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DIRECT EFFECTS OF INCREASING CARBON DIOXIDE ON VEGETATION

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2. METHODS OF EXPOSING PLANTS TO ELEVATED CARBON DIOXIDE

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2.1 INTRODUCTION

The growth enhancement obtained by enriching the air with carbon dioxide (CO_2) has been recognized since the early 19th century. Studies of this effect have been conducted since that time, a period which spans most of the history of modern botany. Much of our present knowledge of this growth stimulation was obtained using crop species in controlled environments, especially greenhouses, to determine how to increase yield with "CO_2 fertilization." In the context of the recent increase in atmospheric CO_2 concentration, we want to know how crops, populations of wild, unmanaged plants, and ecosystem processes will respond. New methods and facilities have been devised to test the broader questions that are now being asked. This chapter, on the methods of exposing plants to atmospheres in which the CO_2 concentration is modified and controlled, is not intended to be exhaustive, but to illustrate the state of the art of the available technologies.

The chapter begins with a discussion of CO_2 control technology and concludes with an evaluation of the possible use of an open field release system. There are also sections describing most of the approaches that have been applied in elevated CO_2 studies, including leaf chambers, sunlit controlled environment facilities, mobile greenhouses, large greenhouses used to study crops, small greenhouses used in studies of natural vegetation, and open top chambers. The sections on open top chambers and open field release of CO_2 are relatively detailed because information on these important approaches has thus far been confined to specialized literature, and there is therefore the need to give these methods more extensive discussion than other more conventional approaches.

There are no published reports of leaf or branch chambers used to treat different leaves or plant parts with elevated CO_2 for periods longer than are needed to study the kinetic properties of photosynthesis in vivo. However, a chamber system that is suitable for such studies is discussed here. The available data on mechanistic changes in the photosynthetic apparatus of intact leaf tissue occurring in response to elevated CO_2 were obtained with leaf chambers and infrared gas analyzers (IRGAs).

Before about 1980, most studies on the effects of elevated CO_2 concentration were carried out in controlled environment facilities including growth chambers and greenhouses. Growth chambers are enclosed spaces in which some or all of the following parameters are controlled or monitored continuously: light quality and quantity, air and soil temperature, water vapor pressure, concentration of atmospheric gases (including water vapor and CO_2), soil nutrients, soil structure and water content, and air movement. Photoperiods and thermoperiods may be separately controlled, and environments may be programmed to change gradually in small time increments or abruptly in larger, square-wave-type increments. For detailed descriptions of the technical aspects of controlled environments the reader is referred to the extensive literature on the subject (Went 1957; Evans 1963; Kramer et al. 1970; Downs et al. 1972; Downs and Hellmers 1975; van Bavel and McCree 1975; Langhans 1978; Tibbitts and Kozlowski 1979; and Downs 1980).

Phytotrons are integrated collections of controlled-growth facilities. The term phytotron (for plant instrument) was first applied to the Earhart Laboratory for Plant Research at the California Institute of Technology in an era when cyclotrons and betatrons were being constructed by physicists to study the behavior of small particles of matter (Downs 1980). A major advantage of the phytotron is that multiple chambers or rooms may be used to create matrices of environmental variables. A matrix of three CO_2 concentrations and three temperatures, for example, requires the use of nine growth rooms. With only two chamber replicates of each condition, this experiment would require 18 growth chambers. Within each growth room, subcells of light intensity or quality, soil nutrients or water status, or certain other environmental manipulations are possible. Separate rooms are needed for each photoperiod or thermoperiod, but if plants are grown on wheeled carts that may be moved from room to room, the number of environmental variables can be greatly increased. With the exception of phytotrons, few plant laboratories have such extensive plant growth facilities.

Carlson and Bazzaz (1980) reported competition experiments in which they used inexpensive growth chambers on wheels. These chambers were small enough for artificial lighting, but mobile so that they could be moved into the greenhouse for use with natural light. Thus they combined some of the best features of growth cabinets, namely, environmental control, with relatively high photosynthetic photon flux density as may be found in a greenhouse. The growth chambers were developed to house experiments to study the effects of various gases, including CO_2, on plants grown under several different conditions.

Naturally sunlit crop growth chambers based on "closed loop" environmental controls have proven very useful for detailed crop studies. At Clemson University, Mississippi State University, and the University of Florida, these systems have been used for short- and long-term experiments on soybean response to CO_2-enriched atmospheres. These systems, because they are closed, lend themselves to the study of water use by crop species. The chambers are divided into two parts,

an upper plant canopy chamber and a lower root zone compartment in which water use and root growth can be measured. The upper chambers are covered with clear glazing, allowing plant exposure to sunlight, and the root compartments are deep enough to allow a more field-like rooting volume than pots can provide.

Greenhouses have had a long history of contribution to agricultural research, and virtually every agricultural research organization has one or more on site. They offer at least partial control over the vagaries of the weather outside and enable an additional crop to be grown in the wintertime in climates where none would be possible in field plots. Only in greenhouses has it been economically practical to use CO_2 enrichment to increase the productivity of crops, and in cooler climates, such as the northeastern United States, it is a recommended horticultural practice (Wittwer and Honma 1969). Numerous CO_2 enrichment studies have been conducted in greenhouses over the last 64 years. Kimball (1983a, 1983b) reviewed over 140 reports and extracted more than 770 observations of the yields or biomass production with CO_2 enrichment of 56 plant species. The majority of these data were obtained from studies conducted in greenhouses. The increase of the mean weight of crop yield was 36%, which shows that CO_2 enrichment is indeed very beneficial to the greenhouse industry.

A new application of the use of greenhouses was tried by Oechel and coworkers (Prudhomme et al. 1984), who designed small greenhouses capable of tracking ambient temperature and humidity while maintaining preset CO_2 concentration to study the effect of elevated CO_2 on Arctic tundra.

The open top field chamber as described by Heagle et al. (1973, 1979) has had extensive use as the plant exposure unit in air pollution/plant effects studies in the field. The system has been used to expose both row crops and plants in pots to a variety of aerial pollutants, and it is currently in use at a number of laboratories throughout the United States. Hardy and Havelka (1975) first used an open top enclosure to expose soybeans to atmospheres enriched with CO_2 for the purpose of studying the effect of increased photosynthate production on symbiotic nitrogen fixation. Rogers et al. (1983) adapted the basic open top chamber system to generate large-scale CO_2 test atmospheres in the field.

The need to study CO_2 effects on vegetation in a natural field environment has led to the concept of artificially elevating CO_2 by release through a network of pipes. The history of this free air CO_2 enrichment (FACE) approach can be traced to studies by agronomists (Kretchman 1969; Baker et al. 1970; Allen 1973; Harper et al. 1973a, 1973b; Baker and Lambert 1980). Field experience with this method is reported in the thesis studies of Harper (1971) and Allen (1973), and technical aspects were extensively reviewed by Allen (1979).

The FACE approach had much in common with methods developed by air pollution ecologists. DeCormis et al. (1975) described a grid release system to study air pollutant effects in vineyards for the French Ministry of Agriculture. The U.S. Environmental Protection Agency Zonal Air Pollution System (ZAPS) released air pollutants through a pipeline network in a prairie grassland (Lee and Lewis 1975; Lee et al. 1978). The U.S. Department of Energy's Argonne National Laboratory developed its own ZAPS capability (Miller et al. 1980), as did the University of British Columbia, Canada (Runeckles et al. 1981), the University of Nottingham School of Agriculture, U.K. (Greenwood et al. 1982), and the U.K. Central Electricity Research Laboratories (McLeod et al. 1983). A related open air fumigation system to provide linear gradients of exposure to a pollutant was designed by Shinn et al. (1977) and modified by Laurence et al. (1982) and by Reich et al. (1982). McLeod and Fackrell (1983) reviewed methods of open air fumigation.

2.2 CO_2 MONITORING AND CONTROL SYSTEMS

Two CO_2 control and monitoring systems are described in this section. The first was designed to be used with growth chambers, and the second utilizes open top chambers.

The CO_2 monitoring and control system for controlled environment studies reported by Norby et al. (1985) included four growth chambers, an infrared CO_2 gas analyzer (IRGA), compressed gas cylinders, a Hewlett-Packard 3497A data acquisition and control unit, and a Hewlett-Packard 9826 desktop computer with printer (Figure 2.1). The entire system was wired into a backup emergency power supply.

The system supplied two walk-in growth chambers with 3.2 m^2 of bench space and two reach-in chambers with 1.1 m^2 of bench space. The computers read CO_2 concentration from the IRGA, accumulated data, printed out statistical reports, calculated CO_2 input requirements, and actuated solenoids for controlling CO_2 sampling and injection.

The CO_2 partial pressure in the growth chambers and the ambient CO_2 outside the building were measured with an Anarad AR500RN IRGA. Gas samples were continuously delivered to the analyzer through heated stainless steel tubing (to prevent condensation). Solenoids, actuated by the HP 3497A on command from the HP 9826, routed

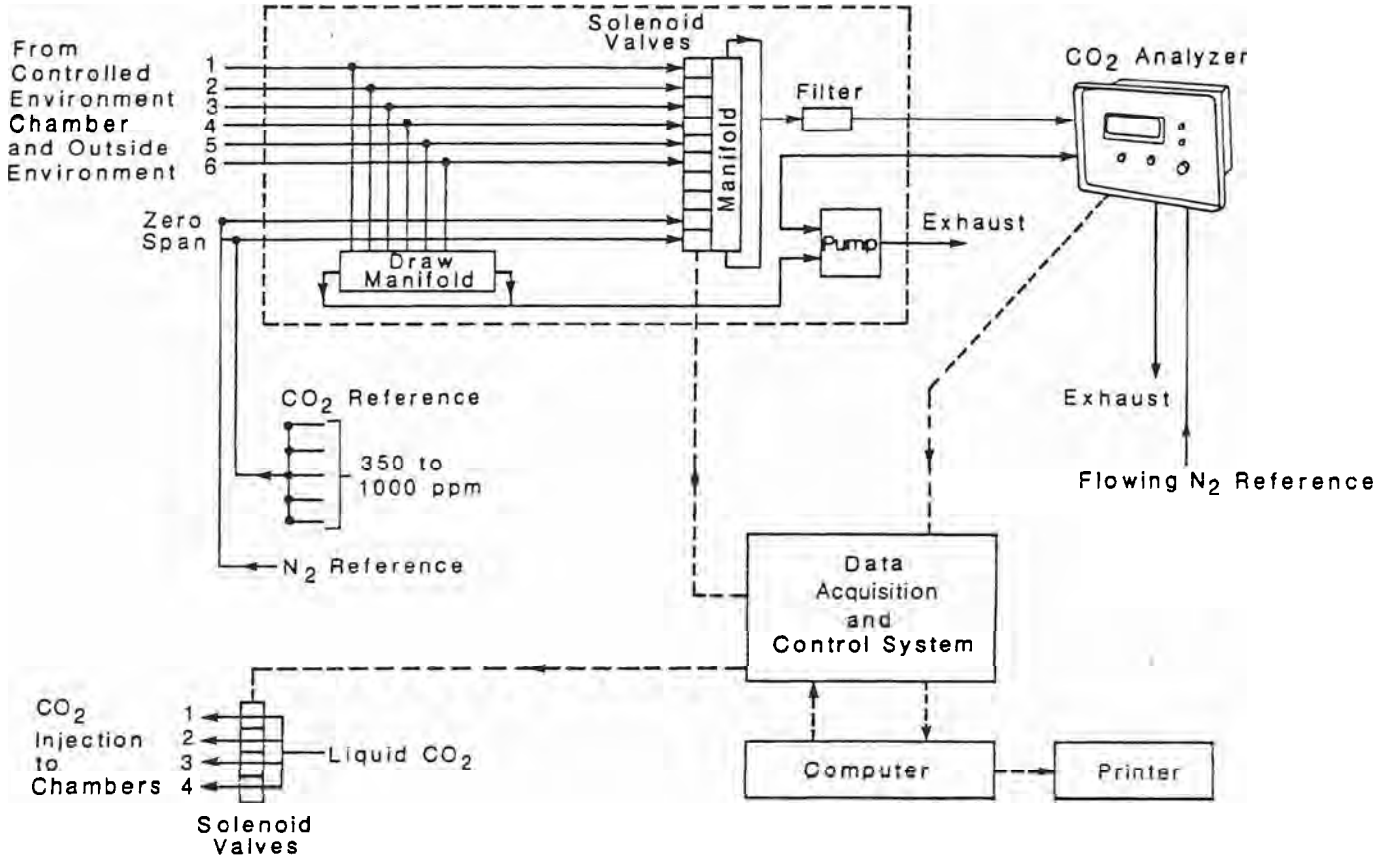

Figure 2.1. Diagram of CO_2 monitoring and control system for four controlled environment chambers. After Norby et al. (1985).

gas to the measuring cell or to a bypass to exhaust. CO_2 concentration was measured as the differential concentration of the sample gas from the reference nitrogen gas (0 parts per million by volume [ppm] CO_2). The four growth chambers and two outside ports were recorded sequentially after a 1-minute delay to allow for analyzer stabilization. Random measurement error after stabilization was approximately 0.6% of the concentration over the range 350 to 1025 ppm.

CO_2 from a cylinder of pressurized liquid CO_2 was injected into the growth chambers in pulses by opening a solenoid valve on command from the HP 3497A. Pulse duration (generally 1 to 8 s) was a function of desired CO_2 concentration and chamber size. Concentration of CO_2 in the chambers was controlled by varying pulse frequency (i.e., the time between pulses), calculated by the HP 9826. Time between pulses was adjusted during every cycle proportionate to the difference between current and desired CO_2 concentrations. The proportionality factor was chosen to provide sufficient sensitivity to changing conditions (such as diurnal changes in ambient CO_2 or opening a growth chamber door) without overcompensating for system "noise" under relatively stable conditions.

The control strategy was satisfactory for short- and long-term experimental use. Coefficients of variation were routinely less than 10%. For example, during January 1984, coefficients of variations for the ambient-, 500-, 700-, and 950-ppm chambers were, respectively, 7.8, 6.0, 3.3, and 6.2%, based on over 10,000 measurements per chamber. During this period, 93% of the individual photoperiod and nyctoperiod means for the three elevated CO_2 chambers were within 5 ppm of the set points, and 80% were within 2 ppm of set points.

Rogers et al. (1983) described a control system for maintaining elevated CO_2 concentrations within open top chambers. A 14-ton (12.7-metric ton) liquid receiver served as a CO_2 supply reservoir. The storage receiver was equipped with an air-cooled condensing unit and a vaporizer. It was operated at between −23° and

−16°C, which gave a pressure of 243 to 307 psig (17 to 22 kg cm^{-2}). CO_2 was delivered from the receiver through 1.27-cm copper tubing to a dispensing manifold. A solenoid valve stopped flow during power failure. Regulators and manually operated flow meters dispensed CO_2 from the manifold to the chambers, where it was added to air in a plenum upstream of an axial fan to ensure thorough mixing (Figure 2.2).

The concentration of CO_2 in each of the open top chambers was monitored twice an hour. Sampling intakes were located in the center of each chamber at 1 m height above the ground. Samples were drawn continuously from the chambers to a point near the water vapor and CO_2 analyzers. Solenoids allowed another pump to divert about 20% of the volume being sampled from the chamber into the measuring circuit.

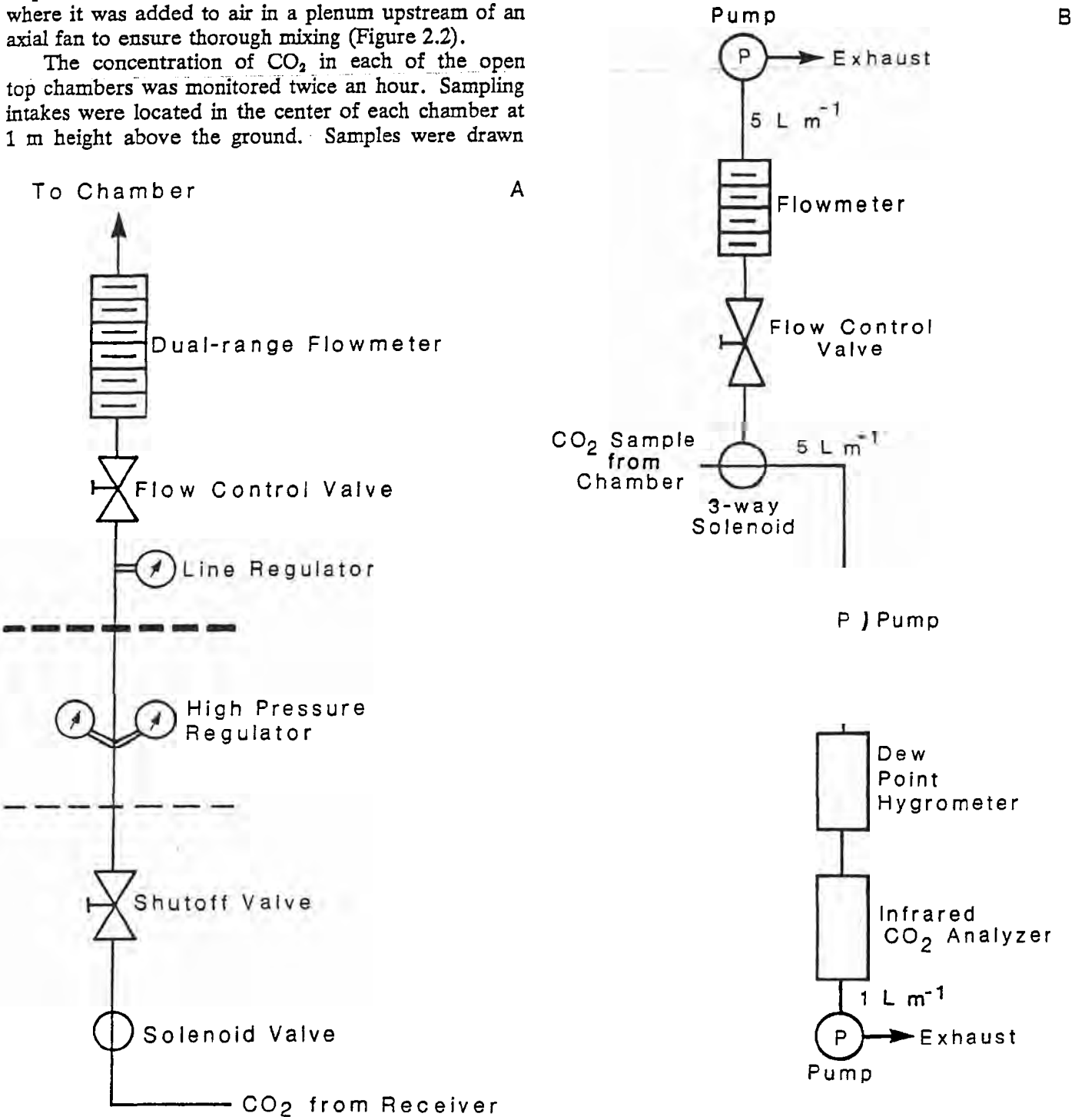

Figure 2.2. Schematic diagrams of the systems to regulate and monitor CO_2 flow. (A) System for regulation of the flow to the plant exposure chambers. (B) Monitoring system for up to 15 chambers. After Rogers et al. (1983).

Although the control of CO_2 concentration was by periodic manual adjustment of flow of CO_2 into the air supply for each chamber, the system maintained mean values for CO_2 concentration within 9% of the desired value.

2.3 LEAF CHAMBERS

There are so many chamber designs for single-leaf gas exchange measurements that an exhaustive discussion of advantages and problems of each one is beyond the scope of this chapter. Representative examples of different chamber designs developed during the past 2 decades can be found in Musgrave and Moss (1961); Mooney et al. (1971); Sestak et al. (1971); Bingham and Coyne (1977); Sinclair et al. (1979); DeJong et al. (1981); Field et al. (1982); Huck et al. (1983); and Valle et al. (1985).

The major design problem of leaf chambers is the same as for growth chambers, namely, how to control the environment around the leaf. Thus, a leaf chamber for measuring gas exchange is only one part of a system which can be subdivided into (1) control of gas composition and the environment around the leaf, (2) measurement of various physical parameters such as changes in gas concentrations, and (3) collection and evaluation of data. Bloom et al. (1980) discuss the effects of materials on water vapor and CO_2 in the gas-exchange circuit. In recently developed systems (e.g., Sinclair et al. 1979; Field et al. 1982; and Valle et al. 1985) computers have been used to integrate all subsystems as well as to manage data and provide hard copy of results.

The simplest systems have measured only CO_2 assimilation. Water vapor loss and CO_2 assimilation, however, must be measured simultaneously to make the analysis of data required to evaluate separately the effect of elevated CO_2 treatment on the supply of CO_2 through stomata to intercellular spaces and the biochemical responses of photosynthesis. von Caemmerer and Farquhar (1981) have summarized the necessary calculations, a discussion of the physical aspects of gas exchange in leaves can be found in Sestak et al. (1971), and the interpretation of gas analysis data is discussed by Sharkey (1985).

Systems for measuring gas exchange between the leaf and its environment are either open or closed. In open systems, air of known composition makes a single pass over the leaf, and the change in CO_2 and water vapor concentration brought about by the leaf is determined. In closed systems, air is continuously circulated around the leaf, and CO_2, water vapor, and other variables of interest are controlled by compensation for exchange between the leaf and the surrounding air. Open systems may ultimately be the simplest to design and control, but they require a high degree of sensitivity in measurement of CO_2 concentration and dewpoint temperature. For example, to determine the flux of CO_2 across the epidermis, Sinclair et al. (1979) used an open system with an IRGA to measure the drop in CO_2 concentration of air as it passed over the leaf. In closed systems, a null-balance approach is used, and the change in concentration of water vapor and CO_2 in the chamber is determined from the rate of injection of water vapor and CO_2 required to maintain a set point concentration. Field et al. (1982) used a closed system and measured the change in pressure across an injection capillary required to maintain CO_2 concentration within the chamber at a set point which was measured by a gas analyzer used in absolute mode.

The leaf gas exchange system (Figure 2.3) described

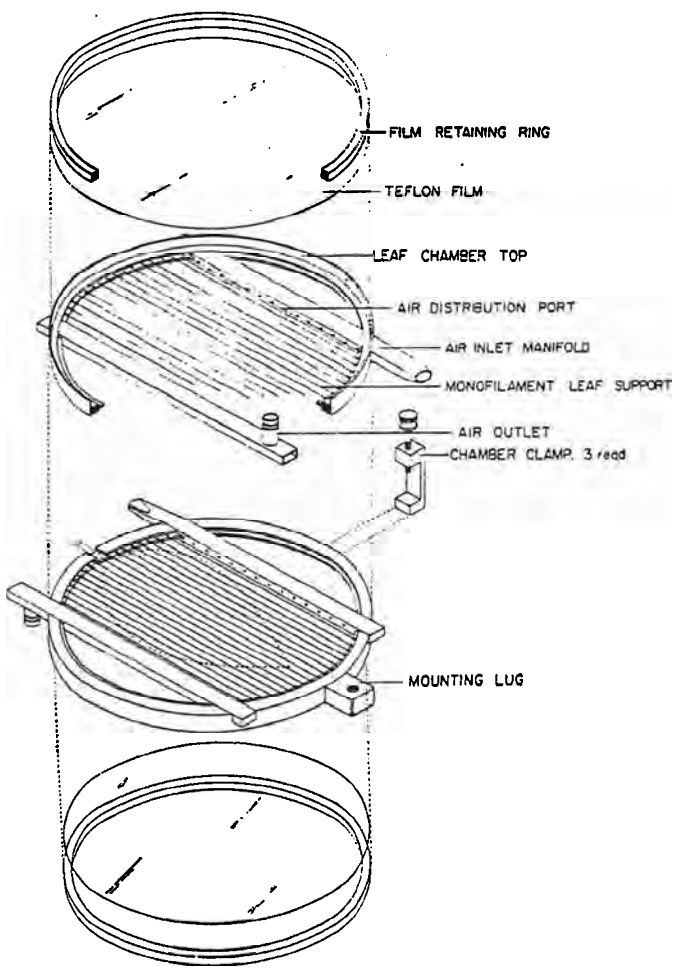

Figure 2.3. Leaf chamber for field measurement of photosynthesis. After Sinclair et al. (1979).

Table 2.1
Environmental Specifications (Minimal Requirements) for Controlled Environments Designed for CO_2 Research

Parameter	Units
Photosynthetic Photon Flux Density	0–2000 μmol m^{-2} s^{-1}
Photoperiod	0–24 h of light continuously programmable in square or sine wave patterns
Air Temperature	5° to 40°C
Thermoperiod	0–24 h phase-separated from photoperiod but in similar wave patterns
Air Water Vapor Concentration	Controllable to give a leaf-to-air vapor pressure gradient of 0.3 – 1.5 nmol m^{-3}
Air Velocity	0–3 m s^{-1} continuously adjustable
CO_2 Concentration	250–2500 ppm

by Sinclair et al. (1979) would be well adapted for use in studies of the effect of prolonged exposure to elevated CO_2 levels on photosynthesis, although to date it has not been used for that purpose. This system was capable of field operation and was able to track environmental temperature, humidity, and solar radiation, as well as continuous measurement of both water vapor and CO_2 exchange and control of CO_2 concentration.

The leaf chamber consisted of two disks of clear Teflon[1] separated by a pair of chrome-plated brass rings. The leaf was inserted between rows of monofilament line on each ring. Leaf temperature was controlled so as to track ambient temperature by passing the air supply line through a waterjacket in the rim of the chamber. In the study of Sinclair et al. (1979), the effect of the chamber on the plants was evaluated on leaves enclosed in the chamber for 6 weeks. There were no visually apparent effects of the chamber nor were there any effects detected in the data on the photosynthetic response when compared with data obtained on neighboring leaves that were of similar age but which had grown outside the chamber. However, leaves inside the chamber did not have insect damage, and senescence was delayed compared with other leaves in the same canopy.

A system similar to the one employed by Sinclair et al. (1979) has been used by Valle et al. (1985) for studying long-term responses of soybean (*Glycine max* [L.] Merr. 'Bragg') leaves to elevated CO_2. The chambers were used to measure photosynthesis and gas exchange in leaves that had been treated with elevated CO_2 in sunlit growth chambers.

2.4 CONTROLLED ENVIRONMENT CHAMBERS

2.4.1 Phytotrons

Integration of environmental control systems and a broad spectrum of controlled environmental variables distinguish phytotrons from greenhouses or growth chambers. Phytotrons are constructed with a redundancy of compressors, pumps, valves, and all systems required to ensure continuous and dependable operation, and warning systems help professional maintenance staff keep the systems functioning properly.

Controlled environments allow the investigator to create any environment or environmental gradient. Because each environmental factor of interest is established and varied at will, one may administer a desired environmental treatment and be assured that the results are the product of treatment alone. In addition, the experiment can be repeated with precision later. This is a decided advantage over field experiments where only selected variables are controllable. In the field, sunlight, air and soil temperatures, precipitation, insects, and diseases are different from site to site, from day to day, and from year to year. Exact duplications of experiments in the field are highly unlikely.

Specifications recommended as minimal requirements for growth chambers to be used in CO_2 research are given in Table 2.1. At the Duke University Phytotron, extensive studies of the effects of elevated CO_2 concentration on a wide range of plant processes have been carried out. Representative examples of different research projects include comparative growth of C_3 and C_4 plants (Patterson and Flint 1980) and the interaction of CO_2 with effects of temperature (Hofstra and Hesketh 1975), photosynthesis (Clough et al. 1981), drought (Paez et al. 1983), and mineral nutrition (Sionit et al. 1981).

2.4.2 Portable Growth Chambers

Portable growth chambers were designed to permit a low-budget approximation of greenhouses and to allow research on air pollutants to utilize the sunlight available inside a greenhouse, or to use a combination of artificial light with sunlight to obtain a flux density

[1]Teflon is a registered trademark.

that approximated natural sunlight (Carlson and Bazzaz 1980).

The sides and tops of the chambers described by Carlson and Bazzaz (1980) were glass and the backs and bottoms were wood. Interior wood surfaces were covered with Formica[2] to minimize sorption of gases including CO_2 and water vapor. They were supported on a wheeled frame of steel, which also carried the refrigeration equipment. Vents in the top of the back wall of each chamber were connected to a plenum. A fan in the plenum circulated air across heat exchangers and back into the growth chamber through a bottom vent. This vent was equipped with movable vanes so that air could be directed anywhere in the chamber to adjust circulation patterns. Air temperature in the chambers was regulated by passing the circulated air around a 600 W heating element and through expansion coils of a refrigeration system. Plants humidify the air rapidly, so humidity control was achieved by condensing moisture from the air.

Pure commercial CO_2 was metered into the chambers to elevate normal ambient CO_2 concentration to the level desired, and air was sampled from the chambers through a system of valves and flowmeters. CO_2 concentration within each chamber was controlled individually. When lamps and sunlight were used together, the plants could be supplied with 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

2.4.3 Sunlit Controlled Environment Chambers

Sunlit controlled environment chambers based on "closed loop" environmental controls were developed in the late 1950s and early 1960s with Mylar[3] polyester film walls. Nondispersive IRGAs allowed rapid measurement of CO_2 concentration, and these analyzers in combination with metered CO_2 allowed direct and continuous measurement of photosynthesis rates. Transpiration was measured by collecting condensate from air conditioning cooling coils. These chambers were used successfully for measuring photosynthesis and transpiration as a function of CO_2 concentration, light, temperature, and soil moisture condition (e.g., Musgrave and Moss 1961; Moss et al. 1961; Baker and Musgrave 1964, Egli et al. 1970). These systems were the predecesssors to the units with controlled root zone as well as canopy zone chambers (Parsons et al. 1980; Phene et al. 1978), which have been further modified for improved systems for CO_2 studies.

Details of the design, functioning, and use of these recently improved chambers have been reported by Jones et al. (1984a, 1984b, 1985a, 1985b). A sketch showing the overall layout of this system is shown in Figure 2.4. This system was based on the original SPAR (soil-plant-atmosphere-research) units reported by Phene et al. (1978) and Parsons et al. (1980).

These chambers were designed to provide accurate, flexible control of dry-bulb temperature, CO_2 concentration, and humidity of the canopy air. In contrast to open flow-through systems, the air mass in closed systems was continuously circulated within the chamber. Temperature, humidity, and CO_2 concentration of the ambient air were monitored and controlled. Specific methods and equipment for controlling chamber conditions varied, but were generally based on (1) sensors that measured temperature, CO_2, and humidity (e.g., dewpoint temperature) levels; (2) feedback mecha nisms such as thermostats or loops in computer logic that compared sensed with desired conditions; and (3) control devices such as heaters that were regulated to produce the desired treatment conditions. Air in these systems was circulated through the canopy from top to bottom and then out through ducts, where the air was reconditioned before flowing back into the canopy chamber. Sensors and control devices were located within the ducts so that the air circulated to the top of the canopy had the experimentally prescribed temperature, CO_2 concentration, and humidity level. Measurement of plant canopy response in a closed chamber system was directly linked to the control of chamber conditions. Changes in CO_2 and humidity levels within the chamber were driven by canopy CO_2 and H_2O gas exchange processes. Thus, in the absence of excessive condensation on walls, blockage of circulation pumps, and other operational problems, successful control actions provided a mirror image of canopy net photosynthesis and transpiration, and the operation of a closed chamber system implicitly provided measurements of canopy response.

Each chamber described by Jones et al. (1984b) consisted of an acrylic plastic top 2.0 by 1.0 m in cross section by 1.5 m tall (volume, 3.0 m^3) secured to a 1.0-m-deep steel lysimeter filled with fine sand. The chambers were located outside and exposed to direct natural sunlight. However, other similar systems may vary in size and in the rooting medium (e.g., Acock et al. in press).

Recent experiments using sunlit controlled environment chambers have focused on short- and long-term effects of elevated CO_2 on soybean growth and yield, photosynthesis, transpiration, and water-use efficiency (Jones et al. 1984a, 1985b), as well as on interactions between elevated CO_2 concentrations and temperature (Jones et al. 1985a) and moisture stress (Jones et al. in press). These are examples of the types of

[2]Formica is a registered trademark.

[3]Mylar is a registered trademark.

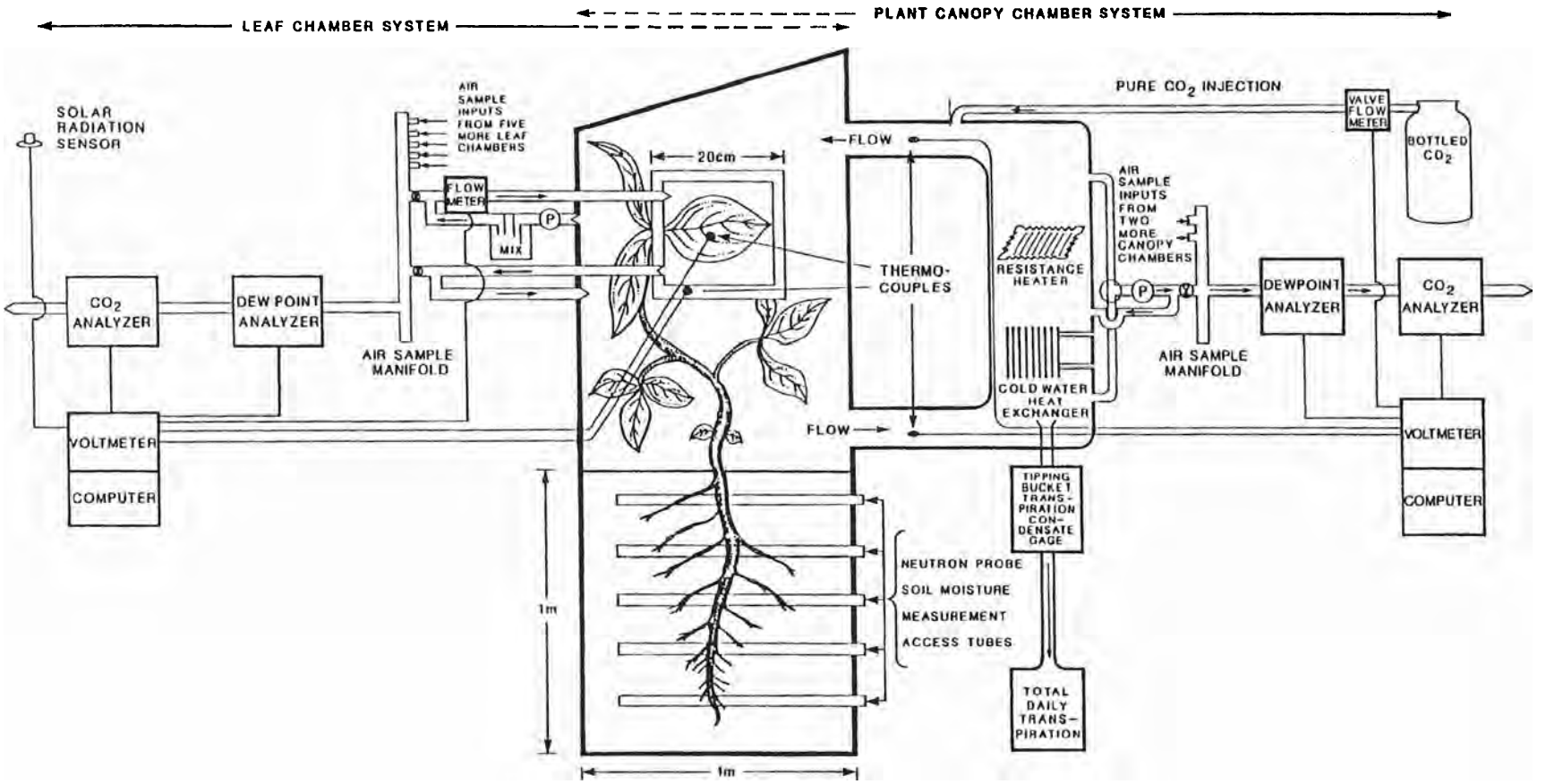

Figure 2.4. Closed system computer-controlled plant environments for CO_2 enrichment study. Plant canopy chamber system described by Jones et al. (1984a, 1984b; 1985a, 1985b). Leaf chamber system described by Valle et al. (1985).

direct CO_2 effects and coupled climate or soil-water interactions that can be obtained in sunlit controlled environment chambers. All of the studies outlined above are discussed in detail in Chapter 4 of this volume.

2.5 GREENHOUSES

Greenhouses are structural frames covered with nearly transparent skins such as glass, fiber glass, polyvinyl chloride, or polyethylene. Because they are manufactured by numerous commercial firms, they are relatively inexpensive compared with custom-built, one-of-a-kind structures. Although constant temperatures are not maintained, greenhouses are usually equipped with heaters and ventilation systems (forced or natural, evaporatively cooled or not) to prevent excessively low or high temperatures. Controls have various degrees of sophistication, but generally provide separate day and night minimum temperature set points to control the heater and a maximum temperature set point to turn on the ventilation. Humidity is usually not controlled, except possibly for some nighttime ventilation at flow rates much smaller than are used for daytime temperature control. Artificial light is generally not used except for the control of photoperiod using low-intensity incandescent light.

Kimball (1983a) has listed the ways the greenhouse environment differs from the open field, and has also reviewed (in press) the data available from the literature on the interactions of CO_2 enrichment with several environmental variables. The most obvious difference between greenhouse and open field environments is that temperatures can be controlled in the former. As in the commercial industry, this feature allows experiments to be done during seasons when it is too cold for crops to grow outside. Furthermore, CO_2 appears to stimulate plant growth by about the same amount across the range of temperatures over which plants are normally grown (Kimball in press).

Another difference between the environment inside a greenhouse and that outside is the considerably lower light intensity inside. Greenhouse coverings typically transmit two-thirds to three-fourths of the available sunlight. Also, greenhouse experiments are often done in the wintertime when the light levels are only one-third to one-tenth of the summertime open field intensities. From theoretical consideration of CO_2 effects on photosynthesis, Kimball (in press) concluded one could expect stimulation of growth by elevated CO_2 compared with growth at present ambient CO_2 concentration at very low and very high light levels with a midrange minimum. Actual observations of growth and yield of CO_2-enriched plants at a range of light intensities did not exhibit any definite discernible pattern within experimental variability. To a first approximation, greenhouse results should apply to the field, but the available data do not support that prediction.

Greenhouses generally have higher humidity and lower windspeed than outside. Thus, greenhouse crops can generally be described as having grown under a more ideal environment with respect to water relations, and they often appear more luxuriant than their shorter, thicker leaved, field-grown counterparts. In his review, Kimball (in press) concluded that most of the previous experiments on the interaction between CO_2 and water stress showed that the stimulation of plant growth with CO_2 enrichment was as large or larger under water stress conditions than under well-watered conditions at present normal ambient CO_2 concentration. Thus, from a water relations standpoint, one could expect responses in the field to be as large as or larger than in a greenhouse. Soil moisture depletion studies can be done in a greenhouse, but the control of the development of tissue water stress is difficult. When large soil volumes are used, stress may develop more slowly than in the field. In small containers, however, stress may develop more rapidly than in the field. Because the wind flow, radiation regimen and humidity are generally different than in the field, it is particularly difficult (and the uncertainty is large) to extrapolate greenhouse water-use measurements to the field situation. Most of these comments apply to salinity stress as well (Kimball in press).

Because most of the prior CO_2-enrichment experiments in greenhouses were conducted using nonlimiting nutrient levels, conclusions based on them probably do not apply to the nutrient-limited unmanaged biosphere. Low nutrient level was the only environmental restraint that appeared to generally limit the relative response of plants to CO_2 enrichment (Kimball in press). Future farmers will need to adjust fertilizer rates to take advantage of the stimulation of yield by the increased atmospheric CO_2 (see also Chapter 9 of this volume).

The economics of doing research are not the same as those of practical horticulture. Thus, for research purposes it is justified to use refrigeration to control the temperature (and even humidity) of closed, CO_2-enriched greenhouses and growth chambers. Alternatively, injecting excessive amounts of CO_2 during ventilation of greenhouses can also be justified for research purposes. This approach uses much CO_2 but little electricity.

2.6 FIELD TRACKING CHAMBERS

The small, field tracking chamber used in studies of Arctic ecosystems (Prudhomme et al. 1984) had a 127-

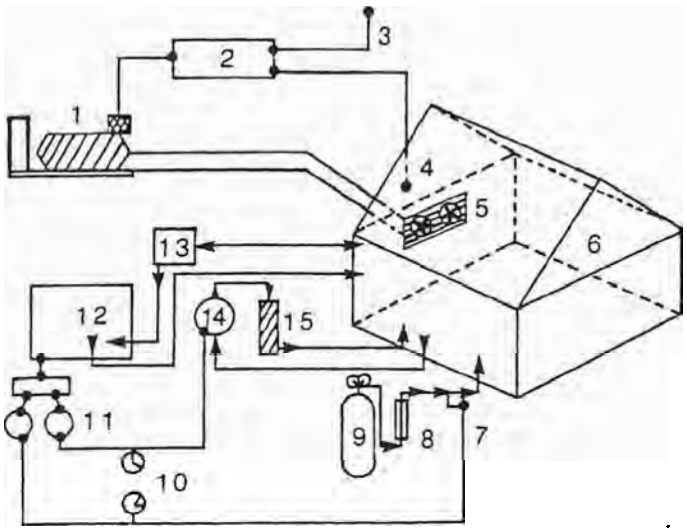

Figure 2.5. Schematic diagram of the field tracking chamber used to measure community CO_2 exchange of tussock tundra at Toolik Lake, Alaska: (1) Compressor, (2) YSI 71A controller, (3) external thermistor, (4) internal thermistor, (5) heat exchanger and fans, (6) chamber, (7) on/off solenoid, (8) flow meter, (9) carbon dioxide cylinder, (10) timers, (11) CO_2 control circuit, (12) ADC infrared gas analyzer, (13) pump, (14) scrub pump, (15) soda lime. Source: Prudhomme et al. (1984).

mm (0.5-in) polyvinyl chloride tubing frame covered with 0.8-mm clear plastic sheeting sealed to a galvanized sheet-metal frame, which was sunk 10 to 15 cm into the soil (Figure 2.5). The chamber enclosed a surface area of 1.65 m^2. CO_2 concentrations inside the enclosure were continuously monitored and maintained at 330 ppm CO_2, representing ambient, or at an elevated level of 600 ppm CO_2 either by adding pure CO_2 gas or by scrubbing the greenhouse air through soda lime.

Air temperature within the chamber was maintained at the desired ambient levels. A temperature controller set to track ambient air temperature activated a compressor unit attached to a remote heat exchanger inside the chamber. The fans inside the greenhouse operated continuously to ensure adequate mixing of the air and CO_2. The entire system was powered by a 6.5 kW generator. Temperatures of the air, moss surface, *Betula nana* leaves, and *Eriophorum vaginatum* stem base, and the soil at 2-cm and 10-cm depths both inside and outside the chamber were measured using thermocouples.

The mass of CO_2 going into the chamber was calculated from the flow rate and the time that CO_2 was injected. The mass of CO_2 removed was calculated based on volume of air removed, its CO_2 concentration, and the length of time that the chamber air was scrubbed. The difference between the mass of CO_2 injected and that removed to maintain ambient or elevated CO_2 levels in the chamber was the net carbon uptake by the community. This measure was expressed as community production as CO_2 exchanged per square meter per day. See Chapter 6 of this volume for a detailed discussion of the results obtained with this system.

2.7 OPEN TOP CHAMBERS

The open top chamber used by Rogers et al. (1983, 1984a, 1984b) was an open-ended cylinder roughly 3 m in diameter by 2.4 m high (Figure 2.6). A high rate of ventilation was assumed to keep the inside temperature and humidity close to those of the outside air. When air in the chamber was mixed with fresh air at normal ambient CO_2 concentration blowing over the top of the chamber, it was difficult to maintain a desired concentration near the top of the plant canopy. Accordingly, a "frustum" was added to the top of the chamber which reduced the size of the top opening to one-half of the ground area inside the chamber (Rogers et al. 1984a). The chambers consisted of a structural aluminum frame covered by panels of clear polyvinyl chloride plastic film. The bottom panel was double-walled; the inside wall was perforated by 2.5-cm holes to serve as a duct to distribute the CO_2-air mixture uniformly into the chamber. Air to this duct was supplied from an axial fan mounted in a sheet metal plenum box with a particulate filter. Pure CO_2 was injected into the plenum box ahead of the fan to ensure thorough mixing.

The open top chamber system performed well in generating and maintaining large-scale test atmospheres in the field and presented no major difficulties once in place. Provisions for delivery of large amounts of liquid CO_2 and electrical power needs were solvable problems.

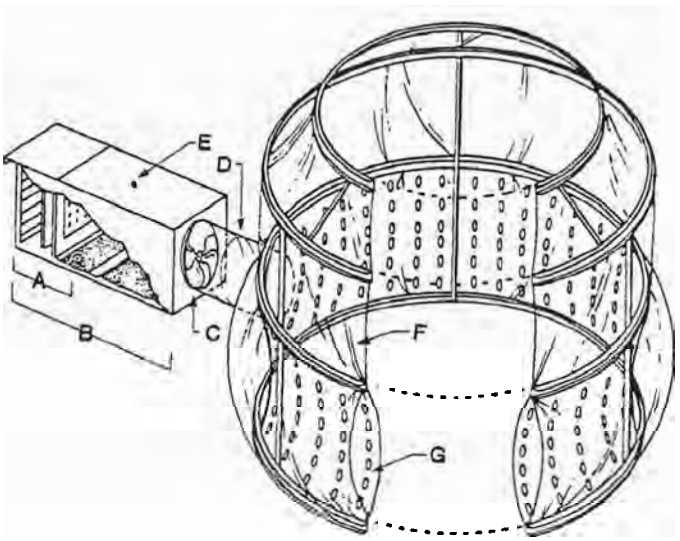

Figure 2.6. Open top field chamber. (A) filters, (B) plenum, (C) blower, (D) air duct, (E) port of injection of CO_2, (F) single-layer wall, (G) double-layer wall with perforations for air entry. After Rogers et al. (1983).

The liquid CO_2 receiver was the best method to store and supply the large quantities of CO_2 (1×10^6 g d^{-1}) needed for a study of approximately 35 chambers. Good separation of treatment CO_2 concentration was obtained for each treatment level up to and even higher than 600 ppm above ambient. Ambient CO_2 levels and levels inside chambers with no added CO_2 were virtually the same.

There have been a number of studies that have evaluated the microenvironment within open top chambers and how it differs from that of unenclosed crop canopies (Heagle et al. 1973, 1979; Mandl et al. 1973; Kats et al. 1976; Shinn et al. 1976; Heck et al. 1979; Olszyk et al. 1980; Thompson 1981; and Winestock et al. 1982). With respect to temperature, some report that air temperature within the canopy of plants inside the chamber does not differ from air temperature in the canopy of plants not enclosed by the chambers. Kats et al. (1976) found no difference up to 38°C; Heagle et al. (1979) found less than 1°C difference; Mandl et. al. (1973) found no differences greater than 1°C over the range 16° to 29°C; and Olszyk et al. (1980) found no differences greater than 2°C. Solar radiation is attenuated by the walls of the chamber: Olszyk et al. (1980) found a reduction of photosynthetically active photon flux density of 10.3%; Heagle et al. (1979) reported a reduction of total solar radiation of about 15%. Relative humidity within the chambers is higher than it is within the canopy of plants outside the chambers, which results in a reduction in daily water use of significant amounts (Olszyk et al. 1980). Shinn et al. (1976) discussed problems associated with maintaining homogeneous pollutant gradients across the chambers, desiccation of some plants, and problems with adequate irrigation.

There is only one reported study of the exchange properties of canopies enclosed within the chambers compared with canopies grown without the chambers. Unsworth et al. (1984) found the canopy boundary layer resistance to O_3 transfer of a mature soybean crop canopy inside the open top chamber to be similar to boundary layer resistance for soybean crop canopies obtained during the day using micrometeorological methods (Wesley et al. 1982).

Do differences observed between the microenvironment of plant canopies inside and outside open top chambers lead to effects on yield? Mandl et al. (1973) saw no significant differences between the rates of germination or the final dry weights of pinto beans inside or outside open top chambers compared at ambient CO_2 concentrations. Howell et al. (1979) reported that yields of plants inside the chambers were sometimes greater than and sometimes less than yields of plants grown outside the chambers. Heagle et al. (1979) reported that the chambers often produced plants that were taller than the same species outside the chambers, but that yields were rarely different from crops in the field without chambers around them. In a later paper they reported no apparent chamber effects for 1979 but a significant effect on mean pod weight in soybean in 1980. Heggestad et al. (1980) reported that significant differences were found in approximately 25% of the 24 comparisons made between mean values of biological parameters such as height, fresh weight, pod weight, and number of pods of plants grown without chambers around them and plants within open top chambers, but there was no distinct trend in favor of either group. Olszyk et al. (1980) came to a similar conclusion, namely, that differences existed between growth statistics inside and outside the chambers, but they tended to be random rather than systematic. This led the authors to conclude that such differences were not directly attributable to the effects of the chamber on physiological or developmental processes. Winestock et al. (1982) also reported that they could find no differences between physiological processes such as stomatal resistance, transpiration, and water potential or the relationship between physiological and microclimatic parameters.

Thus, the conclusion to be drawn is that the climatological differences imposed on plants by the open top chambers do not result in great differences in growth or yield.

2.8 OPEN FIELD RELEASE

The free air CO_2 enrichment (FACE) methodology has been viewed by some as a "real-world" approach which may provide the best test for the effect of the impending CO_2 enrichment on natural ecosystems. The pros and cons of FACE methodology (Shinn and Allen, in press) are presented below at some length because FACE methodology represents a controversial possible step in the development of CO_2 vegetation effects research.

The FACE approach to CO_2 enrichment is to apply a network of pipes or plenums near the ground in such a design as to provide elevated CO_2 to the ambient air of the plants. The object is to avoid the need for an enclosure or chamber around the plants. The major differences between FACE and either outdoor controlled environment chambers or open top chambers, the closest alternatives, are that FACE eliminates the following chamber effects: (1) reduction of the solar radiation environment, and (2) unnatural wind flow, turbulence, and micrometeorological patterns.

Allen (1975, 1979) found that a single line source FACE release in a maize field required a downstream distance about 10 times the height of vegetation (H)

before horizontal gradients approximately vanished (an equilibrium was reached). Air pollution exposure systems tend to verify this. The U.S. Environmental Protection Agency Zonal Air Pollution System (ZAPS) utilized plots with dimensions of 73 m by 85 m, or about 100 H on each side, for a prairie grassland (Lee et al. 1978). The U.S. Department of Energy's Argonne National Laboratory used air pollution exposure plots with dimensions of 29 m by 27 m, about 50 H on each side, for a soybean crop (Miller et al. 1980). The U.K. Central Electricity Research Laboratories (CERL) designed a circular plot array of 27 m in diameter, or about 30 H, for a wheat crop (McLeod et al. 1983).

In computer simulations for a FACE experiment in a tropical forest, it was also found that the plot dimensions approximately scale with the vegetation height (H). Based on the combined theoretical and experimental experiences above, we would estimate that a plot would need to have a minimum area of magnitude 100 H^2, perhaps larger if wind direction changes are also considered. This means plots of about 100 m^2 for a 1-m-tall wheat field, 484 m^2 for a 2.2-m-tall maize field, and 48,400 m^2 (4.84 ha) for a 22-m-tall forest.

A large study area is an advantage when part of the sampling problem is to obtain representative plant material from populations. In natural ecosystems or forests of uneven age this is especially a problem. Ecological studies of effects of elevated CO_2 on cycles of litter production, organic matter accumulations, soil respiration, nutrient cycling, above-ground competition, and phenology require a large area of uniform exposure or treatment. A requirement of a large area with replication of experiments, however, becomes a logistics problem with large numbers of samples to process and analyze and higher associated costs, especially in natural ecosystem studies.

The concentration of CO_2 in a large area supplied through a network of pipes will depend inversely upon wind speed, directly upon the release rate (source) of CO_2 (Allen 1975; McLeod and Fackrell 1983), and inversely with vegetation height when mass consistency is taken into account (Hanna et al. 1982). To hold CO_2 concentration constant on the average, the delivery rate must be increased at higher wind speeds, and this requires a feedback mechanism to be included in the FACE design. Nevertheless, it will be very difficult to maintain constant CO_2 under all weather conditions. Uncontrollably high CO_2 levels may result during a calm in a FACE experiment. Under most conditions only the very center of a circular design will have a uniform horizontal distribution of concentration.

The dilution of gases from the network of pipes has been found to be drastically close to the release points because the major dilution mechanism is by horizontal advection as compared with turbulent diffusion. That is, mean horizontal transport of CO_2 is much greater than vertical diffusion by eddy transport. For that reason an approximate "box budget" can be used to make first-order estimates of horizontal transport.

Use of the box budget is common in air pollution meteorology (e.g., Hanna et al. 1982). Solution of the appropriate equation for the average increase in CO_2 concentration (ΔC) across an area of dimension (ΔX) vegetation of height (H), wind speed (u), and source strength (Q) is:

$$\Delta C = Q\frac{\Delta X}{Hu} \tag{2.1}$$

and we can define the flushing time (t):

$$t = \frac{\Delta X}{u} \tag{2.2}$$

Application of such a box budget permits estimates of the source strength (Q) needed to raise ambient CO_2 concentration by any desired amount (ΔC). To elevate the CO_2 concentration (ΔC) by 100 ppm (183 mg m^{-3}) for a distance (ΔX) of 54 m in a canopy wind speed of 3 m s^{-1} and a maize crop 2.2 m tall, the value of the source Q would be 81 g m^{-2} h^{-1} or 810 kg ha^{-1} h^{-1}. Allen (1975) computed a value of 833 kg ha^{-1} h^{-1} with a much better model but similar boundary conditions. The flushing time using Equation (2.2) for a 54-m plot (ΔX) would be 18 s.

Allen (1975) used a two-dimensional model to calculate the CO_2 concentration distribution from a single line source, or several line sources, perpendicular to the wind direction. It utilized observed wind speed and eddy diffusivity profiles and had a computational grid in a vertical plane parallel to the wind direction. The computed isopleths of CO_2 concentration defined a plume of CO_2 that drifted downwind. Allen found that the model agreed with observations and used it to simulate CO_2 concentrations of a Costa Rican rain forest (Allen and Lemon 1976). The model predicted that CO_2 concentration distribution in the forest would be similar to the distribution in a maize crop but that the 40-m-tall forest would require a source of CO_2 50 to 100 times larger to achieve similar in-canopy CO_2 enrichments.

Experience has shown that in all pipeline release systems including the U.K. Central Electricity Research Laboratories (CERL), FACE, and ZAPS, there were gradients in the mean concentrations. Harper et al. (1973b) observed that to obtain net mean increase (ΔC) in CO_2 of about 100 ppm, the vertical mean gradients near the release pipe (at ground level) had to be about 20 ppm/cm. Although the observed spatial variability is a drawback, it appears that by clever design of

distributed, multilayer, pipeline networks and vertical standpipe releases, coupled with a feedback system of detection and flow controls, some reasonably constant mean CO_2 concentration could be maintained. This increases the complexity of design, however, and it may also demand a custom design for each experimental site to account for local wind conditions and vegetation height and density.

An interplay of spatial and temporal variation in CO_2 concentration also would occur in FACE, not only due to turbulence, but also due to slow fluctuations in the mean wind that change the depth of the CO_2-abundant layer in the plant canopy.

Observations by air pollution ecologists have shown that air concentrations of an added gas (pollutant or CO_2) in an open release system will have a log-normal frequency distribution. McLeod and Fackrell (1983) compared the results of concentration observations by the French Ministry of Agriculture, the U.S. Environmental Protection Agency, the University of Nottingham, the U.S. Department of Energy, the U.K. Central Electricity Research Laboratories, and linear-gradient systems. All of them had a log-normal frequency distribution of concentration at a point for nearly any sample-averaging time scale (a few minutes to a few hours). The geometric standard deviation was such that 10% of the time the observed concentrations were 3 to 5 times greater than the median concentration for any given location in the grid. If the FACE design concentration calls for an added CO_2 concentration (ΔC) of 300 ppm, then about 10% of the time the concentration would exceed 900 to 1500 ppm. Most of these excursions in the CO_2 concentration would be of short duration under typical daytime turbulence conditions (Allen 1973; Desjardins et al. 1978). Furthermore, there can be large variations in average CO_2 concentration from point to point, depending on proximity to the CO_2 release lines or points and on vertical height and horizontal distance downwind in the release array (e.g., experimental data, Allen 1973; model predictions, Allen 1975 and Allen et al. 1985).

Such wide concentration variations may lead to problems of data interpretation in experiments where physiological mechanisms are the subject of investigation. Geometric fluctuations would render certain in situ physiological measurements, such as stomatal diffusion resistance, photosynthesis, and water stress, virtually impossible because they depend on quasi-steady-state conditions. However, variation in long-term average concentration with height or horizontal space may make it difficult to specify the exact CO_2 enrichment level.

The wide concentration variations might be quite acceptable, however, when large numbers of organisms could be readily measured during FACE studies, for example in biomass determinations. There the cumulative effect is perhaps all that is important. This is an important distinction because from the agronomic point of view, as in the early days of FACE studies, yield increases were the significant end product. There may be similar needs in natural ecosystem studies.

The horizontal scale requirement for FACE is a symmetric plot with minimum area of 100 H^2 where H is the height of the vegetation. Scaling up from a 2.2-m-tall maize field to a 22-m-tall forest requires about 100 times the plot area (48,400 m^2 compared with 484 m^2). Using Equation (2.1) to estimate CO_2 requirements, we see that the source Q would not need to be increased to scale-up from maize to forest, when X scales with H. Using Allen's (1975) estimate for Q of 833 kg ha^{-1} h^{-1}, to increase CO_2 by 100 ppm the maize plot of 0.0484 ha requires 40 kg h^{-1}, but the forest plot of 4.84 ha requires 100 times more, 4000 kg h^{-1}.

If the 833 kg ha^{-1} h^{-1} rate of CO_2 were applied to one 4.8-ha forest plot, to attain a 100 ppm increase in concentration above present ambient level the consumption of CO_2 would be about 35,000 t y^{-1} (t=10^6 g). A CO_2 treatment of 300 ppm would require about 105,000 t y^{-1}. A simple experimental design with one each of the above treatments would require about 140,000 t y^{-1}. Clearly scale-up to forests would become a logistics problem, and the calculated daily consumption of 383 t would require large, liquid CO_2-holding reservoirs. About 30 CO_2 receivers, each the size of a tank truck (13 t), would be depleted each day.

As an alternative to using tank receivers, searches have been conducted for naturally occurring geosources of CO_2. Zimmerman and Perry (1979) located for the U.S. Department of Energy several naturally occurring subsurface CO_2 gas accumulations in central Mississippi, West Virginia, west Texas, Colorado, Wyoming, New Mexico, and southeast Utah. The price range used by Zimmerman and Perry (1979) for a profitable development was between \$5 and \$10 t^{-1} at typical temperatures. Wellhead costs were estimated to be about \$20 t^{-1}.

Industrial sources of CO_2 can be found that offer prices comparable to geosources. Coal gasification plants in North Dakota, New Mexico, and Wyoming generate CO_2 at about 700 t h^{-1} with estimated costs of \$12 t^{-1}. This production is to be used for enhanced oil recovery and will apparently not be available for other purposes.

Allen (1975) found that a maize plot (0.3 ha) FACE required 833 kg ha^{-1} h^{-1} to enhance CO_2 concentration of normal ambient air by 100 ppm. Adding a treatment of 300 ppm would be an additional 2500 kg ha^{-1} h^{-1}, and the daily consumption of CO_2 for the combined

treatments, comparable to an open top chamber study reported by Surano and Shinn (1984), would be 24 t d^{-1}. At a bulk price from natural or industrial sources of between \$10 and \$30 t^{-1}, the cost for CO_2 would be \$240 to \$720 d^{-1} for FACE. For comparison, consider the cost of open top chamber experiments in North Carolina (Rogers et al. 1983) and in California (Surano and Shinn 1984), which only consumed about 1 t d^{-1} at a cost of between \$100 to \$150 t^{-1} plus rent on a 13-t CO_2 receiver of \$15 to \$20 d^{-1}. Thus, FACE is currently estimated to cost about four times more than a comparable open top chamber study. However, if the cost per ton of CO_2 for the FACE study were the same as for the open top chamber studies of maize reported by Surano and Shinn (1984), that is, between \$100 to \$150 t^{-1}, then the basic cost for CO_2 used in a FACE study would be between \$2400 and \$2600 d^{-1}. The most optimistic comparison for a 120-day maize growing season would include the assumption that the CO_2 used for FACE would cost 10 to 20% of the cost of CO_2 for open top chambers. In this case the cost of CO_2 for FACE would be \$28,800 to \$86,400 whereas the cost for the same experiment for open top chambers would be \$12,000 to \$18,000.

Scale-up from crops (where FACE has been demonstrated) to forests will require 50 to 100 times as much CO_2 per plot as for crops because of the scale dependency on the square of the vegetation height. The volume rate of CO_2 required in a perennial, FACE forest study would exceed 10^5 t y^{-1}, which is logistically very difficult or impossible. With the cost of CO_2 from natural or industrial sources estimated to be \$10 to \$30 t^{-1}, the cost estimate for a forest study for CO_2 alone would be about \$1.4 to \$4.2 million per year.

2.9 SUMMARY

To learn how crop plants and native plants and ecosystems will respond as atmospheric CO_2 continues to increase will require additional research using all of the approaches described in this chapter. Advantages and disadvantages of the various methods discussed in the chapter are summarized in Table 2.2. The primary benefit of controlled environments in elevated CO_2 research lies with the ability to formulate, test, and improve hypotheses of organism response to environmental conditions. Environmental factors can be manipulated singly or in combinations to critically examine basic effects on organisms. In a growth

Table 2.2
Advantages and Disadvantages of the Methods Described in This Chapter

Method	Advantages	Disadvantages
Leaf Chamber	Single-leaf gas exchange kinetics obtainable.	No whole plant response such as growth; natural environment difficult to duplicate.
Phytotron	Create and control many desired environments; repeat experiments; many environmental conditions possible; biological factors controlled.	Difficult to extrapolate to natural conditions; environmental factors usually constant; plant size limitations; less than sunlight.
Portable Chambers	Small, inexpensive to build; can be used with either natural sunlight or artificial light.	Same as for most controlled environments.
Sunlit Controlled Environments (e.g., SPAR)	High light, similar to natural irradiance; variable conditions; integrated estimates of carbon and water balance; root zone similar to field; same advantages as phytotron.	Complex control; chamber effects (humidity, temperature, wind gradients); limited replication usually.
Greenhouse	Present data base on CO_2 large; natural sunlight.	Difficult to maintain (CO_2) under some conditions; difficult to extrapolate results to the field.
Field Tracking Chamber	Permits study of natural vegetation; track natural variation in the environment; whole ecosystem effects; integrated estimates of carbon and water balance.	Complexity of control functions in a remote setting; possible chamber effects.
Open Top Chambers	Can be used to study crops and natural vegetation in situ; natural sunlight; closely approximates natural environment ease of establishing elevated CO_2 concentrations.	Gradients in humidity and wind produce chamber effects; growth differs inside from outside; many sample chambers needed to deal with natural variability of ecosystems.
FACE	Closest to natural environmental conditions.	Technical feasibility; strong gradients in CO_2 in windy conditions; large sample areas needed; cost is high for large vegetation.

facility, the investigator decides on the magnitude and periodicity of each environmental factor. Results can be interpreted with confidence that the specific effects of each environmental factor are known. If a critical factor is given an inappropriate value or range, erroneous extrapolations may result. The testing of hypotheses generated from controlled environment studies by means of field validation trials is necessary before it is possible to predict field response from laboratory experimentation. van Volkenburgh and Davies (1977) discussed this problem in a report from a study conducted at the Duke University Phytotron.

In all studies of the effects of elevated CO_2 on plants that have been carried out in controlled environments, however, the growth environment differs from the natural environment of plants. Our ability to use present knowledge to predict the probable future effects of CO_2 enrichment of the atmosphere is limited by our ability to account for the differences these test environments produce on plants grown in them as compared with plants grown in the open. Thus the disadvantage of using controlled environments for studying the effects of elevated CO_2 on plant responses is the uncertainty of extrapolating results from chamber environments to field environments.

Requirements for CO_2 research in controlled environments have forced the realization that the light intensity provided in standard commercially available growth chambers is inadequate for many plants. Photosynthetic photon flux density (PPFD) available in growth chambers is typically in the range of 300 to 700 μmol m^{-2} s^{-1}, but most plants require two to three times more photosynthetic energy to respond completely to CO_2 enrichment. High-intensity discharge lamps provide the PPFD required and have been installed in many CO_2 research facilities. Although these better light sources are becoming available, they are expensive to add to existing commercial growth units that were engineered for lower energy inputs. Overheating of plants and desiccation of the air will almost certainly result unless the capacity of the refrigeration systems is also improved.

Greenhouses have a place in CO_2 enrichment research undertaken to understand the response of crops to the open field situation. To a first approximation, some environmental control is possible and the relative stimulation of vegetation by CO_2 is roughly the same over a range of light, temperature, and humidity. In addition, the light quality and intensity in greenhouses more closely approximates natural levels than those in most controlled environment facilities. However, using greenhouses for elevated CO_2 studies has the same type of disadvantage that applies to using controlled environments, namely, that the differences in environment between the greenhouse and the natural, unobstructed environment are a source of inaccuracy that is difficult to estimate.

Sunlit controlled environment chambers and open top chambers give plants exposure to 80 to 100% of natural full sunlight, although there are some differences in the quality of light in the ultraviolet and near-infrared regions. However, the reduction of windflow and turbulence inside chambers influences water loss for both systems.

A general conclusion regarding open top chambers is that the microenvironment around the plant canopy is more humid and slightly warmer than outside the chambers. Surano and Shinn (1984) found that the seasonal rate of increase of growing degree days was higher in open top chambers than for companion plots outside the chambers. Some differences have been reported (cited above) between growth of plants within the chambers and plants not enclosed by chambers in the field when both were exposed to the same atmospheric gas composition. These differences require that control chambers (without elevated CO_2) be included in the experimental design. The chamber effect can be included in the interpretation of results by comparison between growth of the crop or ecosystem being studied in an unenclosed plot and an enclosed plot with normal ambient air supplied to the plants. In the opinion of those who have used this method, these chambers remain the best currently available technology for studying plant responses to a CO_2-enriched atmosphere in the field.

The FACE approach has the advantages of least interference with solar radiation and natural wind flow. Its disadvantages include spatial and temporal variations in CO_2 concentrations, a larger study area than its closest alternatives, and considerably more technical difficulty. Also, at present, it appears that cost will make FACE impractical for tall vegetation, although it may be practical for short crops, forages, pastures, or grasslands (Allen et al. 1985).

2.10 CONCLUSIONS AND RESEARCH RECOMMENDATIONS

There are many technical difficulties in conducting research on CO_2 enhancement. Available facilities include greenhouses, phytotrons, growth chambers, leaf chambers, open top chambers, open air releases, and variations of these. All of these approaches have advantages and disadvantages. Environmental control allows the study of environmental factors alone and in combination. Environmental control, however, induces uncertainty in the extrapolation of results to the variable

natural environments. Controlled environments have space, size, and cost limitations.

Field chambers and open air releases allow the study of the effects of CO_2 under field conditions. Although these techniques do not ideally simulate the field, they currently offer the best available approaches to investigating plant responses to CO_2 under variable "real-world" conditions. The feasibility of FACE techniques for future validation studies of whole crop or entire ecosystem response to atmospheric CO_2 enhancement should be further investigated. Each of the other techniques discussed has an appropriate place, as follows, in CO_2 research:

1. Use controlled environments to study the effects of separate environmental factors on the CO_2 response of organisms and ecosystems.
2. Use single-leaf chambers to study basic details of CO_2 and other environmental changes on photosynthesis and other physiological properties of leaves.
3. Use controlled environment growth chambers to study long-term effects of chronic CO_2 enrichment on whole plants throughout their life cycles. Formulate hypotheses and test understanding by controlling and varying factors singly and in combinations.
4. Use phytotron chambers and refrigerated greenhouses to gain space and multiple factor controls required for larger experiments.
5. Use portable chambers for an inexpensive approach to the development of basic hypotheses.
6. Use sunlit controlled chambers and field tracking chambers to study canopy and ecosystem responses to a combination of variable and controlled-field environments.
7. Use open top chambers to study vegetation and ecosystem responses under field conditions.
8. Continue to examine the feasibility of FACE techniques for future validation studies of whole crop or entire ecosystem responses to atmospheric CO_2 enhancement.

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