Tellus (1990), *42B*, 170–182

A branch chamber system and techniques for simultaneous pollutant exposure experiments and gaseous flux determinations

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

(Manuscript received 29 September 1988; in final form 6 July 1989)

ABSTRACT

We describe an experimental system and techniques for use in simultaneous pollutant exposure experiments and gaseous flux determinations. The system uses flexible Teflon bag-like chambers to enclose entire individual branches of young trees. Five gaseous fluxes (CO_2, H_2O, SO_2, O_3, and H_2O_2) are measured once per hour for each of two branches. Techniques for determining chamber surface loss corrections and methods of estimating needle surface area of conifers are described. A three-week continuous test was run in order to evaluate system performance. Inlet concentrations of the five gases were extremely stable, with uncertainties of $\leqslant 2.2\%$ in all cases and day-to-night differences of $<4\%$. Comparison of two branch chambers, one with clean air and one with the added pollutant gases H_2O_2, SO_2, and O_3, showed that between-chamber differences were $<2\%$ for the average inlet concentrations of H_2O and CO_2. Pollutant losses to chamber surfaces were significant but were generally smaller than losses to the branch itself. Chamber loss corrections were different for daytime and nighttime conditions and also evolved as the three-week experiment proceeded.

1. Introduction

Recent observations of forest decline in the United States, Europe, and other regions of the world have launched a scientific investigation of possible causes. Several excellent reviews of hypotheses have been published (Schütt and Cowling, 1985; Hinrichsen, 1986; Krause et al., 1986; Klein and Perkins, 1987). Many of the hypotheses focus on the role of atmospheric trace gases, such as ozone and photochemical oxidants, SO_2, and organics, which exist at enhanced levels due to anthropogenic influence.

In order to test hypotheses which involve gases as the possible agents of forest damage, some means of interfacing a tree to a chosen gaseous environment must be devised. Research approaches have ranged from exposures of seedlings in laboratory experiments (Yang et al., 1983; Taylor et al., 1986) to field exposures of whole trees in open top chambers (Kats et al., 1985). Any experimental study involves several basic design choices: the subjects may be seedlings or older trees; the enclosure of the tree may be partial or total; and the arena for the experiment may be a laboratory or greenhouse, a managed outdoor facility, or the natural ecosystem.

This paper describes one approach to experimental exposures of trees to gaseous pollutants. It uses branch chambers for conducting laboratory exposures of entire individual branches of trees. The experimental apparatus can accommodate trees up to about 1.6 m in height, and the branch enclosure chambers are of flexible construction to allow for a variety of branch lengths and shapes. Environmental and chemical exposure conditions are controlled and data acquisition is automated.

* The National Center for Atmospheric Research is funded by the National Science Foundation.

Pollutants can be used at concentrations which are representative of the ambient atmosphere of declining forests.

Branch chambers have been used in a few laboratory and field studies. In these applications, branch chambers have been used either 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., 1983; Johansson, 1987; Johansson et al., 1983; Granat and Johansson, 1983). We have chosen to use branch chambers for several reasons. One important consideration is that this approach allows us to use trees rather than seedlings in our exposure experiment. By studying trees, we should get results that pertain more directly to the mature forest. Seedling physiology, being distinct from that of mature trees, has been suspect as a basis for making accurate extrapolations from laboratory exposure studies to the mature forest. Furthermore, observations of damage in the Adirondacks of the northeastern United States have been limited to older trees (A. Johnson, 1987, Univ. of Pennsylvania, personal communication). The use of branch chambers rather than a whole tree enclosure is certainly more expedient and economical, but there are additional important advantages. By conducting experiments on two or more equivalent (same-whorl) branches of a single tree, differences in environmental history and genetics are minimized as experimental variables. Given enough branches, within-tree control and treatment replicates would be possible. The choice of a laboratory rather than a field setting for our experiments involves a tradeoff: a degree of reality is sacrificed, but greater control over environmental and chemical exposure conditions is gained. A laboratory setting also permits the use of more labile gaseous species and thus allows for a higher level of complexity in the studies which can be conducted.

Our approach permits the simultaneous determination of several gaseous fluxes to the tree throughout a pollutant exposure experiment. Deposition of pollutants, transpiration, and net photosynthesis/dark respiration are continuously measured. CO_2 exchange and transpiration serve as valuable indicators of the tree's physiological response to the exposure experiment. Pollutant flux data are important input for models of gas exchange at the biosphere-atmosphere interface, and can provide insight into the tree's biochemical response to the pollutant exposure.

This paper gives details of the exposure system and branch chamber designs. In addition, we discuss experimental techniques used in determining gaseous fluxes from the measured concentrations of gases. System performance during a 3-week continuous run is described.

2. Experimental apparatus

2.1. Exposure system design

Fig. 1 shows a photograph of the exposure system. An environmental chamber provides the tree with lighting, humidity control, and temperature control. The frame of this chamber is rectangular and is constructed from slotted angle iron. Suspended from the top of the 2.6-m frame is a 1000-W metal halide lamp (Philips). An open, clear glass tray (73 cm × 73 cm) containing ~5 cm of water is placed a few centimeters below the reflector and helps to dissipate thermal radiation from the lamp. A sheet of polycarbonate plastic can be placed between the lamp and the tray to remove the ultraviolet ($\lambda < 400$ nm) component of the radiation, if desired. The experimental tree is placed inside the frame below the water tray, in a region that is approximately 76 cm (l) × 76 cm (w) × 165 cm (h). Floor height inside the frame is adjusted so that light flux incident on the experimental branches is a selected value in the range of 300–1500 $\mu E\ m^{-2}\ s^{-1}$ (PAR). A curtain of heavy plastic surrounds the frame, extending from the water tray to the floor to enclose the tree. Laboratory air is cooled and humidified via a commercial evaporative cooler and then is passed through a duct into the plastic enclosure. Using this arrangement, temperature and humidity inside the tree chamber range from ~25°C and 40% (day) to ~18°C and 65% (night).

Controlled delivery of gas mixtures to the branch chambers is accomplished using mass flow controllers (Tylan Corp., models FC260 and FC261) and PFA Teflon tubing and fittings. Fig. 2 shows the flow arrangement used to deliver clean air to a control branch chamber and air

Fig. 1. Exposure system and branch chambers.

containing a three-pollutant mixture of O_3, SO_2, and H_2O_2 to a second branch chamber. Gas flow begins with clean air from a pure air generator (Aadco Instruments, Model 737). The generator processes "house" compressed air and produces pollutant-free zero air which contains <1 ppb (parts per billion by volume) of O_3, SO_2, NO/NO_x, H_2S, COS, and fluorocarbons. Since the output air is depleted in CO_2 relative to the ambient atmosphere, a flow of 5% CO_2 in air from a compressed gas cylinder is added to the clean gas stream. For the control branch, the resulting air containing ~ 360 ppm CO_2 (parts per million by volume) is humidified by passage through a fritted glass bubbler containing water at 15°C. For the pollutant chamber, pollutants are added to the humidified clean air stream, using methods illustrated in Fig. 2 and listed in Table 1A.

Fig. 2 illustrates the scheme used in sampling the inlets and outlets of the two branch chambers. A 5-way stream selecting ball valve (Whitey Co., model 43ZF2) is plumbed with its common outlet connected to the detector sampling manifold and its 4 selectable inlets connected to the branch chambers' inlets and outlets. The valve samples each of the four positions sequentially, in the order shown in Fig. 2. Valve position is driven by a motor controlled by the data acquisition system (described below). The chosen sampling period is

Table 1. *Experimental methods*

A. Gas generation methods

Gas	Source
SO_2	cylinder, 10 ppm SO_2 in N_2 (Linde, custom grade)
O_3	UV photolysis of pure O_2 (Linde, extra dry grade)
H_2O_2	Henry's Law generator (Lind and Kok, 1986)

B. Gas detection methods

Gas	Detection method	Detection limit	Sampling delay time	Calibration method
SO_2	pulsed fluorescence	0.6 ppb	4 min	permeation tube
O_3	UV absorption	2 ppb	10 s	NBS photometer
H_2O_2	enzyme fluorometer	100 ppt	5 min	aqueous standards
H_2O	chilled mirror dewpoint	−10°C	10 s	annual factory calibration
CO_2	infrared absorption	0.5 ppm	5 s	dynamic dilution of NBS standard CO_2

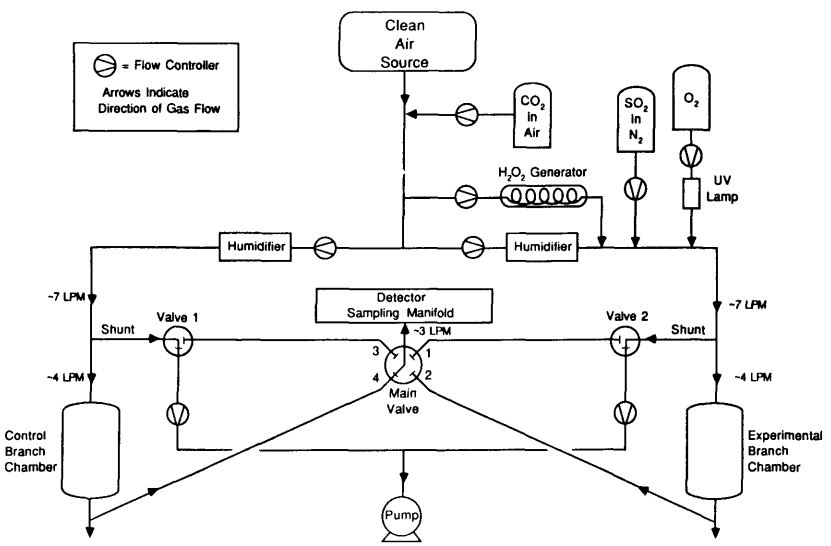

Fig. 2. Flow arrangement for gas delivery to two branch chambers. All gas flows are controlled by mass flow controllers of appropriate ranges. Humidifiers are in a temperature-controlled water bath at 15°C. Sampling of inlets and outlets of the two chambers is controlled by the main valve, which is a 4-position stream selecting valve with common outlet. The figure shows the case where the outlet of the control chamber is being sampled. The flow rate through each chamber is kept approximately constant throughout the sampling cycle by shunting a portion of the inlet gas flow (valves 1 and 2, 3-position solenoid valves) at times when the inlet stream is not being sampled. Branch chambers are represented schematically for clarity.

15 min, so that the inlet and outlet of each branch chamber are sampled once per hour. Samples are drawn actively through this valve by the sampling pumps which are integral to each of the detectors. Throughout the sampling cycle, the flow rate through each branch chamber is kept approximately constant by shunting a portion of each inlet stream at times when the inlet is not being sampled by the detectors (valves 1 and 2 in Fig. 2).

Five detectors are connected to the PFA Teflon sampling manifold. Dewpoint is measured by a chilled mirror instrument (EG & G Environmental Equipment, model 911). This instrument also contains a platinum resistance thermometer, which monitors the interior air temperature of the control branch chamber. Carbon dioxide is measured by infrared absorption (Analytical Development Corp., Model 225 Mk3), SO_2 is quantified using a pulsed fluorescence technique (Thermo Electron Co., Model 43A) and O_3 is determined via ultraviolet absorption (Thermo Electron Co., Model 49). Hydrogen peroxide is measured using the enzyme-based fluorometric system developed at the National Center for Atmospheric Research (Lazrus et al., 1985, 1986). For this experiment, the H_2O_2 detector was operated in the single-channel mode (no catalase) and all detected peroxide is assumed to be hydrogen peroxide. Table 1B lists sampling delay times, detection limits, and calibration procedures for the instruments.

Data acquisition is handled by a datalogger (Monitor Labs, Model 9350). Analog signals from the detectors are sampled and digitized every 8 s. In most cases, the signals are averaged for each 15-min sampling period. For SO_2 and H_2O_2 only the second of two 7.5-min averaging periods is used, to allow for the sampling delay times of the instrument (Table 1B). A separate channel records the sampling position of the main valve. Averages are stored on cassette tape (Techtran Industries, Model 818) for later analysis. Calibration information for each detector is programmed into the datalogger so that signals are stored in meaningful units (parts per billion by volume, abbreviated ppb here, for pollutants; parts per million by volume, ppm, for CO_2; percent relative humidity and dewpoint in °C for H_2O).

2.2. *Branch chamber design*

Fig. 3 shows the branch chamber design. The chamber is a non-rigid bag-like enclosure, made out of PFA Teflon film (thickness ~ 50 μm). This material is selected for its chemical inertness and its transparency at visible and ultraviolet wavelengths. One open end of the Teflon film is placed around the edge of an oval-shaped back plate and is fastened to the plate with an O-ring. This plate is machined from 1-cm FEP Teflon and measures 20.5 cm and 12.7 cm at its longest and widest points. The back plate supports the gas inlet and outlet fittings, a fan motor, and hardware used to attach the branch chamber to the exposure system frame. Six stainless steel 3.2-mm ($\frac{1}{8}$-inch) diameter rods extend from around the perimeter of the back plate and provide internal skeletal support for the flexible chamber material. The rods are sheathed in heat-shrink Teflon to minimize surface losses of gases. Directly opposite the back plate, Velcro strips seal the other end of the chamber around the base of the branch stem.

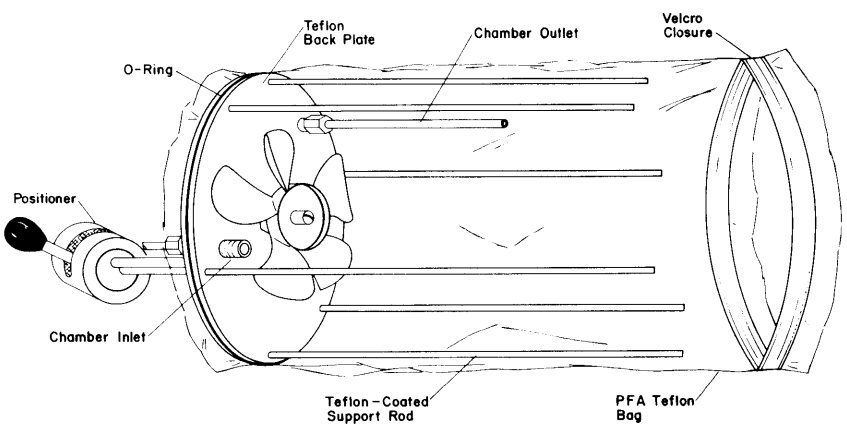

Fig. 3. Details of the branch chamber construction.

Small leaks at this closure are unavoidable but are not important in our configuration because we are pushing air through the bag and thus operating under conditions of slight positive pressure (Jarvis and Čatský, 1971).

Gas is introduced to the chamber directly behind the nylon fan blade and is blown over the branch. The fan ensures thorough mixing of the incoming air stream and minimizes the boundary layer at needle surfaces. The fan speed is adjusted to give an air velocity at the branch of approximately 10 cm s^{-1}, as measured by a hot wire anemometer placed in the center of the chamber. The outlet sampling tube extends into the middle of the chamber, near the branch.

In the application described here, the volume of the branch chamber was ~6 liters and the branch was about 25 cm in length and 13 cm in width. Smaller or larger branches can be accommodated by changing the size of the Teflon bag and bending, shortening, or lengthening the internal support rods.

The natural alignment of the branch itself is undisturbed by the branch chamber because an adjustable positioner attached to the back plate can be used to adjust the chamber's location in all three dimensions. The entire weight of the branch chamber is supported by the exposure system frame via an attachment at the back of this positioner. Because of these design features, the presence of the branch chamber causes little or no mechanical stress to the tree. We have had no difficulty in placing two branch chambers on a single tree 1–1.4 m in height.

3. Gaseous flux calculations

3.1. Correction for chamber losses

From the data of our exposure experiment, we wish to calculate the fluxes of five gases (H_2O_2, O_3, SO_2, H_2O, and CO_2) to or from the branch. The branch is placed in a stirred chamber, with gas flowing at a constant rate through this chamber and concentrations of all 5 species being measured at the inlet (C_{in}) and outlet (C_{out}). Each flux (J) is evaluated from the general expression

$$J = \frac{F \Delta C_{branch}}{A}, \tag{1}$$

where F is the total gas flow rate, A is the surface area of the branch, and ΔC_{branch} is the concentration difference (between inlet and outlet) produced by the branch itself. The flow rate F is determined from the calibrated flow controller settings, and A is measured after the exposure experiment using methods discussed in the following section. If the chamber is perfectly inert and thus does not influence the concentration of the gas, ΔC_{branch} is simply calculated as $(C_{in} - C_{out})$. In our experiment, this simplest analysis applied in the case of CO_2. For other gases, however, the chamber itself does influence the measured concentration difference, so that $(C_{in} - C_{out}) = \Delta C_{total} \neq \Delta C_{branch}$. The derivation below yields expressions for ΔC_{branch} for the case in which the chamber surfaces act as a sink for the gas of interest (H_2O, SO_2, O_3, and H_2O_2 in our experiment).

We consider the general case of a continuously stirred branch chamber, where the concentration of a gas at the outlet is equal to its concentration inside the chamber. We first write the mass balance equation for an individual gaseous species as

$$\left\{\begin{matrix}\text{rate of entry}\\ \text{into chamber}\end{matrix}\right\} - \left\{\begin{matrix}\text{rate of exit}\\ \text{from chamber}\end{matrix}\right\} + \left\{\begin{matrix}\text{rate of production}\\ \text{in chamber}\end{matrix}\right\} - \left\{\begin{matrix}\text{rate of loss}\\ \text{in chamber}\end{matrix}\right\} = \left\{\begin{matrix}\text{rate of accumulation}\\ \text{in chamber}\end{matrix}\right\}, \tag{2}$$

where all rates are in units of molecules per second. We represent the volumetric flow rates ($cm^3\ s^{-1}$) into and out of the chamber as F_{in} and F_{out}, and the chamber volume as V (cm^3). With P as the net rate of all production processes and L as the net rate of all loss processes, eq. (2) may be rewritten as

$$F_{in} C_{in} - F_{out} C_{out} + P - L = V \frac{dC_{out}}{dt}. \tag{3}$$

At steady state during an exposure experiment, $F_{in} = F_{out} \equiv F$ and $dC_{out}/dt = 0$. We assume that: (1) the surfaces of both the chamber and branch serve as independent, first-order sinks for the gas; (2) the branch is the only possible source of the gas within the chamber; (3) no homogeneous chemical reactions occur in the chamber to produce or consume the gas. Eq. (3) then becomes

$$F C_{in} - F C_{out} + \{P - k_{branch} C_{out} V\} - k_{chamber} C_{out} V = 0, \tag{4}$$

where k_{branch} and $k_{chamber}$ are the first-order rate constants (s^{-1}) for the two loss processes. Recognizing the quantity in braces as the net effect of the branch, we re-express eq. (4) as

$$FC_{in} - FC_{out} - F\Delta C_{branch} - k_{chamber} C_{out} V = 0, \quad (5)$$

where ΔC_{branch} is the quantity in the flux eq. (1) which represents the net concentration change (inlet minus outlet) caused by the branch.

To solve for ΔC_{branch} from eq. (5), we must evaluate the term for chamber loss rate, $k_{chamber} C_{out} V$. This is accomplished by making measurements without the branch present, keeping C_{in}, F, and V constant. We define chamber loss, CL, as the measured fractional loss of the gas during the chamber-only measurement:

$$CL \equiv \frac{C'_{in} - C'_{out}}{C'_{in}} = \frac{C_{in} - C'_{out}}{C_{in}}. \quad (6)$$

Mass balance applied to the empty chamber measurement then yields:

$$\frac{k_{chamber} V}{F} = \frac{CL}{1 - CL}. \quad (7)$$

When the branch is present, we define total loss, TL, as the measured fractional loss of the gas:

$$TL \equiv \frac{C_{in} - C_{out}}{C_{in}}. \quad (8)$$

Algebraic manipulation of eqs. (5), (7), and (8) then yields the expression for fractional branch loss:

$$BL \equiv \frac{\Delta C_{branch}}{C_{in}} = \frac{TL - CL}{1 - CL}. \quad (9)$$

Since TL, CL, and C_{in} are all measured quantities, eq. (9) may be used to calculate ΔC_{branch}. Substitution into eq. (1) then gives the branch flux, which by our notation is positive for fluxes to the branch and negative for fluxes from the branch.

If the chamber loss term is small, eq. (9) can be approximated by the simple additive model, BL + CL = TL. This approach has been followed by previous investigators (Elkiey and Ormrod, 1981, 1987). For O_3 and SO_2 in our system, values of CL are small and eq. (9) and the additive model agree within about 5% and 15%, respectively. For gases which react readily with surfaces, however, the additive approximation is not adequate. This effect is illustrated by the case of H_2O_2, a labile gas which in our experiment has a chamber loss fraction of about 0.40 and a total loss fraction of 0.60. The branch loss fraction of 0.33 calculated using eq. (9) is 65% higher than the additive approximation, 0.20.

During our exposure experiments, chamber loss is measured periodically by removing the branch from the chamber. All flow rates, environmental conditions, and sampling procedures are identical to those of the exposure. The chamber water vapor concentration is decreased only slightly by the removal of the branch for this measurement (~6% daytime and ~0.5% nighttime). We have found that chamber loss is different for daytime and nighttime conditions, so separate measurements of CL are made. Fig. 4 shows chamber loss fractions as a function of time for H_2O_2, H_2O, SO_2, and O_3 during a 24-day exposure of a red spruce branch. Values of CL change rapidly at first, when the chamber surfaces are cleanest, and then generally level off. Frequent measurements of chamber loss are important in determining accurate branch fluxes during a continuous exposure. Especially for SO_2 and H_2O_2, the presence or absence of light is a critical variable. Changes in temperature and relative humidity also may contribute to the difference between daytime and nighttime values of CL.

To determine branch fluxes from our measured concentration data, eqs. (9) and (1) are applied for each chemical species. Interpolation of data such as in Fig. 4 gives daily values of the chamber loss term (CL) for each gas. In eq. (1), the gas flow rate (F) is determined from mass flow controller readings which are calibrated using bubble flowmeters. Needle surface area (A) is estimated by the methods described below.

3.2. *Determination of needle surface area and biomass*

For our experiments, we wish to estimate needle surface area and biomass of conifers in a manner which is nondestructive to the experimental branches. Our method assumes that branches on the same whorl of the tree are physiologically and morphologically equivalent, since they are of the same age. At the end of the exposure experiment, small twig samples are cut from an unenclosed branch which is on the same

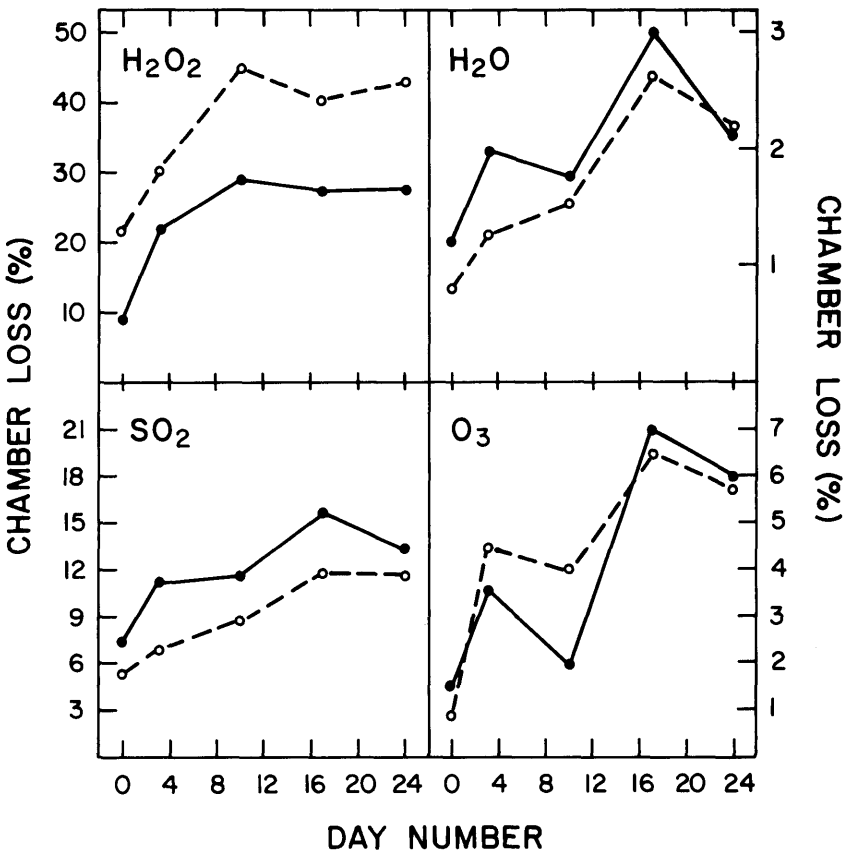

Fig. 4. Chamber loss (CL, %) for the empty branch chamber as a function of time during a 24-day exposure experiment. Values for both light-on (dashed) and light-off (solid) conditions are given. The chamber loss fraction for CO_2 was equal to zero within measurement error.

whorl as the experimental branches. The mass and surface area measurements are made on these samples. The measurements yield correlation factors for mass per unit twig length and area per unit twig length. We have found that these correlation factors depend on the location of the sample on the branch, so we determine correlation factors for three separate categories of twigs: current-year growth tips, central branch stem, and lateral stems. For the experimental branches, total twig length of each category is measured and the correlation factors are applied to calculate total surface area and biomass.

Two measures of needle surface area and two measures of needle biomass are obtained. Total needle surface area is determined by a glass bead technique, adapted from the procedures of several investigators (Thompson and Leyton, 1971; Drew and Running, 1975; Davies and Benecke, 1980; Koppel and Frey, 1984). A monolayer of glass beads (80–115 μm in diameter, Jencons Scientific Limited) is applied to the sample surface using a 1:8 volume mixture of spray adhesive (Scotch brand Photomount, 3M Company) and hexane. The calibration factor, weight gain per unit surface area, is determined using a coiled wire that has a diameter similar to that of the needles. Application of the beads to the complex surface of the twig is improved by the use of a simple fluidized bed technique: the glass beads are placed in a fritted glass funnel, and a flow of a few liters per minute of air is

pushed through the bottom of the funnel. Beads are first applied to the intact twig, and the surface area of the needles-plus-stem is determined. Beads and adhesive are then removed by placing the twig in pure hexane and vibrating the sample in an ultrasonic cleaning unit for about 30 s. Needles are detached from the stem, and the procedure is repeated to determine the surface area of the stem only. Needle surface area is determined by subtraction (Thompson and Leyton, 1971; Koppel and Frey, 1984). Repeated applications of the bead coating procedure to the same intact twig were found to have a standard deviation of 2%. Projected area of the needles is determined using a video camera system (Delta T Devices, model AMS). Needle fresh weight and dry weight are also obtained.

4. System performance

System performance was evaluated during a 24-day continuous experiment on a 10- to 15-year-old red spruce tree. Two branch chambers operated simultaneously on two separate branches of the tree, with one chamber serving as a clean air control and the other chamber receiving clean air for days 1–3 and a mixture of O_3, SO_2, and H_2O_2 for days 4–24. Results of this experiment will be presented in a separate paper. Here, we report only on the reliability and stability of the branch chambers and exposure system during this extended test. The concentrations of the gases at the chamber inlets will serve as the primary indicator of system stability. Inlet concentrations of CO_2 and H_2O for both chambers and O_3, SO_2 and H_2O_2 for the experimental chamber were determined once per hour using the sampling and data acquisition methods described above. Calibrations of the detectors were completed just prior to the experiment. The H_2O_2 instrument was calibrated daily during the experiment, and the calibration of the SO_2 detector was checked on days 6 and 16, and found to need no adjustment. In addition to the gas measurements, the relative humidity and temperature inside the control chamber were measured.

The branch chambers operated continuously and without incident during the 24-day experiment. In the discussion below, all error estimates are 95% confidence limits, calculated as twice the standard error of the mean for 97 hourly values (daytime) and 117 hourly values (nighttime) during the 21 days of pollutant exposure (days 4–24). The daytime temperature of the control chamber ranged from 23.5°C to 29.4°C, with an average of 25.8 ± 0.3°C. The daytime relative humidity in the control chamber was in the range of 46.8% to 63.3%, with an average of 56.4 ± 0.8%. The nighttime control chamber temperature averaged 20.2 ± 0.2°C with a range of 18.8°C to 22.5°C. The nighttime relative humidity was 72.6 ± 0.6%, with maximum and minimum values of 79.5% and 64.4%. Daytime chamber temperature and relative humidity were much like summertime field conditions in red spruce stands of the Adirondacks. Nighttime chamber conditions were slightly warmer (by ~5°C) and less humid (by ~18%) than the summertime field conditions (R. Falconer, 1987. Personal communication of data from Marble Lodge, Whiteface Mountain, New York, USA).

Table 2. *Daytime inlet concentrations, 21-day average values*

Gas	Daytime average*	Relative error (%)	Maximum	Minimum
O_3	101.4 ± 0.4 ppb	0.4	107.2 ppb	96.3 ppb
H_2O_2	9.2 ± 0.2 ppb	2.2	11.3 ppb	7.4 ppb
SO_2	14.27 ± 0.07 ppb	0.5	14.92 ppb	13.35 ppb
CO_2 (experimental)	362.7 ± 1.5 ppm	0.4	379.8 ppm	347.9 ppm
H_2O (experimental)	$(21.74 \pm 0.08) \times 10^3$ ppm	0.4	22.55×10^3 ppm	21.04×10^3 ppm
CO_2 (control)	364.9 ± 1.5 ppm	0.4	383.8 ppm	349.9 ppm
H_2O (control)	$(21.38 \pm 0.07) \times 10^3$ ppm	0.3	22.39×10^3 ppm	20.61×10^3 ppm

* Average of 97 hourly measurements throughout 21 days. Error limits are 2 times the standard error of the mean.

Although measurements of the total tree enclosure environment were not made continuously, spot checks indicated that the temperature was probably within a few degrees Centigrade and relative humidity was probably ~30% lower (day) and ~8% lower (night) than the values recorded for the branch chambers.

Table 2 reports daytime values for the average, maximum, and minimum inlet concentrations for the 21-day pollutant exposure period. Inlet concentrations for both branch chambers were very stable, with 95% confidence limits of less than 1% for all but H_2O_2. The higher 2.2% uncertainty of inlet H_2O_2 most likely reflects variations in the detector calibration, rather than source instability. The nighttime averages (not shown) had similar uncertainties and differed from the daytime averages by less than 4% in all cases.

Table 3. *Percent pollutant loss due to branch**

		O_3	SO_2	H_2O_2
Daytime	Average	61	66	61
	S.D.	8.5	14	4
	Range	48–78	42–89	49–70
Nighttime	Average	45	72	72
	S.D.	21	12	2
	Range	6–90	50–90	65–76

* Average of 97 hourly values (daytime) or 117 hourly values (night-time) during the 21-day exposure. S.D. gives the standard deviation of the sample. The remainder of the pollutant loss is due to surfaces of the branch chamber and gas delivery system.

There was excellent agreement between the two branch chambers for the H_2O and CO_2 average inlet concentrations, with observed differences of 1.7% or less for both day and night averages.

In most cases, chamber loss was found to be the smallest component of the total pollutant loss during the experiment. Table 3 gives the percent of pollutant loss due to the branch. In 5 out of 6 cases, branch loss averaged more than 60% of the total observed loss of pollutants. The nighttime branch loss of ozone was 45% of the total loss on average and ranged from 6–90% of the total loss. Table 4 demonstrates that inlet concentrations were easily resolved from outlet concentrations for all five gases. Listed are the average daytime and nighttime inlet-outlet concentration difference observed for each gas during the exposure experiment, the instrument noise, and the calculated *t*-statistic which tests the significance of the difference between the measured inlet and outlet concentrations. In all cases, the differences were significant at the level of $P \gg 99.9999\%$.

The detectors performed reliably, with only one failure (of the H_2O_2 detector) causing a loss of data on day 12 of the experiment. Continuous operation of detectors and gas sources posed no serious difficulties.

The branch chambers were removed from the tree twice during the pollutant exposure, for approximately 5 h each on days 10 and 17, so that measurements could be made on the branches. Chamber loss values (e.g., (6) and Fig. 4) were also measured during these times. Removal of each chamber requires only about 1 min, and replacement requires ~5 min. Because the

Table 4. *Measurability of hourly inlet–outlet differences*

		CO_2	O_3	H_2O_2	SO_2	Dewpoint
$[C_{in} - C_{out}]$*	day	10.08 ppm	12.81 ppb	5.971 ppb	3.83 ppb	0.67°C
	night	1.76 ppm	8.39 ppb	5.333 ppb	5.36 ppb	0.24°C
Instrument† noise (s)		0.5 ppm	1 ppb	0.02 ppb	0.3 ppb	0.06°C
t-statistic‡	day	151	96	1580	68	84
	night	26	63	1411	95	30

* Average hourly inlet/outlet concentration difference observed in a 21-day exposure experiment.

† Manufacturer's stated instrument noise.

‡ Test for the significance of the difference between two means, C_{in} and C_{out}; as in J. E. Freund (1979). The number samples (n) in each hourly value of C_{in} and C_{out} is 112 for CO_2, O_3, and dewpoint; $n = 56$ for H_2O_2 and SO_2. The *t*-statistic is calculated as $(|C_{in} - C_{out}|/s)\sqrt{\frac{1}{2}n}$. A value of $t > 5$ indicates that the difference is significant at the level $P > 99.9999\%$.

chamber is non-rigid, we evaluated the variation in chamber volume due to this reassembly. We determined chamber volume for each segment of the exposure period by measuring the dilution ratio of a sample of a 32 ppb standard SF_6 mixture (Scott-Marrin, Inc.) injected into the sealed chamber. The SF_6 analysis was performed using a gas chromatograph equipped with an electron capture detector. The detector response was calibrated using a 100.2 ppt (parts per trillion by volume) standard SF_6 mixture (Scott-Marrin, Inc.). For the three one-week segments of the exposure period, the control chamber volume was 5700, 6900, and 6600 ml. The experimental branch chamber was 5400, 6300, and 6500 ml in volume. Thus, a range of approximately $\pm 10\%$ was seen about the average volume for each chamber. The precision of the volume measurement technique was found to be about $\pm 2\%$.

5. Discussion

Performance of the exposure system and branch chambers was satisfactory, as judged by the stability of gas inlet concentrations and the small between-chamber differences for CO_2 and H_2O concentrations. Pollutant losses to chamber surfaces were significant but were generally smaller than losses to the branch itself. Environmental conditions of temperature and relative humidity in the chambers were in a range that was reasonable for the tree, producing no visible ill effects on the vigor of the enclosed branches. The exposure system will be improved by the addition of sensors to monitor simultaneously the temperature of each branch chamber and the larger tree enclosure.

As described in a companion paper (Ennis et al., 1990), the exposure system, branch chambers, and methods presented here have been used to determine concurrent physiological response and gaseous pollutant fluxes in exposure experiments on red spruce. Results from such experiments will prove useful in (1) testing hypotheses concerning the forest damage problem and (2) quantifying dry deposition of gaseous pollutants to vegetation. The branch chambers should readily accommodate experiments on other tree species and with other gases, both in the laboratory and in the field.

The use of branch chambers in exposure experiments presupposes that the individual branches of a tree can be treated as independent units ("branch autonomy", van der Wal, 1985). The idea of branch autonomy is important since the results of an exposure experiment would be compromised if the tree is able to compensate for pollutant stress to one of its branches. Although this issue is hardly resolved, some experimental evidence from ^{14}C and wood growth studies has supported the notion that individual branches are largely autonomous after the first year of growth (Forward and Nolan, 1961; Rangnekar et al., 1969; Isebrands, 1982). It is unknown if autonomy is maintained during periods of short-term stress to one or more branches. Insight into these questions will be gained from branch exposure experiments which make concurrent observations of within-tree control branches and unenclosed branches.

6. Acknowledgements

We are grateful to C. Claiborn and J. Smith for helpful discussions concerning the correction of data for chamber losses. We wish to thank J. Smith, T. Fredrick, and K. Sieckman for writing programs to analyze the gaseous flux data. A. Johnson and D. Vann provided red spruce saplings. C. Ennis gratefully acknowledges the support of a postdoctoral fellowship from the Advanced Study Program of the National Center for Atmospheric Research. The National Center for Atmospheric Research is sponsored by the National Science Foundation. This research was co-sponsored by NCAR and the United States Environmental Protection Agency, Corvallis, Oregon, under NCAR-EPA Interagency Agreement No. DW499325-01-0.

Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency through Interagency Agreement No. DW499325-01-0 to NCAR, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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