In clouds, sulfuric acid (H_2SO_4) is generated when dissolved SO_2 is oxidized (Calvert et al., 1985; Penkett et al., 1979). Ozone and H_2O_2 act in concerted fashion as the oxidants to bring about this transformation. Ozone is the most important oxidant at the high initial values of cloud droplet pH. As acid is generated, the pH of the droplet falls, reaction of dissolved SO_2 with O_3 slows down, and H_2O_2 becomes the principal oxidant of SO_2 below about pH 5 (Penkett et al., 1979). Thus, SO_2 is more completely converted to H_2SO_4 when both oxidants are present simultaneously. We postulate that the same chemistry could apply to SO_2 oxidation in the aqueous media of plant tissue. In this view, the gases would gain entry to the plant via stomata, dissolve in water covering the cell wall surfaces in the substomatal cavity, and then participate in the pH-dependent oxidation reactions discussed above to generate acidity. If H_2O_2 reacts with SO_2 before it is scavenged by peroxidase or other plant antioxidants, its presence makes possible the attainment of extremely low pH values. Certainly some biological buffering of acidity produced in such reactions will occur. If the buffering capacity were to be exhausted, however, the highly pH-dependent physiological functions of the plant would be disrupted.

Aside from the fact that H_2O_2 plays a key role in the production of H_2SO_4, consideration of physical and chemical properties suggests that H_2O_2 itself is potentially harmful to plants. Hydrogen peroxide is approximately equivalent to ozone in its oxidant capabilities, and is several orders of magnitude more soluble in water (Calvert et al., 1985; Penkett et al., 1979; CRC Handbook, 1987). It is produced photochemically in the atmosphere by reactions which involve NO_x and hydrocarbons, so that its presence should be widespread and increasing as atmospheric levels of NO_x and hydrocarbons increase. Several investigators have measured tropospheric H_2O_2 in ground-based and airborne field studies (Heikes et al., 1987a; Heikes et al., 1988; Heikes et al., 1987b; Van Valin et al., 1987; Kok, 1987). Measurements at remote boundary layer sites give clean-air background values of about 1 ppb for H_2O_2 in summer (Heikes, 1988. Personal communication of data from Niwot Ridge, Colorado, and Mauna Loa Observatory, Hawaii).

In the eastern US, maximum concentrations of ~8 ppb have been measured in the summertime; on the summit of Whiteface Mountain, summertime values are typically in the range of 0.5–2 ppb. Aircraft measurements have often shown that the concentration of H_2O_2 increases with altitude above the boundary layer (Heikes et al., 1987a), an observation which may be relevant to the occurrence of greater red spruce damage in high-elevation forests of the northeastern US.

We find no reported studies of the effects of gaseous H_2O_2 on plants, but two studies of H_2O_2-laden mist have been conducted on conifers. Masuch et al. (1986) investigated the effect of H_2O_2-containing fog on 3-year-old Norway spruce trees over a 6-week period. Analysis of needles showed some evidence of the types of damage seen in the European forest: phloem cells collapsed in younger needles, and a buildup of tannins occurred in the vacuoles of mesophyll cells. However, McLaughlin et al. (1988) exposed red spruce seedlings to episodes of H_2O_2 fog for 16 weeks and saw no foliar damage or alterations in gas exchange capabilities and seedling growth.

Following the presentation of experimental methods and data, our analysis explores possible relationships between our experimental results and observations of red spruce decline in the forests of the northeastern US. In addition, the evolution and relationships of pollutant fluxes are related to the chemistry and dynamics of external and internal uptake by the branch.

2. Experimental methods

2.1. Apparatus and approach

The tree used in the experiment was a 10- to 15-year-old red spruce, obtained from the forest of the Adirondack Mountains in New York at about 1000 m elevation. No symptoms typical of the forest decline syndrome (needle loss, chlorosis, etc.) were evident. The tree was rootballed in October, 1986, and remained in the Adirondacks for the winter. It was transported to Boulder, Colorado, in March of 1987, where it was potted in soil from the Adirondack Mountains site and held in cold storage at 5°C for one month. After transfer to a warmer environment at NCAR, budbreak was noted in May, 1987. Environmental conditions were maintained at ~18°C and ~60% relative humidity until the experiment was conducted in October, 1987. A low-flux photoperiod of $15\frac{1}{2}$ h was provided by fluorescent growth lights (Vitalites), and was supplemented for 6 h per day with a high-flux 1000-watt metal halide lamp (PAR ~600 μE m^{-2} s^{-1}).

The exposure experiment and flux measurements were conducted using our branch chamber system, which consists of an environmentally-controlled tree enclosure and flexible Teflon branch chambers (Ennis et al., 1990). For the experiment, 2 branches from the third whorl were entirely enclosed in separate branch chambers. The experiment was conducted continuously for 24 days, 24 h a day, and consisted of two phases. For the first 3 days, both branches received clean air from a laboratory pure air generator. This three-day period provided baseline data to assess the initial variability between the two branches. For days 4 through 24, the control branch continued to receive clean air, while the experimental branch received air with the added pollutants SO_2, O_3, and H_2O_2. Pollutant concentrations were chosen so that the chamber atmosphere would mimic maximum summertime conditions observed at Whiteface Mountain (S. McLaren, 1987. Personal communication). Concentrations of 5 gases (CO_2, H_2O, O_3, SO_2, H_2O_2) were measured at the inlet and outlet of each branch chamber hourly during the exposure experiment. Table 1 gives the average inlet concentrations for pollutants (ppb, parts per billion by volume), CO_2 (ppm, parts per million by volume), and H_2O (ppm). These concentrations had a precision (± 2 times the standard error of the mean) of ~2% or better during the experiment. Daytime and nighttime inlet averages differed by 4% or less. Also listed for each gas in Table 1 is a representative outlet concentration, which gives the actual exposure concentration inside the chamber. The outlet concentration is a function of the flux to or from the branch and chamber, and varies for each gas during the course of the experiment and also on a diurnal basis.

During the 24-day exposure experiment, a 1000-watt metal halide lamp provided a daily 7-h photoperiod, with a flux of ~500 μE m^{-2} s^{-1} (PAR) incident at the branch chambers. A sheet of polycarbonate plastic was used as a filter to

Table 1. *Branch chamber conditions*

A. Branch chamber concentrations

	Daytime concentration		Nighttime concentration	
	inlet	outlet	inlet	outlet
Control chamber				
CO_2 (ppm)	365.3 ± 1.4	353.2	353.8 ± 0.9	355.0
H_2O (10^3 ppm)	21.37 ± 0.06	22.76	20.96 ± 0.04	20.87
Experimental chamber				
CO_2 (ppm)	363.4 ± 1.4	353.7	351.2 ± 1.1	353.0
H_2O (10^3 ppm)	21.65 ± 0.09	22.58	21.01 ± 0.06	20.68
O_3 (ppb)*	101.4 ± 0.4	88.6	100.5 ± 0.3	92.1
SO_2 (ppb)*	14.27 ± 0.07	10.43	13.95 ± 0.06	8.56
H_2O_2 (ppb)*	9.2 ± 0.2	3.3	9.3 ± 0.2	3.9

B. Branch chamber environmental conditions

	Daytime			Nighttime		
	average	maximum	minimum	average	maximum	minimum
T (°C)	26.0 ± 0.3	29.4	23.5	20.5 ± 0.2	23.1	18.8
RH (%)	56.0 ± 0.8	63.3	46.8	71.4 ± 0.7	79.5	61.8

* Average of 97 (daytime) and 117 (nighttime) hourly measurements from days 4–24. All other entries are based on 100 (daytime) and 135 (nighttime) hourly measurements from days 1–24. Error limits are 2 times the standard error of the mean.

remove the ultraviolet component ($\lambda < 400$ nm) of the lamp spectrum. The tree enclosure provided an environment of ~25°C and 40% relative humidity (day) and ~18°C and 65% relative humidity (night). Conditions inside the control branch chamber are listed in Table 1B. Temperature and humidity of the experimental branch chamber were not measured, but were likely very similar. Fans maintained an air velocity of ~10 cm s^{-1} in the branch chambers. Although measurements of the total tree enclosure environment were not made continuously, spot checks indicated that the enclosure temperature was within a few degrees Centigrade and the relative humidity was ~30% lower (day) and ~8% lower (night) than the values recorded for the branch chambers.

The tree was watered at the end of days 5, 10, 14, and 20 of the 24-day experiment.

2.2. *Gaseous flux measurements*

Gas fluxes to or from the tree were determined from the flow rate, needle surface area, and the difference in concentrations measured at the inlet and outlet of each chamber. For each gas, losses to the surfaces of the empty chamber itself were determined in separate experiments before and after the 24-day exposure and also on days 3, 10, and 17 of the exposure. Losses changed during the experiment and were highly dependent on the chemical species and the presence or absence of light. They ranged from a low of 0% ± 0.4% (within measurement error) for CO_2 to a high of 45% ± 0.3% for H_2O_2 in the presence of light. Data from these empty-chamber measurements and computational methods for their use in the calculation of branch fluxes from the raw data have been presented (Ennis et al., 1990). The flow rate was determined from mass flow controller readouts which were calibrated prior to the exposure experiment using bubble flowmeters. After the exposure experiment, four estimates of needle surface area and biomass were made by the methods given in Ennis et al. (1990); results are shown in Table 2.

During the 24 days of the experiment, daytime and nighttime fluxes for CO_2 and water were observed for both branches. In addition, pollutant fluxes (O_3, SO_2, H_2O_2) were observed

Table 2. *Needle area and biomass*

Measurement	Experimental branch	Control branch
total surface area (cm^2)	209.3	204.8
projected area (cm^2)	83.75	82.05
fresh weight (g)	3.295	3.202
dry weight (g)	1.544	1.505

for the experimental branch during days 4–24. The sampling protocol allowed these flux measurements to be made once per hour for each branch chamber. In most cases, data from the last 5 h of the 7-h photoperiod were averaged to compute an average daytime flux for each day of the exposure. The last 6 h of the dark period were averaged to give the corresponding nighttime flux. Total needle area (row 1, Table 2) was used to compute the fluxes, and the flux to the stem itself was assumed to be zero. Other investigators have most often measured only projected needle area, shown for our experiment in row 2 of Table 2. Our fluxes should be increased by a factor of about 2.5 for comparison with such studies. Flux units of moles m^{-2} s^{-1} were chosen so that direct comparisons of fluxes could be made for the various gases. A failure of one detector (H_2O_2) caused the loss of all flux data for day 12 of the experiment.

2.3. *Episodic measurements: hydrocarbon emissions, porometry, and chlorophyll fluorescence*

The continuous gaseous flux measurements were supplemented with other kinds of response analyses which were made periodically during the 24 days of the experiment. These measurements caused only brief interruptions in the acquisition of flux data. Hydrocarbon emissions were measured for each branch near the end of the photoperiod on days 3 (post-clean phase), 7 (week 1 pollutants), 14 (week 2 pollutants) and 21 (week 3 pollutants). The technique was used as described in Greenberg and Zimmerman (1984). Each sample of chamber air was pumped from the outlet of the branch chamber into an electropolished stainless steel canister and later analyzed using a gas chromatograph equipped with a flame ionization detector. Isoprene and monoterpenes were quantified using NBS standard n-propane as a reference material. Sampling required approximately 10 min per branch chamber and was completed in situ, with no disturbance to the tree, branch chambers, or gas delivery.

Other measures of branch response were made on days 0 (pre-experiment), 3 (post-clean phase), 10 (end, week 1 pollutants), 17 (end, week 2 pollutants), and 24 (end, week 3 pollutants). These measurements required that the branch chambers be removed from the tree for approximately 5–6 h on each of the occasions. In each case, the chambers were removed at the end of the photoperiod and were tested for surface losses of SO_2, O_3, H_2O_2, CO_2, and H_2O while the assays of tree response were completed. These assays were porometry and chlorophyll fluorescence. Measurements of stomatal resistance and transpiration were made using a steady-state porometer (Model 1600, LI-COR, Inc.). Measurements were made on 1-year-old needles of a 55-mm segment of the outermost central stem section. Surface area of the segment was calculated using area-per-unit-length correlation factors, determined as previously described (Ennis et al., 1990). In addition to the experimental and control branches, an unenclosed branch from the same whorl was monitored for comparison. Non-destructive chlorophyll fluorescence measurements were made on intact branch segments using a pulse amplitude modulation fluorometer (model PAM 101 Chlorophyll Fluorometer; H. Walz, Effeltrich, Germany). Slow fluorescence induction curves, with periodic superimposed saturating flashes, were obtained for current-year needles on the central stem tip of each branch. Rapid induction curves were obtained for current-year and 1-year-old needles of lateral stems, using a digital storage oscilloscope (Model SE 571, BBC Goerz Metrawatt). In many cases, measurements were also made on an equivalent unenclosed branch, for comparison with the control and experimental branches. Because of the complexities of needle morphology and orientation, a known surface area of photosynthetic material could not be placed a reproducible distance from the fluorescence excitation source. For this reason, different fluorescence curves are analyzed and compared by making use of ratios, rather than absolute values, of the curve parameters.

3. Results

No visual damage to the experimental branch was noted after the 3-week exposure to gaseous H_2O_2, SO_2, and O_3. Comparisons of porometer and chlorophyll fluorescence measurements for enclosed and unenclosed branches (described below) indicate that the presence of the enclosure chambers themselves did not perturb branch photosynthetic and stomatal processes.

3.1. Gaseous flux measurements

For each day of the 24-day experiment, Figs. 1–3 present daytime and nighttime averages of the fluxes of CO_2, H_2O, and pollutants to the

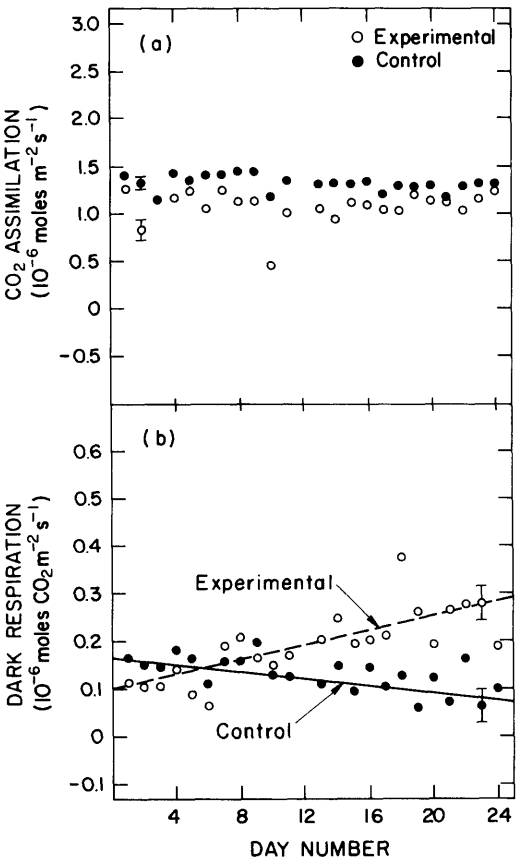

Fig. 1. Daytime and nighttime fluxes of CO_2 for the experimental and the control branch as a function of day of the experiment. Each point is an average of generally 5 hourly measurements (day) and 6 hourly measurements (night). The daily standard deviation of the mean of the hourly fluxes was calculated for each point ($\sigma_{\bar{x}}$). Error bars represent the average of all the daily $\sigma_{\bar{x}}$ values obtained during the 24-day experiment ($\pm 1\,\bar{\sigma}_{\bar{x}}$), and are shown on only four points in the interest of clarity. Trend lines are shown for cases where linear regression gave a nonzero slope (Table 3; text). Dark respiration (b) had a significant trend upward for the experimental branch and downward for the control branch. Net photosynthesis (a) showed no change for either branch. Pollutant exposure of the experimental branch began on day 4.

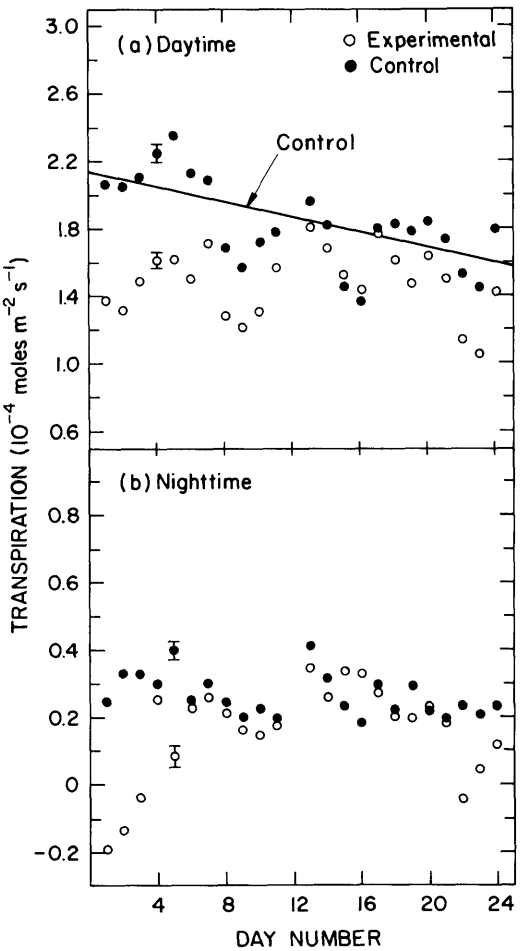

Fig. 2. Average daily transpiration from the control and experimental branches during the experiment. The daily standard deviation of the mean of the hourly fluxes was calculated for each point ($\sigma_{\bar{x}}$). Error bars represent the average of all the daily $\sigma_{\bar{x}}$ values obtained during the 24-day experiment ($\pm 1\,\bar{\sigma}_{\bar{x}}$), and are shown on only four points in the interest of clarity. Trend lines are shown for cases where linear regression gave a nonzero slope. See Table 3 and text for description of trends, oscillations, and anomalous negative points in (b).

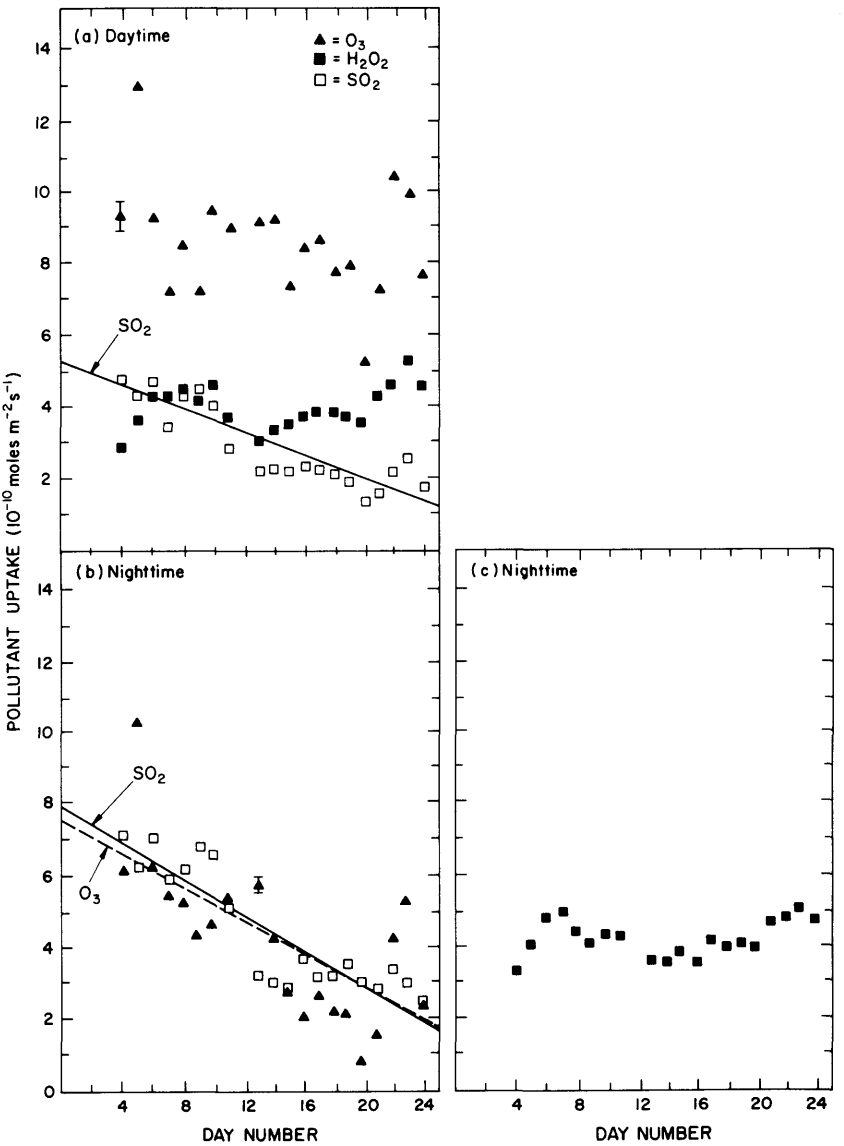

Fig. 3. Average daily fluxes of pollutants to the experimental branch, as a function of day of the experiment, for daytime and nighttime conditions. The daily standard deviation of the mean of the hourly fluxes was calculated for each point ($\sigma_{\bar{x}}$). Error bars represent the average of all the daily $\sigma_{\bar{x}}$ values obtained during the 21-day pollutant exposure ($\pm 1\ \overline{\sigma}_{\bar{x}}$), and are shown on only two points in the interest of clarity. Error bars for SO_2 and H_2O_2 were smaller than the symbols used to represent the average flux in the figure. Trend lines are shown for cases where linear regression gave a nonzero slope.

enclosed branches. The presence of a trend in any given flux is detected by means of a regression analysis of flux versus day number. A trend is reported if the slope of the regression line differs from zero by at least three times the standard error of the slope ($|t| \geq 3$; $P > 0.99$). Table 3 presents overall average fluxes and parameters of the linear regression for each flux.

Fig. 1a shows the average daytime CO_2 fluxes to each branch for the 24 days of the enclosure.

Table 3. *Flux data summary: overall average and linear regression parameters**

Daytime	Average flux	Slope	Intercept	Standard error of slope	t	r^2
experimental CO_2	1.09×10^{-6}	2.235×10^{-9}	1.061×10^{-6}	5.337×10^{-9}	0.42	0.009
control CO_2	1.34×10^{-6}	-4.286×10^{-9}	1.388×10^{-6}	2.389×10^{-9}	−1.8	0.133
experimental H_2O	-1.49×10^{-4}	2.899×10^{-7}	-1.522×10^{-4}	5.906×10^{-7}	0.49	0.011
control H_2O	-1.84×10^{-4}	2.398×10^{-6}	-2.140×10^{-4}	5.920×10^{-7}	4.1	0.439
SO_2	2.85×10^{-10}	-1.594×10^{-11}	5.100×10^{-10}	2.077×10^{-12}	−7.7	0.766
H_2O_2	3.97×10^{-10}	3.308×10^{-12}	3.502×10^{-10}	2.153×10^{-12}	1.5	0.116
O_3	8.58×10^{-10}	-7.566×10^{-12}	9.643×10^{-10}	5.638×10^{-12}	−1.3	0.091
Nighttime						
experimental CO_2	-0.194×10^{-6}	-7.786×10^{-9}	-9.667×10^{-8}	1.436×10^{-9}	−5.4	0.583
control CO_2	-0.133×10^{-6}	3.106×10^{-9}	-1.723×10^{-7}	8.688×10^{-10}	3.6	0.378
experimental H_2O	-0.162×10^{-4}	5.986×10^{-7}	-8.720×10^{-6}	4.218×10^{-7}	−1.4	0.087
control H_2O	-0.268×10^{-4}	3.680×10^{-7}	-3.141×10^{-5}	1.688×10^{-7}	2.2	0.185
SO_2	4.41×10^{-10}	-2.372×10^{-11}	7.756×10^{-10}	2.881×10^{-12}	−8.2	0.790
H_2O_2	4.23×10^{-10}	2.331×10^{-12}	3.896×10^{-10}	1.820×10^{-12}	1.3	0.084
O_3	4.20×10^{-10}	-2.381×10^{-11}	7.556×10^{-10}	5.767×10^{-12}	−4.1	0.486

* Average fluxes are in units of moles m^{-2} s^{-1}. Fluxes from the branch are negative; fluxes to the branch are positive. Regression analyses were performed on the variables flux (moles m^{-2} s^{-1}) versus day number. The entire 24-day enclosure period was used to compute CO_2 and H_2O entries. The 21-day exposure period was used to compute SO_2, O_3, and H_2O_2 entries.

The experimental branch had a lower (~20%) average rate of CO_2 assimilation than the control branch, but neither branch showed a significant change in assimilation rate as the experiment progressed. Thus, net photosynthesis was not affected by either enclosure in the branch chamber or exposure to the pollutants.

Fig. 1b shows average nighttime CO_2 fluxes from each branch (respiration). The experimental branch initially had a lower respiration rate than the control branch. For days 7–10, the respiration rate of the two branches had become comparable and from day 11 to the conclusion of the experiment, the experimental branch had the higher respiration rate. Regressions on the entire 24-h period showed a significant upward trend for the experimental branch respiration. The regression equation gave a doubling time of 14 ± 3.5 days for the respiration rate of the experimental branch. A smaller but still significant downward trend observed for the control branch respiration was perhaps a response to the exposure of the branch to clean air rather than unfiltered laboratory air.

Fig. 2a presents daytime transpiration rates for each branch over the course of the experiment. Significant branch-to-branch variability was observed, with the control branch ~45% higher than the experimental branch during the initial 3-day clean air phase of the experiment. The transpiration rates of the two branches tended to become more similar as the experiment proceeded, but the general tendency for the control branch rate to be higher persisted. Oscillations were seen in this flux data, with each branch showing low points on days 9, 16, and 23. The similarity in overall appearance of the plots for each branch suggests that the oscillatory behavior may be reflective of variation in the overall water status of the tree. The observed oscillations did not correspond with the tree's watering schedule (days 5, 10, 14, 20) or the days on which the chambers were removed from the branches for episodic measurements (days 3, 10, 17). The control branch transpiration exhibited a downward trend over the entire exposure period, while the experimental branch displayed no overall trend in transpiration.

A small amount of water was transpired by each branch at night (Fig. 2b). This nighttime transpiration was roughly 15% of the daytime value. Neither the control branch nor the experimental branch displayed a significant trend in transpiration rate by our criterion. Oscillatory

behavior, seen for the daytime transpiration, was not as evident in the nighttime data. For most of the experiment, the nighttime transpiration rates of the experimental and control branches were comparable (days 4, 6–21). For days 1–3, when both branches received clean air, the data for the experimental branch indicated a water flux to, rather than from, the branch. We suspect an experimental error of some kind is involved in this anomalous result. Detector failure is not likely, since the control branch data are unaffected. The problem may have been due to incomplete equilibration of some sampling lines leading to the dewpoint analyzer. Another possible source of the error lies with the correction for loss of water to the chamber surfaces at night. The correction factor was determined before the experiment, and was remeasured after days 3, 10, 17, and 24. For reasons unknown, the chamber loss correction for the experimental branch chamber may have been inadequate for the initial segment of the experiment.

Average values for daytime and nighttime fluxes of SO_2, H_2O_2, and O_3 to the experimental branch are presented in Fig. 3. Ozone consistently displayed the highest of the daytime pollutant fluxes (Fig. 3a). This flux had an average value of 8.6×10^{-10} moles m^{-2} s^{-1} and showed no significant trend throughout the exposure period. The H_2O_2 and SO_2 daytime fluxes were initially comparable (days 4–11) and were each about half the magnitude of the O_3 flux. On day 11 the two fluxes diverged, and the H_2O_2 flux exceeded the SO_2 flux for the remainder of the exposure.

Regression analyses on the SO_2 and H_2O_2 daytime fluxes showed no significant trend for H_2O_2 over the entire exposure period, and a significant downward trend for the SO_2 flux. In fact, the SO_2 flux presented the most significant trend seen for any daytime flux, declining by about a factor of 3.5 during the 21-day exposure period. Although no cumulative trend was seen for the daytime H_2O_2 fluxes over the entire exposure period, the data exhibit interesting structure on shorter timescales. Regression analysis gives a significant upward trend ($t = 5.6$) for the last 12 days of the exposure period. Regression of the O_3 and SO_2 daytime fluxes do not yield significant trends for these days. Another striking feature of the H_2O_2 flux data in

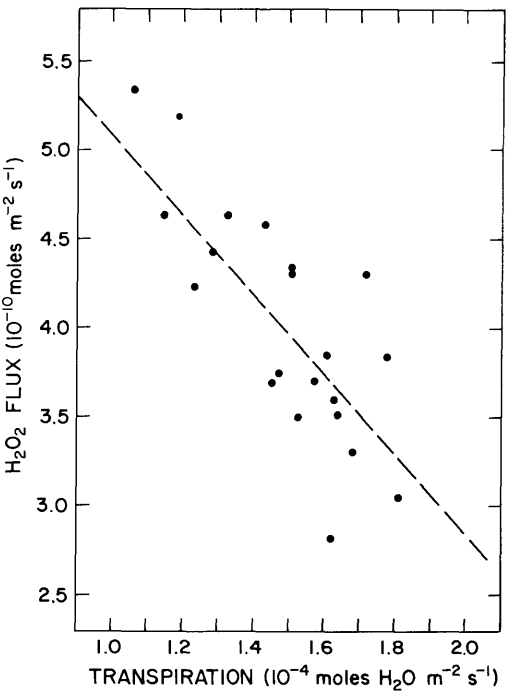

Fig. 4. Linear regression of daytime H_2O_2 flux to the experimental branch (Fig. 3a) versus daytime transpiration (Fig. 2a). Regression results: $t = 4.88$, $r^2 = 0.57$.

Fig. 3a is the occurrence of large-scale oscillations. Local minima appeared on days 4, 13, and 20, straddling peaks on days 8, 17–18, and 23. The data displayed nearly monotonic behavior between these minima and maxima. The periodicity would appear to be about 8–9 days, so that 2 complete cycles and an incomplete third cycle were seen in the 21 days. The periodicity did not correspond with the tree's watering cycle ($\sim$5 days) or with the weekly removal of the branch chambers for episodic measurements. The oscillations in the daytime H_2O_2 flux did tend to correlate negatively with oscillations in the daytime transpiration rate (Figs. 2a, 3a). Fig. 4 presents the linear regression of H_2O_2 flux versus transpiration (daytime data) to illustrate the relationship of these two fluxes.

Figs. 3b and 3c show the average nighttime fluxes of SO_2, O_3, and H_2O_2 to the branch. Compared to the daytime data in Fig. 3a, the three nighttime fluxes were more similar in magnitude throughout the exposure period. Each flux had an average of about 4×10^{-10} moles m^{-2} s^{-1} (Table 3). Large downward trends were

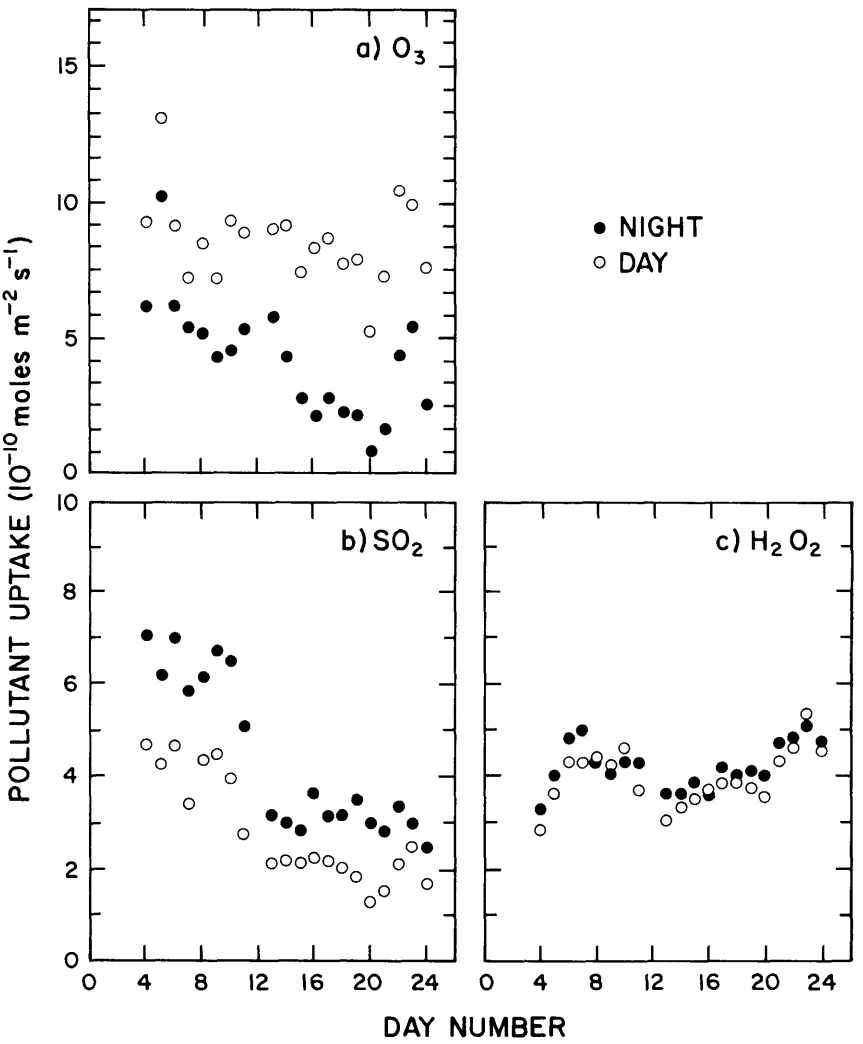

Fig. 5. Pollutant fluxes of Fig. 3, plotted to compare the daytime (open circles) and nighttime (closed circles) fluxes for each individual pollutant.

seen in the SO_2 and O_3 fluxes, with each flux decreasing by more than a factor of 3 over the whole exposure period. The H_2O_2 flux exhibited no trend for the 21 days, but showed evidence of a slow oscillation that closely resembled the daytime H_2O_2 flux behavior (Figs. 3a, 5c). Regression analyses on the data for just the last 8 days of the experiment show that the H_2O_2 flux was trending upward ($t = 3.6$) at the end of the exposure, whereas the SO_2 and O_3 fluxes had flattened out.

Fig. 5 presents the same data of Fig. 3, plotted to compare the daytime and nighttime fluxes of each individual pollutant. The day/night behavior was unique for each gas. Ozone fluxes were consistently higher during the day; SO_2 fluxes were consistently higher during the night; and the daytime and nighttime fluxes of H_2O_2 were nearly identical.

3.2. Episodic measurements

Fig. 6 presents the porometer measurements of stomatal resistance and transpiration for the experimental, control, and unenclosed branches. Measurements for the unenclosed branch showed no significant trends and were comparable to

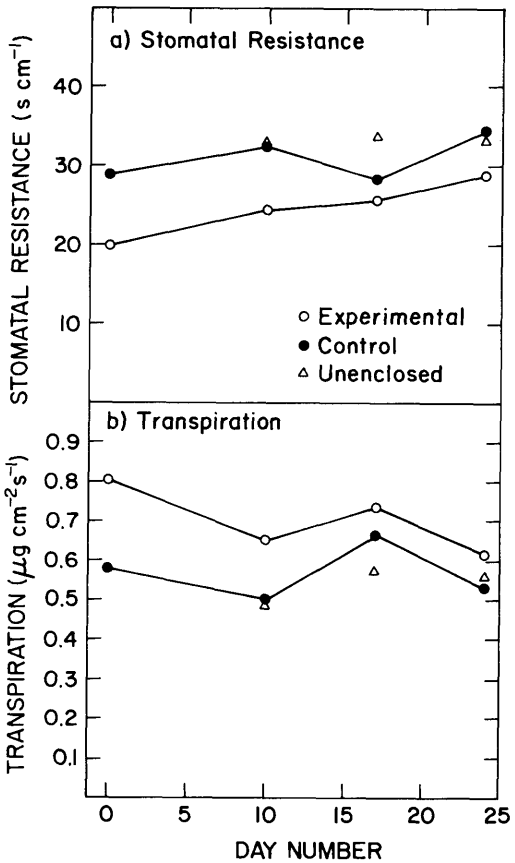

Fig. 6. Results of porometer measurements on one-year-old needles of the experimental and control branches and of a third unenclosed branch from the same whorl. For the experimental and control branches, each point represents the average of three replicate measurements; for the unenclosed branch, each point is the average of two measurements. Regressions show significant trends only for the experimental branch data (see text).

those for the enclosed branches. The unenclosed branch looked most like the control branch, suggesting that the presence of the branch enclosure chamber did not significantly impact stomatal function. Regression analyses show significant ($|t| \geqslant 3$) trends for the experimental branch. Stomatal resistance increased for this branch, while the transpiration rate decreased.

Measurements of isoprene and monoterpenes are listed in Table 4 and plotted in Fig. 7. These measurements were made late in the photoperiod of days 3, 7, 14, and 21. A one-time measurement during the dark was made $3\frac{1}{2}$ h after the end of

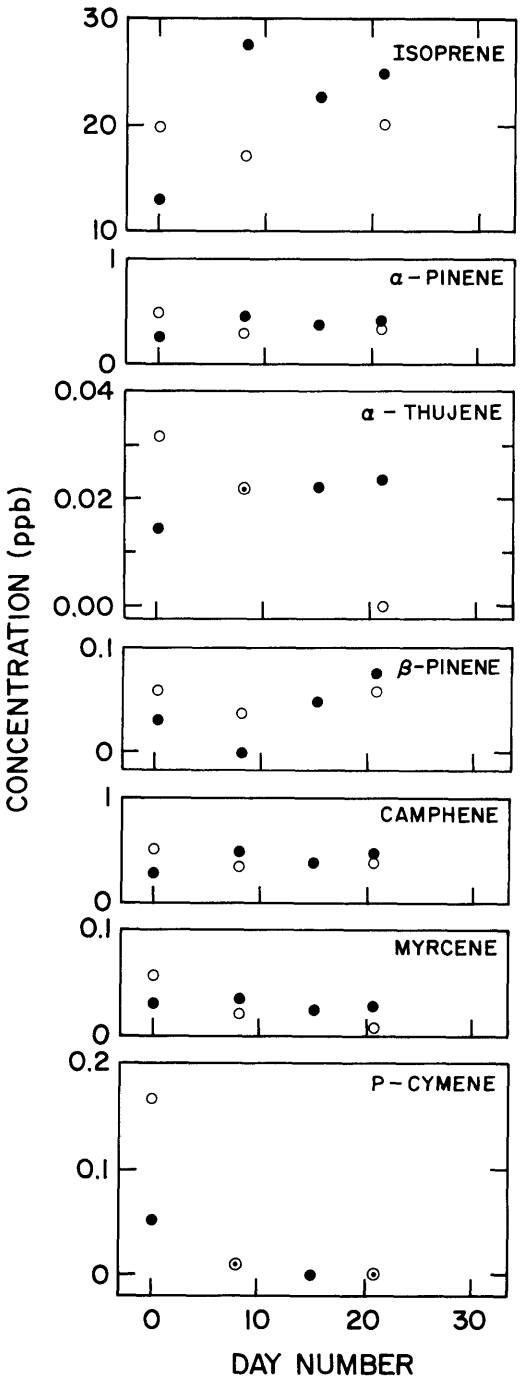

Fig. 7. Concentrations of isoprene and monoterpenes measured in the two branch chambers for the experimental (open circles) and control (closed circles) branches.

the photoperiod on day 21, and gave the expected result of much lower hydrocarbon concentrations. A blank measurement on the two empty chambers, made with the light on and all gases flowing as usual, yielded no measurable amounts of any of the hydrocarbons. The dominant emission observed for each branch was isoprene, by a large margin. To our knowledge, these are the first measurements demonstrating that isoprene is emitted by red spruce. The concentrations listed in Table 4 can be used to calculate an emission rate (moles m^{-2} s^{-1}) by multiplying the concentration, in ppb, by a factor of 1.4682×10^{-10} (experimental branch) or 1.5004×10^{-10} (control branch). Pooling all data for both branches, an approximate emission rate of 3×10^{-9} moles m^{-2} s^{-1} is obtained for isoprene. Regression analyses on the data showed only one significant trend, for α-thujene of the experimental branch ($t = 17.5$).

Chlorophyll fluorescence induction curves were analyzed in several ways to determine if changes occurred in either the control or the experimental branch due to the exposure experiment. The application of periodic saturating pulses during slow induction permitted calculation of quenching coefficients, Q_Q and Q_E, for the photochemical and energy-dependent quenching mechanisms (Schreiber et al., 1986). The curves for Q_Q and Q_E were quite similar for the control and experimental branches and for a third unenclosed branch. No significant differences emerged by the end of the experiment; in fact, the 2 enclosed branches became more similar as the experiment proceeded. After an initial rise time of ~2 min, curves for Q_Q reached a steady-state value of near 1 for all branches, indicating efficient reoxidation of the electron acceptor Q in PS II. This rise-time and steady-state value for Q_Q did not change significantly (data not shown). Q_E curves are presented in Fig. 8. Branch-to-branch variability is indicated by the pre-pollutant data (day 0 and day 3 plots) of Fig. 8. As the experiment proceeded, the Q_E curves became virtually coincident for the control and experimental branches. Relaxation of Q_E occurred at slightly less than 2 min for all branches and was invariant during the experiment.

Ratios of other induction curve parameters also showed no large differences between branches and no systematic development for any individual branch. The ratio $(F_v)_{max}/F_o$ was computed to evaluate the integrity of the water splitting apparatus of PS II (Schreiber et al., 1978). The values of this ratio were within ±5% for the experimental, control, and unenclosed branches at the end of the exposure. Calculated $(F_v)_{peak}/F_o$ ratios were within ±10% for the three branches, suggesting that electron transport through PS II and PS I was unaffected by the exposure experiment. The ratio of peak to steady-state fluorescence $(F_v)_{peak}/(F_v)_{ss}$ displayed none of the dramatic differences seen in comparisons of damaged and healthy trees in the German forest (Lichtenthaler and Buschmann, 1984). Relaxation times for peak variable fluorescence, which reflect the activation of Calvin cycle enzymes, were within 10% for the control and experimental branches at the end of the experiment. Finally, the O-to-I rise times of the fast fluorescence transients were compared as a diagnostic of the onset of PS II light reactions.

Table 4. *Concentration of hydrocarbons (ppb) in branch chambers*

	Experimental branch					Control branch				
Day no.	0	8	15	22	22 (dark)	0	8	15	22	22 (dark)
isoprene	19.850	17.274	—	19.898	0.040	12.970	27.421	22.462	24.794	0.110
α-pinene	0.505	0.306	—	0.359	0.020	0.284	0.475	0.387	0.420	0.041
α-thujene	0.032	0.022	—	*	0.012	0.015	0.022	0.022	0.024	0.004
β-pinene	0.058	0.038	—	0.058	*	0.031	*	0.051	0.076	0.010
camphene	0.516	0.361	—	0.424	0.030	0.275	0.474	0.375	0.403	0.056
mycene	0.057	0.022	—	0.009	*	0.032	0.034	0.024	0.029	0.006
p-cymene	0.164	0.010	—	*	0.031	0.050	0.010	*	*	*

—, Not available.
* Below detection limit.

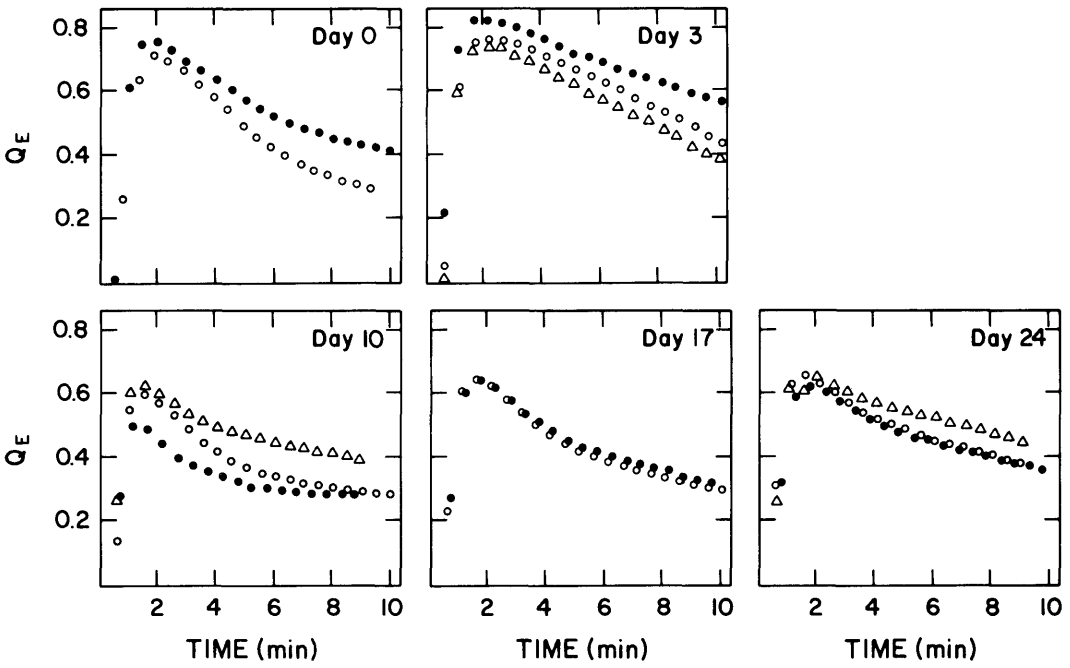

Fig. 8. Energy-dependent quenching curves, as determined by chlorophyll fluorescence measurements, for the experimental (open circles), control (closed circles) and unenclosed (triangles) branches.

These times were about 80 msec for both current-year and one-year-old needles, with a range of about $\pm 10\%$ for the three branches.

4. Discussion

No visible damage occurred due to the exposure experiment, and therefore we concentrated our efforts on the search for any evidence of incipient or "hidden" (Heath, 1980) injury. Our results show that exposure of red spruce to the gaseous pollutants SO_2, H_2O_2, and O_3 elicited one clear-cut physiological response, an increase in nighttime respiration (Fig. 1b). Increased respiration has been seen in other conifers in response to O_3 and SO_2 fumigations (Barnes, 1972; Skärby et al., 1987; Katainen et al., 1987; Vogels et al., 1986). Since respiration provides energy and several molecular precursors for cellular repair processes, enhanced respiration is recognized as a plant response that often follows injury (McLaughlin and Shriner, 1980). The exact site of damage cannot be pinpointed by our experiment, but our results suggest that the chloroplast is not likely to be a site of injury. Photosynthetic capacity, as indicated by net CO_2 assimilation in Fig. 1a, was not diminished in the experimental branch. The chlorophyll fluorescence measurements support this conclusion, since they indicated no significant deviations in the photosynthetic function of the exposed branch. In their study of the effects of a month-long ozone fumigation of 20-year-old Scots pine shoots, Skärby et al. (1987) also observed an increasing respiration rate and an unchanging net photosynthetic rate during the exposure. Field surveys have demonstrated that conifers showing signs of visible damage have higher rates of respiration than healthy conifers (McLaughlin et al., 1982; Vogels et al., 1986). Recent field observations of 30- to 40-year-old red spruce saplings in the eastern US showed higher respiration rates for trees at higher elevation, where damage is generally greatest (McLaughlin et al., 1988). Since net photosynthesis was unchanged in our experiment, the observed increase in respiration implies that the net carbon flux to the exposed

branch was declining. Ultimately, this could lead to the reallocation of carbon from secondary compounds or to reduced growth (Evans, 1975; McLaughlin and Shriner, 1980).

Our in-chamber measurements of whole-branch water flux did not reveal any overall trend in transpiration for the exposed branch (Fig. 2). This measurement gives the integrated response of all age classes of needles to the exposure experiment. Thus, exposure to the combination of SO_2, O_3, and H_2O_2 did not elicit stomatal closure as an avoidance response, as has been seen for O_3 and high doses of SO_2. An alternative response, loss of stomatal control and concomitant increased transpiration, has been observed in some exposure studies but was not seen in our investigation.

The pollutant flux behavior seen during the 3-week experiment (Figs. 3 and 5) illustrates that pollutant fluxes are not static, but evolve with time. Most notable were the daytime and nighttime SO_2 fluxes, each of which declined as the experiment proceeded. This decline cannot be attributed to stomatal closure. Similar behavior has been seen by other investigators for SO_2 (Jensen and Kozlowski, 1975; Elkiey and Ormrod, 1987) and NO_2 (Srivastava et al., 1975).

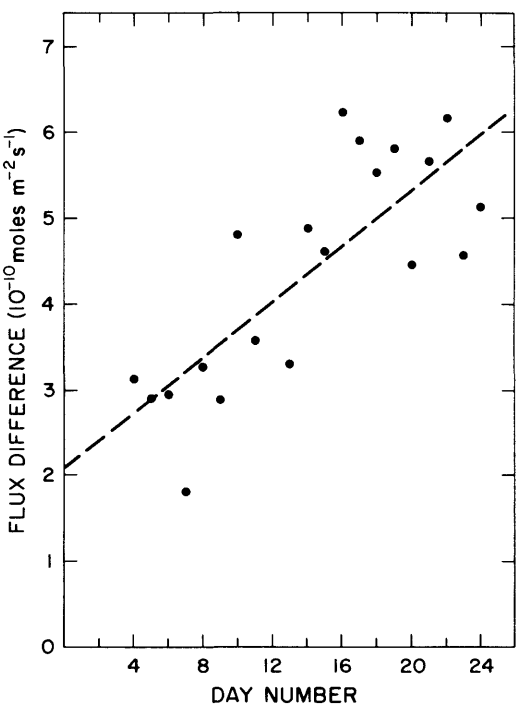

Fig. 9. Internal ozone flux to the experimental branch as a function of time during the experiment. Linear regression shows a significant trend upward ($r^2 = 0.618$, doubling time = 17 ± 4 days).

Pollutant fluxes are plotted in Fig. 5 to contrast the day/night behavior of each flux, and allow for some general observations about mechanisms of uptake for each gas. In broad terms, pollutant flux can be considered to consist of two primary components: uptake by exposed surfaces (dry deposition to needles and stems in our case) and internal flux via stomatal and, to a lesser extent, cuticular entry. The daytime total flux is comprised of both surface and stomatal fluxes, with the stomatal component maximized relative to the nighttime case. As our nighttime transpiration measurements demonstrate (Fig. 2b), it cannot be assumed that stomates are completely closed at night. But, to a first approximation, surface fluxes might be considered to dominate at night. With this simple model in mind (Winner et al., 1985), we can estimate the relative importance of internal and external fluxes for each pollutant.

The day/night behavior of ozone is most readily interpreted, exhibiting consistently higher fluxes in the daytime when stomates are open. If nighttime flux is assumed to represent the surface uptake, Fig. 5a shows that stomatal uptake represents about one-third of the daytime flux at the start of the experiment. By about the midpoint of the exposure, this fraction had increased to approximately one-half, and the absolute magnitude of the stomatal component had doubled. Fig. 9 plots the internal O_3 flux (the difference between daytime and nighttime O_3 fluxes) versus day number to illustrate the increase in internal ozone dose. Linear regression gives a doubling time of 17 ± 4 days ($r^2 = 0.618$) for internal ozone flux. One possible explanation for this development, which could have serious long-term implications for the tree, is that mesophyll resistance to ozone uptake decreased after several days of the exposure. This is the most likely explanation because the other major resistances to ozone flux (aerodynamic, boundary, and stomatal) were unchanged during the experiment. Increased production of antioxidants, such as ascorbic acid, peroxidase, and glutathione, has been observed as a response to

ozone exposures and could account for the decreased mesophyll resistance to ozone uptake. Whether the presence of SO_2 and H_2O_2 influenced the O_3 flux is unknown. An interesting question, raised but not answered by our experiment, is whether the apparent change in mesophyll resistance is reversible. It is important to note that the internal O_3 flux showed no signs of leveling off after 21 days of pollutant exposure. In longer exposures, ozone and its toxic byproducts (oxyanions and oxyradicals such as O_2^-, OH, and HO_2) could become increasingly important agents for inducing physiological damage in the tree. Further experiments are needed to determine whether the upward trend in internal O_3 flux is related to the respiration response of the experimental branch.

The simplest analysis of pollutant uptake assumes that the surface component of the total flux is approximately equal to the nighttime flux and, furthermore, that this component is the same for the daytime conditions of higher light flux, higher temperature, and lower relative humidity. This assumption apparently is invalid in the case of SO_2, which has highest fluxes at night (Fig. 5b). We believe this phenomenon is most likely due to the branch chamber's higher relative humidity at night (Table 1B). Other investigators have demonstrated that SO_2 flux to vegetation is extremely sensitive to relative humidity and that the relationship is positive and nonlinear (Spedding, 1969; McLaughlin and Taylor, 1981). Thus, we cannot resolve the total SO_2 flux into its component surface and internal fluxes from our experiment. Our results were obtained under environmental conditions which approximate the natural diurnal cycling of temperature and relative humidity, and therefore suggest that real-world nighttime fluxes of SO_2 could be significant in conditions where wind-induced turbulence reduces the normally-dominant nighttime aerodynamic resistance. Under such conditions it is possible that the largest fraction of surface deposition of SO_2, in particular, occurs at night.

Hydrogen peroxide fluxes, shown in Fig. 5c, were nearly identical from day to night. Assuming that relative humidity is not a confounding variable for this flux, the implication of this unexpected result is that stomatal uptake of H_2O_2 is possibly negligible. It seems that the extreme reactivity and solubility of this gas, which originally led us to suspect its potential toxicity to vegetation, may result in the near-total deposition of H_2O_2 to external plant surfaces. If supported by further experiments, this finding would refute our hypothesis that within-plant generation of sulfuric acid could occur by a concerted mechanism of SO_2 oxidation by O_3 and H_2O_2. For the tree, surface deposition rather than internal uptake of H_2O_2 is probably advantageous, as cuticular waxes might be expected to be more resistant to attack than cellular membranes, enzymes, etc. Since our observed end-of-experiment trends were showing significant increases for both the daytime and nighttime H_2O_2 fluxes, it is possible that more extended exposures to H_2O_2 could eventually damage the needle surface.

Although many conifers are not isoprene emitters, we observed that isoprene is emitted by red spruce. Because isoprene and other hydrocarbons undergo reactions that produce ozone when NO_x levels are high (Zimmerman et al., 1988; Crutzen, 1987), it is possible that isoprene emission by red spruce sets up a mechanism for enhancing local ozone concentrations in the forest (Elstner et al., 1985). Such a situation could accelerate the occurrence of forest damage. If damage elicits an increase in hydrocarbon emissions, the result is destabilizing in that further increases in ozone would follow. However, if the response of the damaged red spruce is to reduce its hydrocarbon emissions (perhaps in an effort to conserve carbon for repair processes), the response would have a stabilizing effect. Our experiment did not provide clear evidence of either an increase or a decrease in hydrocarbon emission in response to the pollutant exposure, so we are able only to suggest the above range of possibilities.

5. Conclusion

Our experiment suggests several ideas which require further testing in laboratory and field investigations. Exposure of red spruce to the combination of H_2O_2, SO_2, and O_3 induced an increase in dark respiration, but we are unable to isolate which gas or gases produced the effect. An increase in stomatal uptake of ozone also

occurred during the exposure, and may be related to the respiration response. Experiments involving the individual pollutants and pairs of the pollutants will test this possibility. Deposition of H_2O_2 and SO_2 to the branch surfaces was significant at night; the effects of prolonged deposition of H_2O_2 and SO_2 on needle surface integrity require additional study. Further experiments are planned to determine whether the effects we observed are repeatable and can be extended to larger populations of red spruce. Our findings are consistent with field observations of enhanced respiration and reduced growth in high-elevation red spruce stands in the eastern US.

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