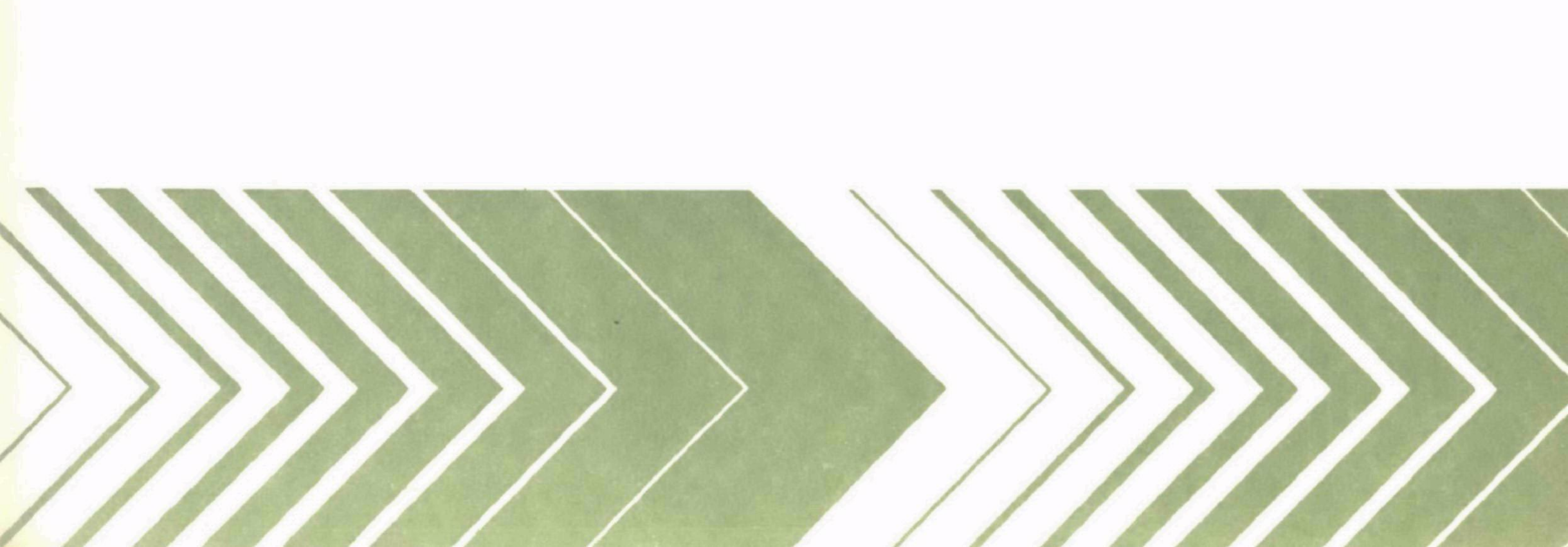
United States
Environmental Protection
Agency

Environmental Research
Laboratory
Corvallis OR 97330

EPA-600/3-79-108
October 1979

Research and Development

A Gas-Exchange System for Assessing Plant Performance in Response to Environmental Stress



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EPA-600/3-79-108
October 1979

A GAS-EXCHANGE SYSTEM FOR ASSESSING PLANT PERFORMANCE IN RESPONSE TO ENVIRONMENTAL STRESS

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lakes and streams; and the development of predictive models on the movement of pollutants in the biosphere.

The performance of plants in pollution-stressed natural and agroecosystems is a concern of the Corvallis Laboratory. This report describes an experimental laboratory system designed to assess how pollutants may affect plant growth and productivity. The adaptability of the system makes it applicable to a wide range of research efforts focusing on the response of vegetation to either soil or atmospheric pollutants.

Thomas A Murphy
Director, CERL

ABSTRACT

Anthropogenic stresses are increasingly common as environmental factors affecting the performance of plants in both natural and agro-ecosystems. There is a need to determine how these stresses may influence vital physiological processes in plants. This report documents the design, construction and performance of a whole-plant, gas-exchange system that can accurately monitor gas flux (e.g., carbon dioxide, water vapor, pollutants) between plants and the atmospheric environment. From these data, rates of key physiological processes - photosynthesis, transpiration, gaseous uptake and emission - can be assessed. Example studies are reported on the uptake of sulfur dioxide by plants and emissions of monoterpenes from plants.

CONTENTS

INTRODUCTION

Gases such as carbon dioxide and water vapor are key constituents in many plant physiological processes. The influx of carbon dioxide from the atmosphere into the leaf is required for photosynthesis, and the efflux of water vapor during transpiration helps dissipate heat build-up within the leaf. This gaseous exchange process between a plant and its environment is intimately associated with the plant's physiological status. Therefore, the study of gas flux provides an experimental means of assessing the physiological performance of plants.

Irrespective of the direction of flow, gas movement between leaves and the surrounding atmosphere is a consequence of diffusion. Net flux (J) or rate that a gas (i) is emitted or absorbed is proportional to the ratio of the steepness of the concentration gradient (ΔC) to distance (x_i) over which diffusion occurs.

$$J_i = D_i \, \Delta C_i / x_i . \qquad (1)$$

D_i is the diffusion coefficient of the gas and is a function of the molecular weight and diffusion medium (Nobel 1974). Since the driving force of gas exchange is the concentration gradient, any gas exhibiting a concentration differential will tend to diffuse along the gradient, and this movement occurs irrespective of the physiological importance of the gas to the plant. In addition to the concentration gradient, gas flux is regulated by resistances (R) along the diffusion pathway. Incorporating this component into Equation (1) yields the following:

$$J_i = \Delta C_i / R_L \qquad (2)$$

This relationship, depicted in Figure 1, is a basis for understanding the gas-exchange process in plants (Gaastra 1959).

It is possible to assess the rates of net photosynthesis and transpiration with an analysis of carbon dioxide and water vapor fluxes. Further analysis, coupled with appropriate experimental design, can relate changes in carbon dioxide and or water vapor flux to corresponding changes in leaf resistance components, including boundary layer (R_a), stomatal (R_s), and residual (R_r). With these capabilities the researcher can monitor the effects of varied environmental conditions (gaseous pollutants, water stress, toxics, light, temperature, etc.) on the plant, with an objective of addressing how each affects specific physiological processes. The purpose of this research was to design, fabricate and test a gas-exchange system that could perform these functions.

GAS-EXCHANGE SYSTEM

The gas-exchange system (Figure 2) is designed to assay quantitative changes in gas composition that result from plant activity; the system is based upon the theory of mass balance of gases. This approach requires quantification of each of the three fates of a gas entering the chamber: (i)

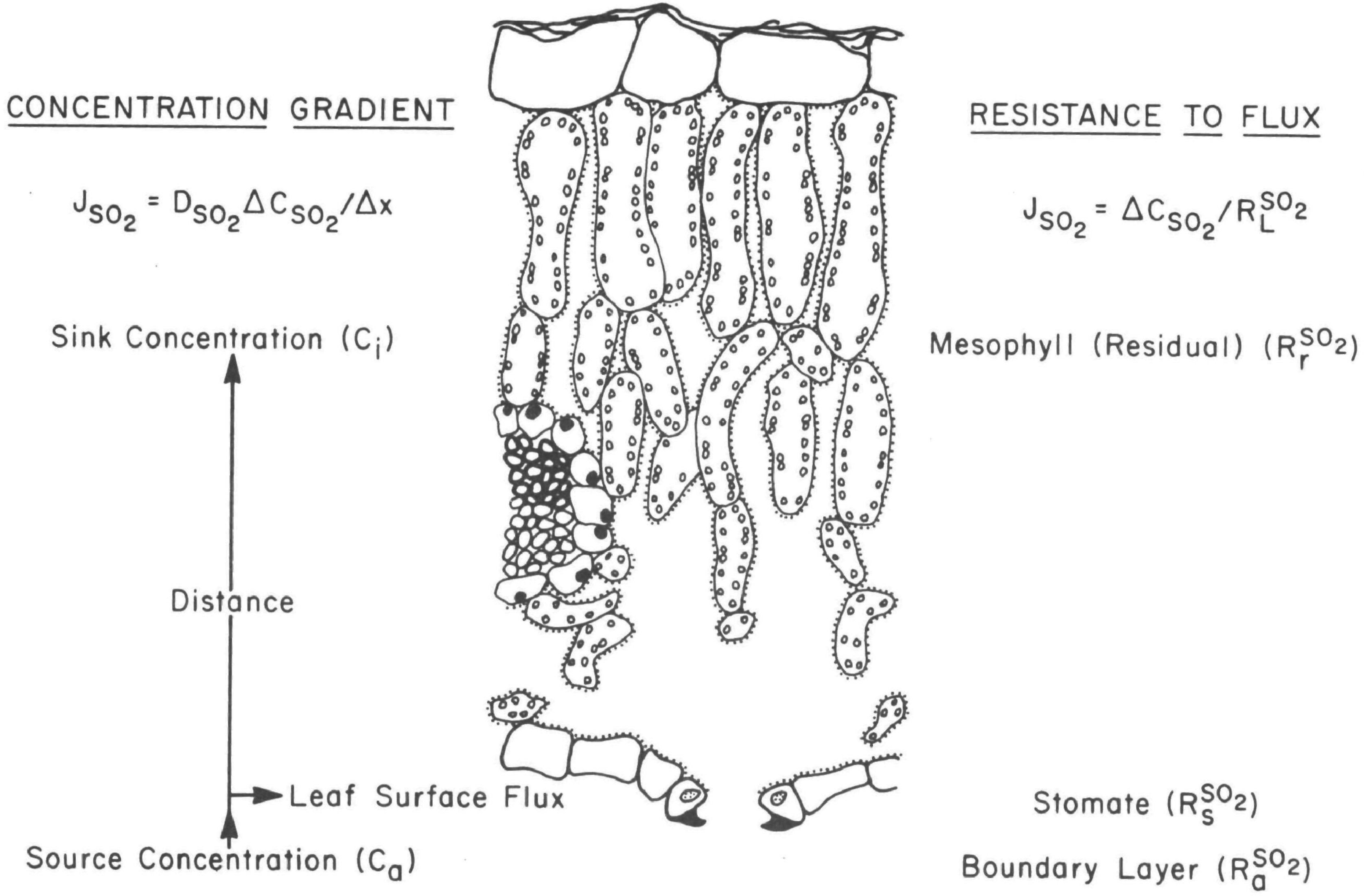

Figure 1. Diagram illustrating the factors affecting the flux of gases between a leaf and its environment.

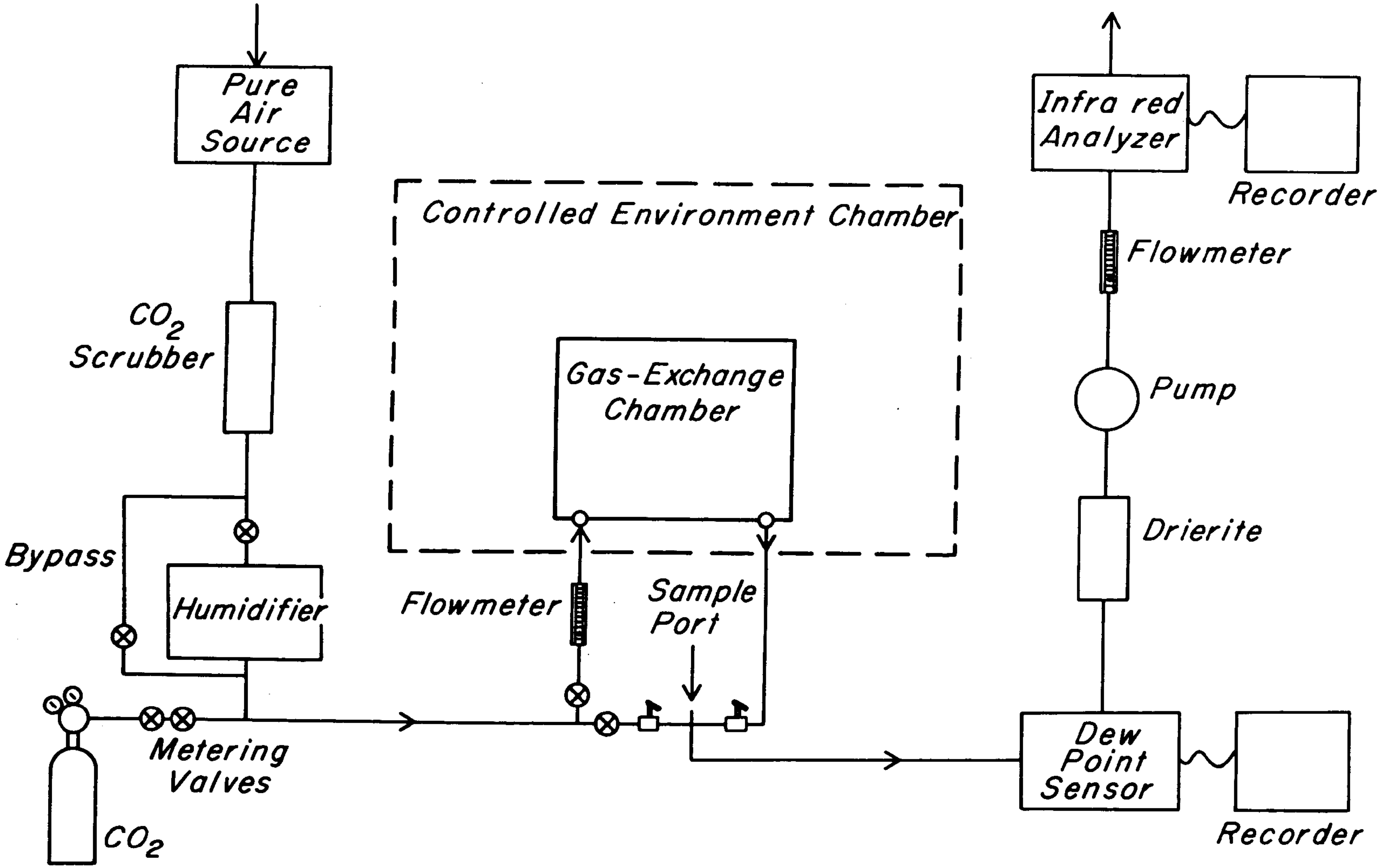

Figure 2. Diagram of the gas-exchange system.

adsorption to the chamber walls and equipment, (ii) reaction (adsorption and absorption) with the plant, and (iii) exit by the outlet. By knowing the inlet and outlet mass of the gas plus the loss rate to the chamber (*via* experimentation), the mass reacting with the plant can be determined.

The flux of any gas can be modeled as follows (Sestak, Catsky and Jarvis, 1971):

$$J = (F \times C)/n \qquad (3)$$

where J = flux of gas to the plant,

F = flow of air through the chamber,

C = change in gas concentration between the chamber inlet and outlet,

n = leaf area.

The model is applicable to gas being taken up (e.g., carbon dioxide and sulfur dioxide) as well as that diffusing out of the leaf (e.g., water vapor and hydrocarbons).

A schematic of the gas-exchange chamber is shown in Figure 3. The cylindrical, plexiglass unit consists of two compartments partitioned by a horizontal, removable baseplate. The lower compartment houses the plant pot and root mass, and the upper unit encloses the above-ground vegetation. The baseplate is sectioned through the diameter with a small opening that allows the two halves of the plate to encircle the stem so that the above- and below-ground plant parts are isolated. Any cracks or openings are sealed with modeling clay, thus completely separating the two compartments. The geometry of the respective compartments is specified in Table 1.

TABLE 1. DIMENSIONS OF EXPERIMENTAL GAS-EXCHANGE CHAMBER

Compartment	Diameter (m)	Height (m)	Volume (m^3)	Height/Diameter
Upper Compartment				
Large Unit	0.375	0.415	0.046	1.11
Small Unit	0.375	0.155	0.017	0.41
Lower Compartment	0.260	0.240	0.013	0.92

The chamber is supported in a plexiglass frame as shown in Figure 3.

Many features of the upper compartment are incorporated to optimize rapid mixing of the air mass so that stratification and pocketing are minimized. Engineering theory (Rogers *et al*. 1977) suggests that instantaneous mixing is achieved in a cylindrical enclosure in which the height:diameter ratio does not exceed 2. Chamber dimensions for the two upper compartments (Table 1),

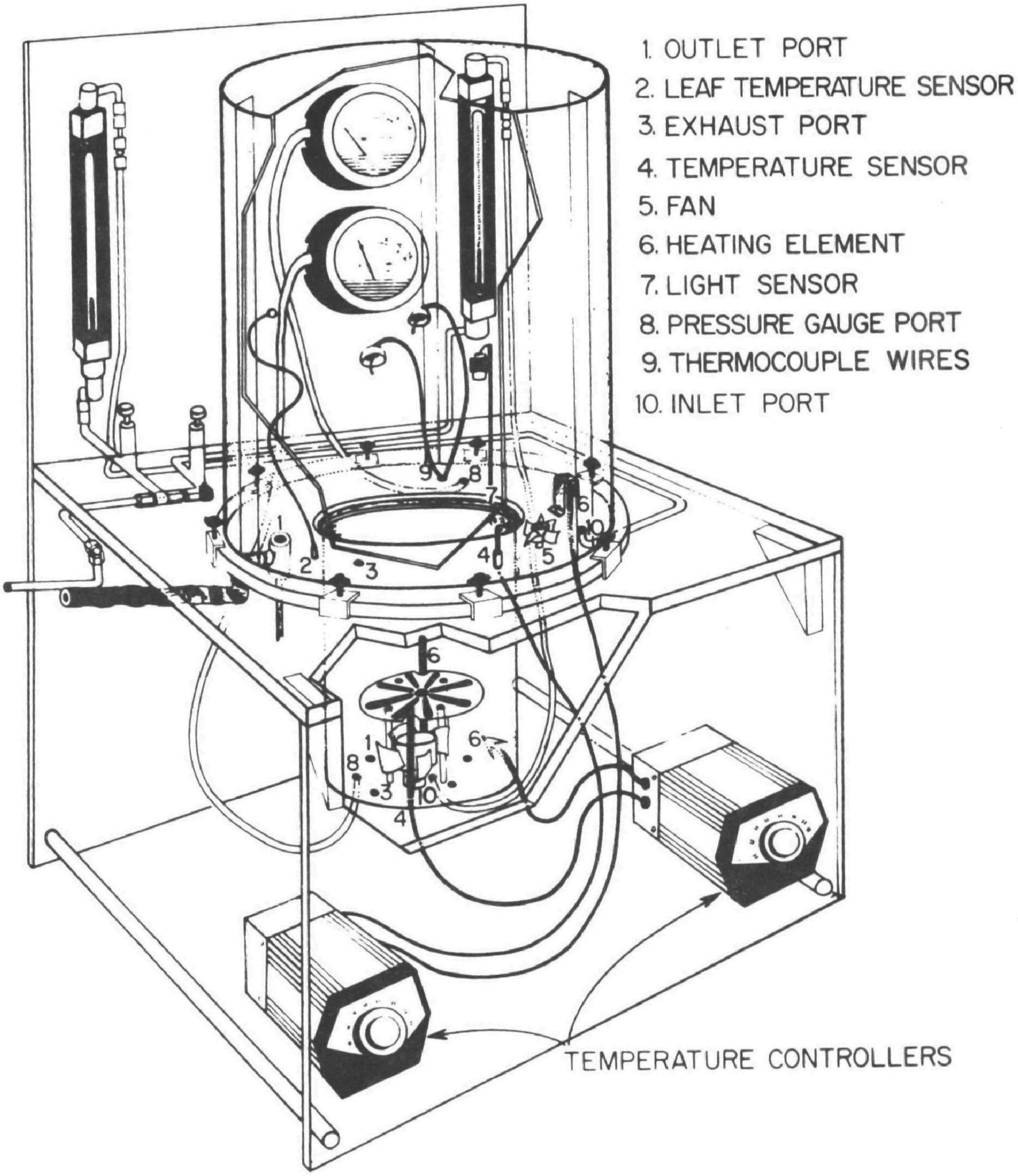

Figure 3. Diagram of the gas-exchange chamber.

which were dictated by plant size and physical constraints of the controlled environment chamber, provide a ratio of 1.11 and 0.41. Turbulence is created by two devices. First, six impeller blades rotated by a variable speed electric motor (outside the chamber) rapidly mix the incoming air with the existing air reservoir. Second, three vertically-arranged baffles (2.5 cm high) are aligned equidistant around the sides of the chamber. Several ports in the floor of the upper and lower chambers provide access for inlet and outlet air lines as well as equipment probes. The construction of the lower compartment is identical to the upper one except for the height:diameter ratio and the absence of wall baffles.

Air flow into the chamber is regulated by variable flow rotameters. Air temperature regulation is provided by a floor-mounted, temperature sensor and heating pole integrated to a proportioning controller (Love Controls Corp., Model 49). Since the system requires continuous positive pressure, a differential pressure gage is plumbed to the chamber and mounted on the housing cabinet. Air temperature is monitored by a temperature probe mounted at the chamber outlet and shielded from incident light. Light irradiance is monitored at canopy level (Lambda Instruments Co., Inc., Quantum Sensor, Model LI-105). All wavelengths of visible light are transmitted equally through the chamber ceiling and walls; however a uniform reduction of 8% at each wavelength occurs (Rohm and Haas, Inc.). Leaf temperature is monitored continuously with an in situ thermocouple as described by Lange (1965), and temperature (±0.2°C or °F) is reported on a multi-point digital thermometer (Omega Engineering, Inc., Model 2176A with Analog Processor) and recorder.

All gas lines are 1/4-inch stainless steel or copper tubing. Ambient air is pumped through a reactor (AADCO 737 Series Pure Air Generator) which produces pressurized, clean air (Figure 2). At several locations along the inlet air lines, ports are provided for bleeding in gas mixtures such as carbon dioxide, pollutants or dry and moist air. A series of regulatory valves situated in the lines prior to the gas-exchange chamber permit manual redirection of the gas flow so that alternative flow of inlet and outlet sample air to the analyzers can be achieved. When required, syringe ports are placed in the gas lines at the chamber's inlet and outlet. Air is forced through a dewpoint and temperature water bath system in which varying dewpoint levels are achieved by changing the temperature of the water bath or by mixing humid with dry air.

The quantitative analysis of gas stream composition between inlet and outlet air is the basis of the gas-exchange system. The choice of specific analyzers is dictated by the objectives and will likely vary between experiments; consequently discussion of individual analyzer units is not provided.

PERFORMANCE OF THE GAS-EXCHANGE SYSTEM

The gas-exchange system requires instantaneous mixing so that composition of all aliquots of the air reservoir are equivalent. This criterion is assessed using first order chemical reaction kinetics (Rogers et al. 1977). The time for an outlet concentration (e.g., 350 ppm CO_2) of a chamber gas to decrease to a specific value (given an inlet concentration of 0) is monitored experimentally and compared with that predicted from theory. The expected time is derived from the following equation:

$$C_t = C_{t_o} (1 - e^{-Qt}) \tag{4}$$

where C_t = the outlet gas concentrations after some time t,

C_{t_o} = initial outlet gas concentration at time t_o

Q = 1/residence time or the ratio of flow to volume.

Solving for t,

$$t = -\ln (1 - C_t/C_{t_o})/Q. \tag{5}$$

If the gas-exchange system exhibits instantaneous mixing, time calculated from the above equation will approximate that observed under experimental conditions. This test (using CO_2 as the test gas) was performed in an empty chamber (large upper compartment) at six different flow rates ranging from 2 to 6 l min^{-1} with a dewpoint and chamber temperature of 4.5 and 26.7°C, respectively.

Irrespective of flow rate, the profile of carbon dioxide decay within the chamber is an inverse function of flow rate (Figure 4A). A linear regression analysis (Figure 4B) of observed versus expected times accounts for 95% of the variation, and the slope (1.14) is not statistically different from 1.0. This suggests that instantaneous mixing is achieved.

GAS-EXCHANGE SYSTEM IN OPERATION

Two examples of studies utilizing the gas-exchange system are reported: (i) flux of gaseous pollutants to plants and (ii) the emission of monoterpenes from plants.

FLUX OF GASEOUS POLLUTANTS

Populations of Geranium carolinianum, a winter annual weed, vary in their foliar response to acute sulfur dioxide (SO_2) exposure, and population differences are genetically controlled (Taylor 1978). Flux of sulfur dioxide to plants was monitored to determine whether pollutant resistance is associated with reduced SO_2 uptake into the plant. Seeds were germinated and seedlings grown in a Jiffy Mix: Perlite (1:2;V:V) mixture. Plants were cultured in a greenhouse with maximum day/night temperatures of 28° and 20°C, respectively. The photoperiod was extended to 16 hours per day. A modified Hoagland's nutrient solution (1/2 strength) was applied daily. At least two weeks prior to experimentation, plants were transferred to a growth chamber having an environmental regime similar to that of the gas-exchange chamber (see legend, Figure 6).

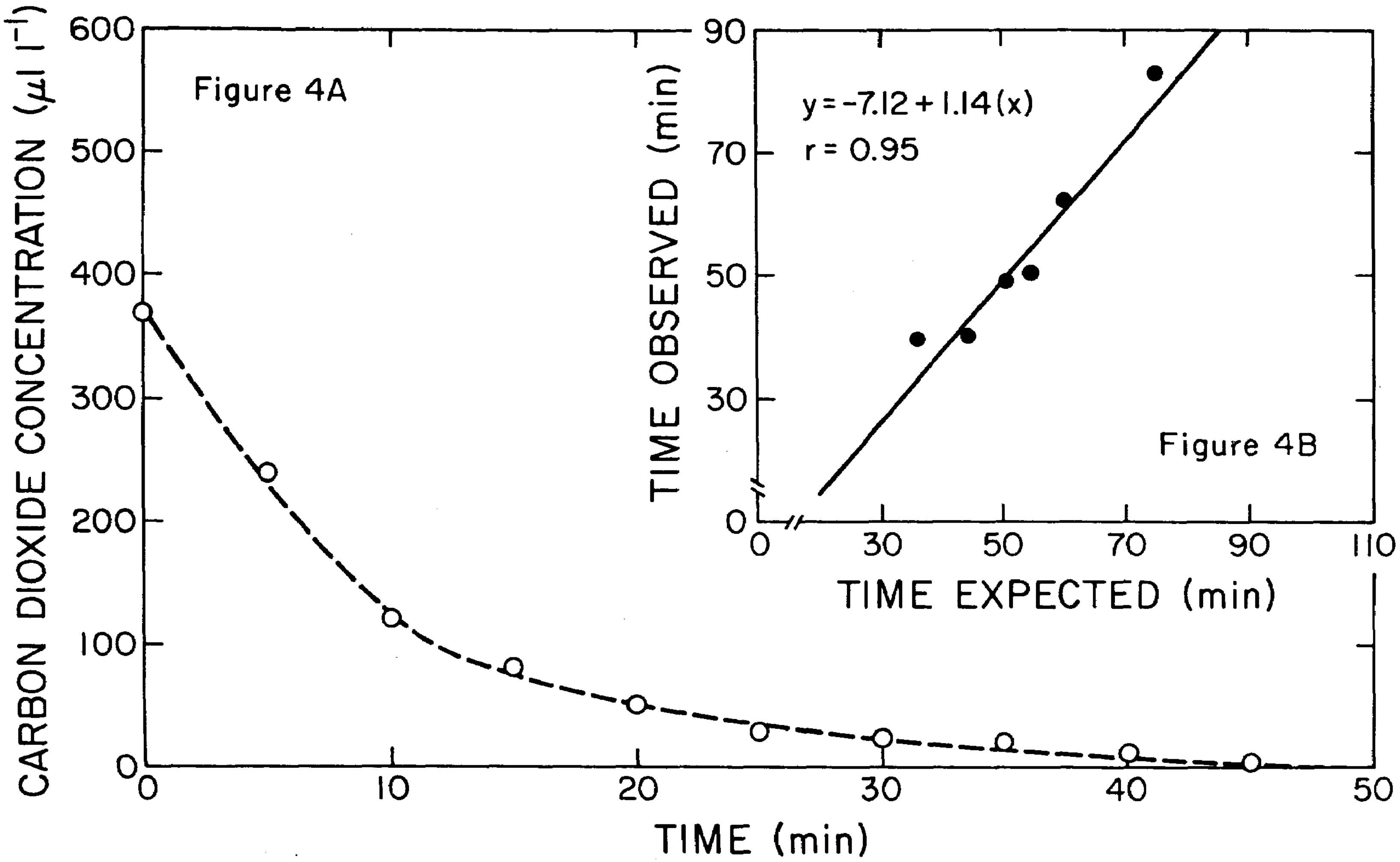

Figure 4A. Carbon dioxide decay rate within the chamber given an air flow rate of 4.5 l min^{-1} and initial outlet and inlet CO_2 concentrations of 365 and 0 ppm, respectively.

Figure 4B. Relationship between observed and expected time for outlet CO_2 concentrations to approach 0 ppm at varying flow rates.

In using the mass balance approach to analyze gaseous exchange, the various fates of SO_2 molecules entering the chamber must be quantified. The potential of chamber surfaces to serve as a sink for various gases depends upon the humidity of the chamber air since any surface water film adhering to the walls will scavenge water-soluble pollutant molecules. To assess this sink potential, an artificial transpiration system was designed to inject continuously a known volume of moist air. Inlet and outlet lines were monitored for SO_2 and water vapor concentrations, and the latter was varied by regulating manually the volume of steam injected. The expected outlet concentration of SO_2 was determined by calculating the dilution effect of the steam air on the SO_2 entering the chamber, and any discrepancy between expected and observed sample line concentrations was attributed to pollutant adsorption to the chamber walls.

As the sample dewpoint increased, the adsorption of SO_2 to the chamber increased linearly (Figure 5). Using linear regression analysis this relationship is expressed as:

$$\text{chamber loss} = 2.42\ DP_{OUT} - 26.32$$

where chamber loss = percentage of the total SO_2 concentration differential reacting with the chamber interior and

DP_{OUT} = the outlet dewpoint in °C.

Although the SO_2 loss to the chamber at lower dewpoints is small, the percentage adsorbed at higher levels may exceed 20%. Consequently, the chamber's capacity to scavenge SO_2 molecules must be incorporated into the data analysis in order to assess accurately SO_2 flux to the plant.

Total leaf flux of SO_2 is the sum of loss to the leaf surface (adsorption) and leaf interior (absorption), and it is important to quantify the significance of each since SO_2 absorption is responsible for foliar necrosis. Plants were exposed to 0.4 $\mu l\ l^{-1}$ SO_2 (outlet concentration) in the dark for three hours (adequate to achieve a SO_2 steady state flux). This exposure regime was followed by an equivalent SO_2 concentration (outlet SO_2 level of 0.4 $\mu l\ l^{-1}$) after the lights were turned on. The pollutant dose was not sufficient to cause visible leaf injury. Concurrent measures of dewpoint and leaf temperature were recorded so that leaf resistance to water vapor flux could be calculated (Nobel 1974). Following this protocol, the plant was removed and unifacial leaf area measured. With these data, values were obtained for steady state SO_2 flux to the plant ($\mu g\ m^{-2}\ hr^{-1}$) and concurrent leaf resistances ($sec\ cm^{-1}$) to water vapor and SO_2 flux for each plant in both light and dark. The SO_2 flux data incorporated appropriate calculations to exclude that fraction of the pollutant lost to the chamber walls.

For all plants the pattern of leaf resistance and the flux of SO_2 to the plant throughout the night-day exposure regime was similar; an example is shown in Figure 6. In the dark, total leaf resistance to SO_2 flux remained constant (30 $sec\ cm^{-1}$) and with light, decreased precipitously to a lower steady state value (4 $sec\ cm^{-1}$). This response pattern, showing distinct dark and light plateaus, is mirrored by total leaf flux of SO_2, which exhibits

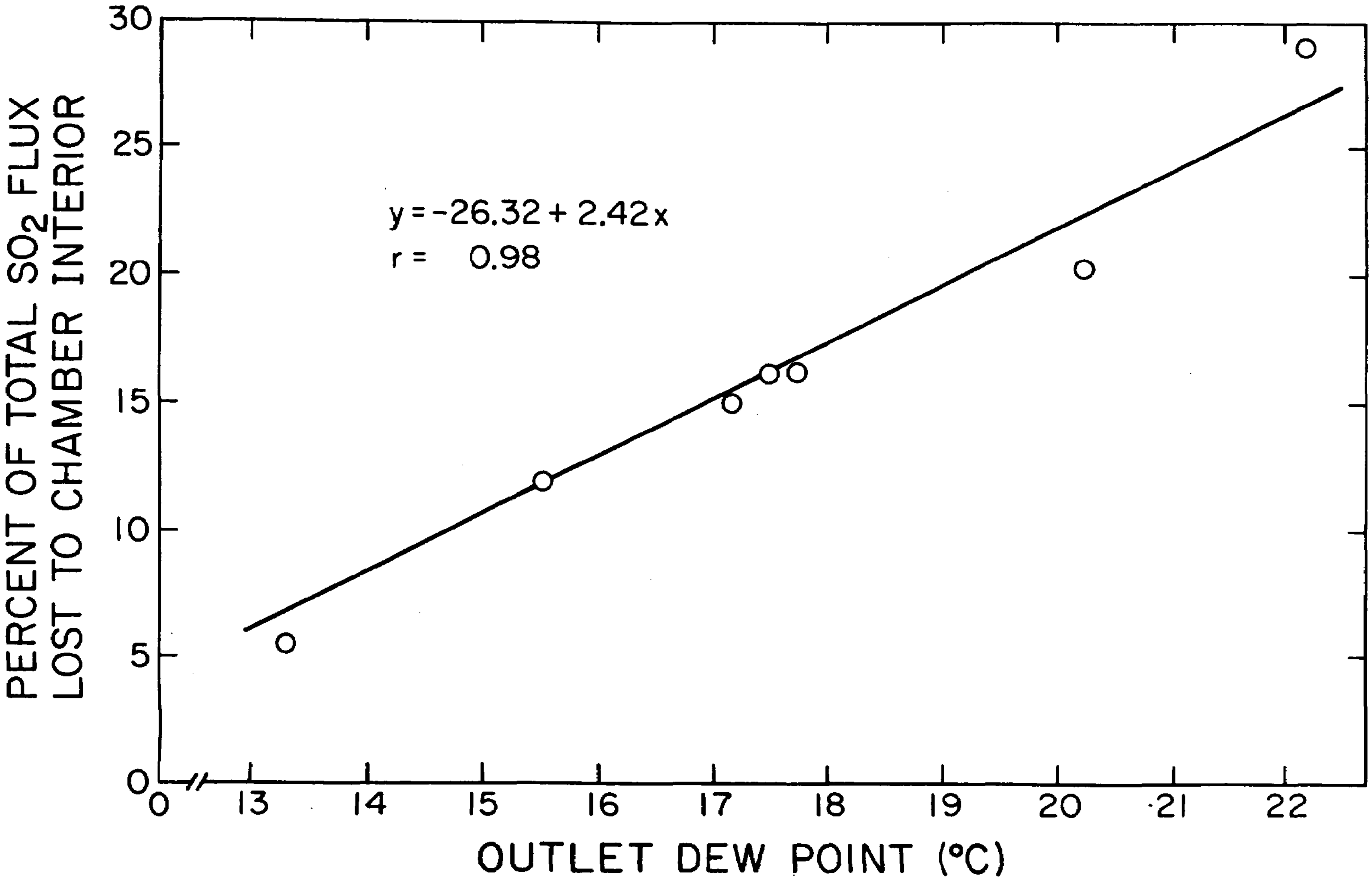

Figure 5. Influence of outlet dewpoint on SO_2 flux to the chamber's internal surface and equipment. The chamber conditions were equivalent to the day environmental regime described in the legend to Figure 6.

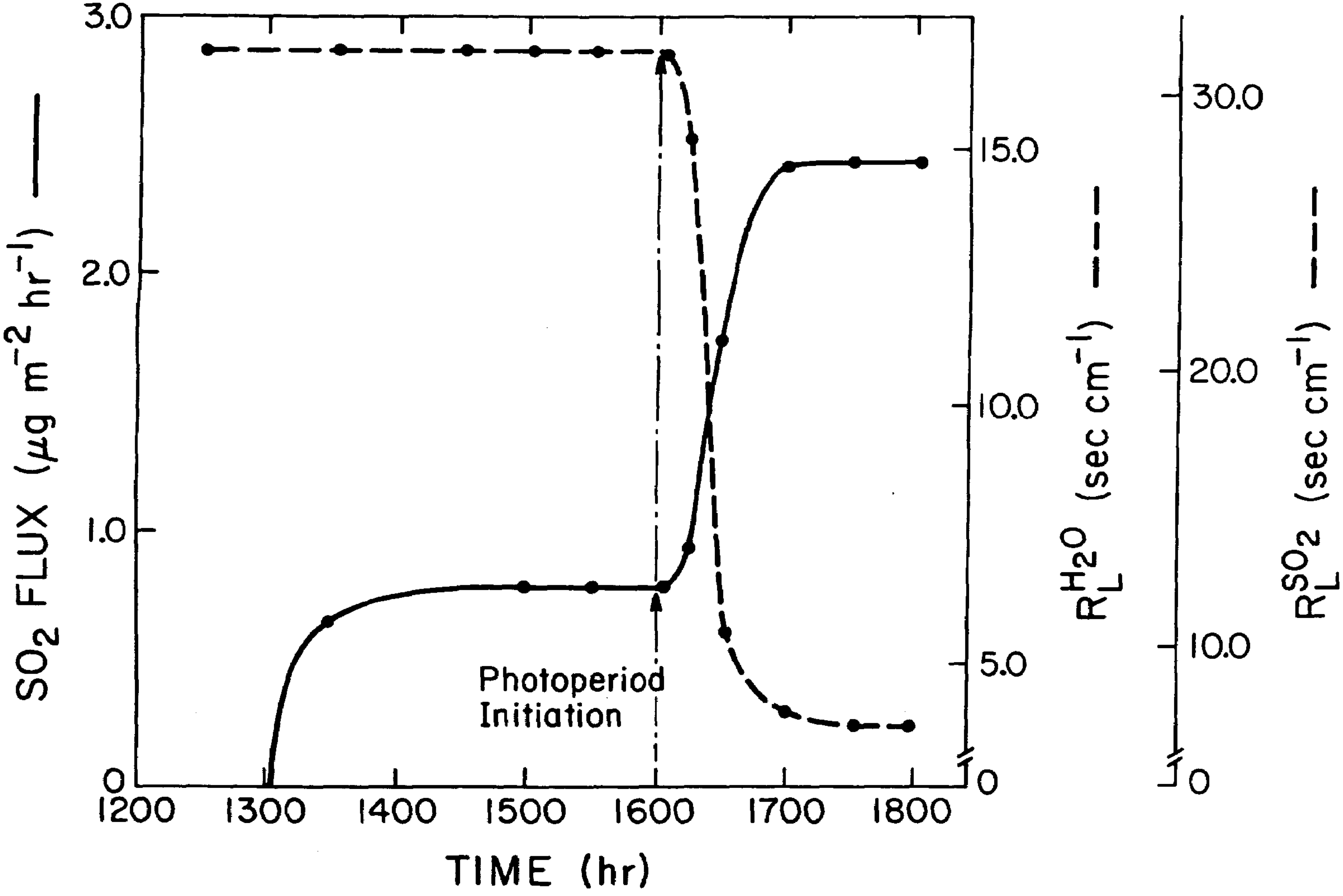

Figure 6. Relationship of SO_2 flux and leaf resistance to water vapor flux ($R_L^{H_2O}$) and SO_2 flux ($R_L^{SO_2}$) as a function of time in the dark and light. Chamber conditions in the day and night environments were: air temperature = 27°C, inlet CO_2 concentration = 320-345 μl l^{-1} and outlet SO_2 concentration = 0.4 μl l^{-1}. Light irradiance during photoperiod was 490 μE m^{-2} sec^{-1}.

steady state uptake rates of 0.8 and 2.5 (μg m^{-2} hr^{-1} in the dark and light, respectively).

Total SO_2 flux and leaf resistance at steady state for each plant under both light and dark conditions are plotted with SO_2 flux as the dependent variable (Figure 7). Total pollutant flux to the plant, including both adsorption and absorption, is related inversely to leaf resistance and is asymptotic at both leaf resistance extremes. Consequently, SO_2 flux into the leaf decreases with increasing leaf resistance. Using linear regression analysis of the log-transformed values for each variable, a model for SO_2 flux as a function of leaf resistance in both sensitive and resistant plants was developed (Figure 8). Respective regression lines and slopes for resistant and sensitive plants do not differ statistically. These results indicate that given equivalent leaf resistance values for gaseous flux, SO_2 resistant and sensitive plants do not differ in the leaf uptake of the pollutant.

Analysis of total leaf flux of SO_2 shows that under conditions promoting absorption into the leaf (i.e., light), the percentage of the total SO_2 flux lost to the leaf surface is approximately 20%. This determination is derived from a comparison of the asymptotic SO_2 flux values at high versus low leaf resistances (Figure 7) and assumes that the capacity of the leaf surface to extract SO_2 is constant and unaffected by the opening of the stomates.

HYDROCARBON EMISSIONS FROM PLANTS: EFFECT OF TEMPERATURE

The specific objectives of this study were (i) to identify specific monoterpenes being emitted by a species of pine, (ii) to determine monoterpene emission rates under rigidly controlled environments, and (iii) to assess how leaf temperature affects the emission rate. A detailed report of this project is available (Tingey et al. 1978).

Seedlings of slash pine (Pinus elliotti) were grown in a greenhouse and transferred, at least four weeks before experimentation, to a controlled environment chamber similar to that in which the gas-exchange chamber was housed. The gas-exchange system was modified slightly to accommodate analysis of hydrocarbons by gas chromatography. Syringe ports were placed in both inlet and outlet lines so that air samples of 25- to 50-ml could be collected with gas-tight syringes. Samples were injected into a gas chromatograph designed to cryogenically concentrate, separate and quantify the levels of monoterpenes. After experimentation each plant's needles were oven-dried and assayed for dry weight. The data were analyzed using regression techniques, and the results are presented graphically. Since emission rates were log-normally distributed, the data were transformed to logarithms for calculation (Tingey et al. 1978).

Qualitatively, five monoterpenes were identified in the outlet air (parenthetical data are average emission rates in μg C/gm dry wt/hr at 35°C): α-pinene (4.46), β-pinene (3.44), myrcene (0.32), limonene (0.06) and β-phellandrene (0.22). Leaf temperature exerted demonstrable effects on the rate of monoterpenes emitted (Figure 9). The data for the sum of the five monoterpenes (Figure 9A) show a log-linear relationship between emission rate

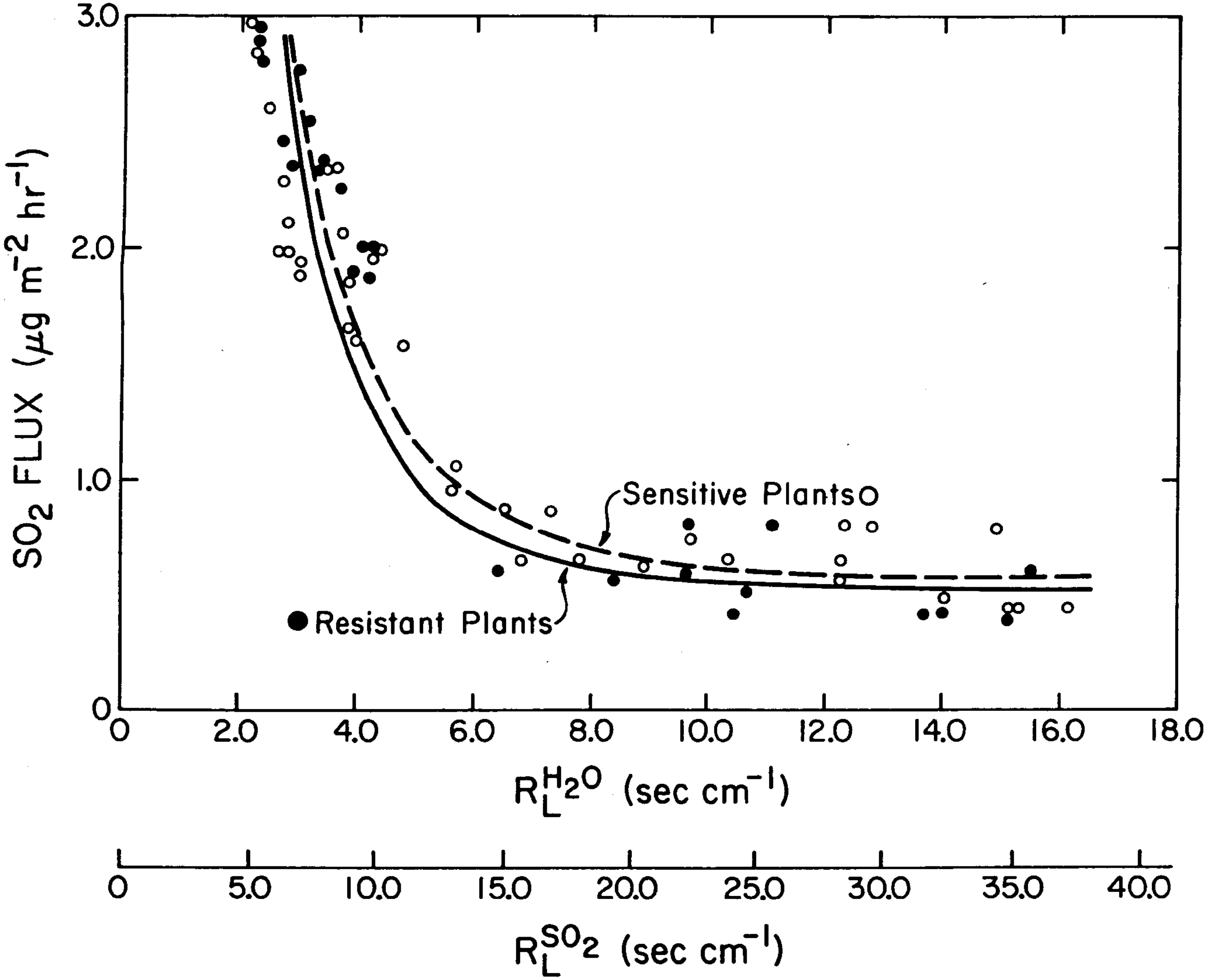

Figure 7. Relationship between leaf resistance to gaseous flux ($R_L^{SO_2}$ and $R_L^{H_2O}$) and SO_2 flux to the plant in both SO_2 resistant and sensitive plants.

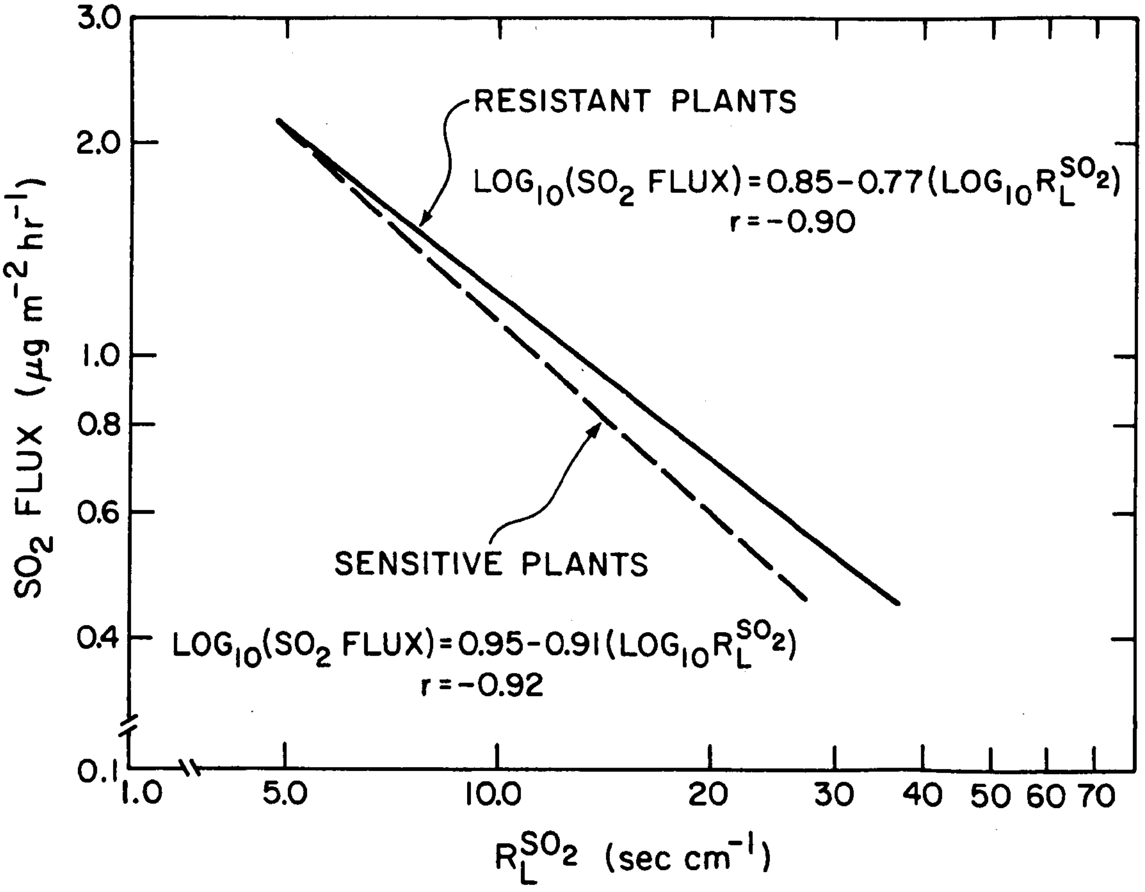

Figure 8. Regression analysis depicting linear relationship between $\log_{10}$ (leaf resistance) and $\log_{10}$ (SO_2 flux) for SO_2 resistant (n = 44 and S_b = 0.071) and SO_2 sensitive (n = 36 and S_b = 0.083) plants.

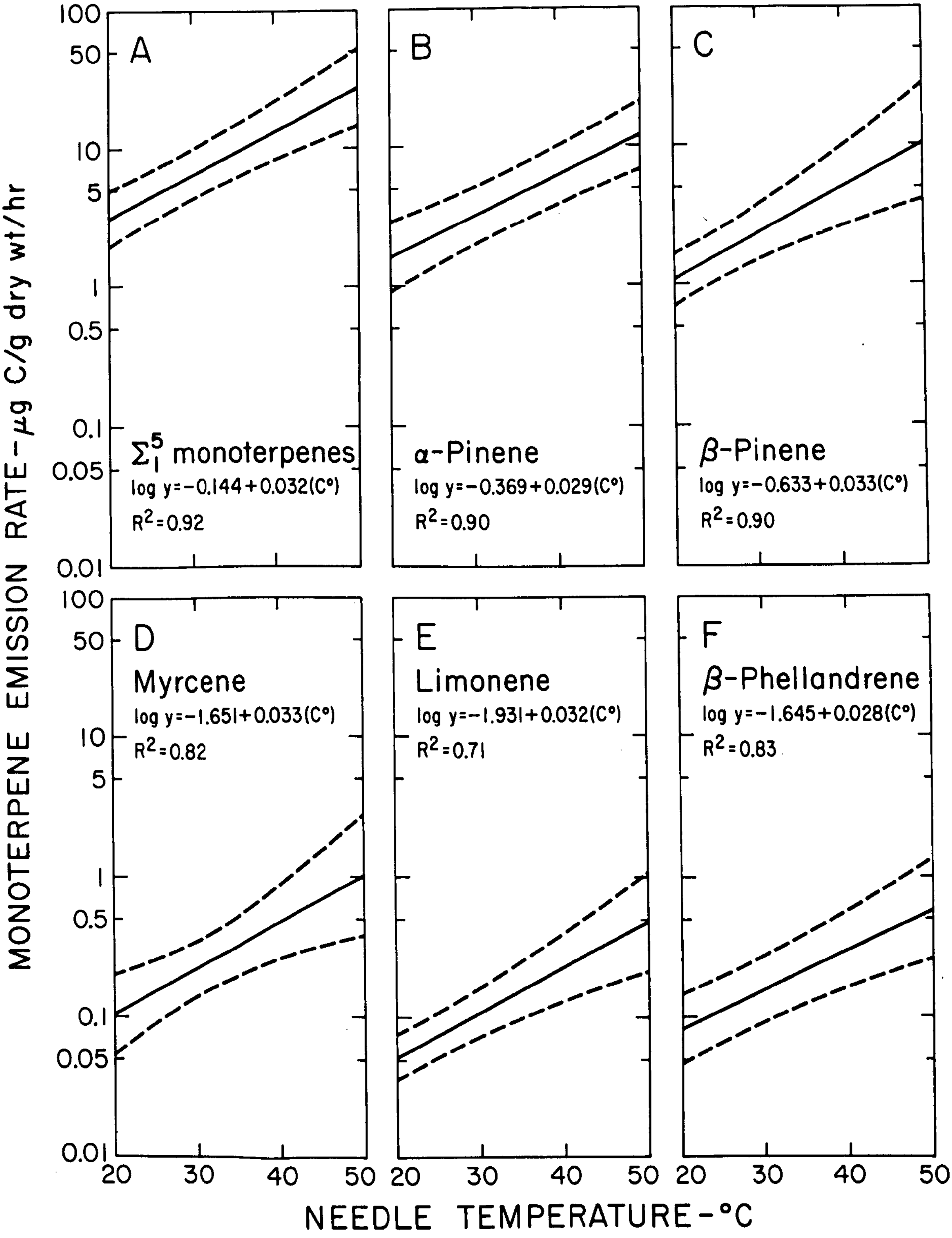

Figure 9. The influence of varying temperatures on monoterpene emission rates in slash pine.

and temperature so that the mean value increased exponentially with temperature. As temperature increased from 20 to 46°C, the rate of the sum of monoterpenes emitted increased from 3 to 21 μg C/g dry wt/hr. The regression equation accounted for 92% of the observed variation (Figure 9A).

CONCLUSIONS

The objective of this research was to establish a gas-exchange system capable of assessing indices of physiological activity that are closely linked to overall plant performance. Two types of data are presented to support this objective. First, the performance trials show that the system conforms to necessary design criteria of gas conditioning, delivery, instantaneous mixing and monitoring. Second, the results of two studies, measurement of SO_2 uptake and hydrocarbon emissions, indicate that with appropriate experimental designs, research can evaluate the effects of the environment on vital gas-exchange processes in plants.

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-79-108	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE A Gas-Exchange System for Assessing Plant Performance in Response to Environmental Stress		5. REPORT DATE October 1979 issuing date 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G.E. Taylor, Jr. D.T. Tingey		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS 1. National Research Council 2. Terrestrial Division Corvallis Environmental Research Laboratory		10. PROGRAM ELEMENT NO. 1AA602 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Corvallis Environmental Research Laboratory Office of Research & Development U.S. Environmental Protection Agency Corvallis, OR 97330		13. TYPE OF REPORT AND PERIOD COVERED inhouse 14. SPONSORING AGENCY CODE EPA/600/02
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Environmental Simulator Plant Physiology	Gas-Exchange System	06/F

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* unclassified	21. NO. OF PAGES 24
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE