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Chapter 3

Field Techniques for Exposure of Plants and Ecosystems to Elevated CO_2 and Other Trace Gases

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I. INTRODUCTION

Carbon dioxide (CO_2) concentration of the atmosphere has varied considerably over geological time. Ice core data from the USSR Vostok Station showed that the Earth's atmospheric CO_2 ranged from as low as 180 to 200 μmol mol^{-1} during the last two glacial maxima (13,000 to 40,000 and 130,000 to 160,000 years before present, Barnola *et al.* 1987). However, following the rapid glacial melting, the concentration rose quickly to about 260 to 270 μmol mol^{-1}. Since around 1700 AD, the CO_2 concentration has increased from about 270 μmol mol^{-1} to 315 μmol mol^{-1} in 1958 and 355 μmol mol^{-1} in 1991.

Recognition of the importance of CO_2 for photosynthesis and growth of green plants had a long period of development during the 18th century (review by Krikorian 1975). Stephan Hales showed that wood could generate many times its volume of air. Joseph Priestley first recognized that the action of green plants could reverse the process of animal respiration and of combustion. Jan Ingenhousz elaborated further the findings of Priestley. However, these early workers were more interested in the uptake of oxygen than in the uptake of CO_2.

The importance of CO_2 for growth of green plants became clear during the early 19th century. According to de Saussure (1804), peas exposed to 8% CO_2 grew better than those in ambient air. The effect of the concentration of CO_2 (as well as light and temperature) on photosynthesis of green leaves was quantified by Matthaei (1904) and Blackman and Matthaei (1905).

The history of CO_2 enrichment has been reviewed by Wittwer (1986). Some of the earliest work was reported in Vermont by Cummings and Jones (1918). Kimball (1983a) reviewed 430 observations and reported average yield increases of various types of crops to be 33 ± 6% for a doubling of CO_2 above ambient levels. Much of our present knowledge of the stimulation of photosynthesis and growth was obtained using crop species in controlled environments, especially greenhouses, to determine how CO_2 "fertilization" would increase yield (Wittwer and Robb 1964). In the context of the recent accelerating increases in atmospheric CO_2, as well as potential climate change, we want to know how crops,

unmanaged vegetation, and ecosystem processes will respond. New methods and facilities have been devised to test the broader questions that are now being asked. Field exposure techniques, especially those that do not modify microclimate, are being developed and improved for exposure of large areas of plants to elevated CO_2 and other trace gases.

This chapter contains sections describing most of the approaches that have been applied in elevated CO_2 (and gaseous air pollutant) studies, including leaf chambers, sunlit controlled environment facilities, mobile greenhouses, large greenhouses used to study crops, small greenhouses used in studies of natural vegetation, open-top chambers, air exclusion systems, and open-air field release systems. 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 few 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, branch chamber systems for air pollution research that are also suitable for CO_2 studies have been developed (Houpis *et al.* 1991; Teskey *et al.* 1991). The available data on mechanistic changes in the photosynthetic apparatus of intact leaf tissue occurring in response to elevated CO_2 have been 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 leaf cuvettes, whole-plant 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, 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. 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 conditions.

Naturally sunlit crop growth chambers based on "closed-loop", air circulation systems with computer-managed environmental controls, such as those developed at Florence, South Carolina (Phene *et al.* 1978; Baker *et al.* 1982) and at

Gainesville, Florida (Jones *et al.* 1984a; 1984b) have proven very useful for detailed crop studies. These units are sometimes referred to as SPAR (Soil-Plant-Atmosphere Research) chambers. At Clemson University, Mississippi State University, and the University of Florida, these systems have been used for short- and long-term experiments on responses of cotton, maize, soybean, wheat, citrus, tomato, and rice to CO_2-enriched atmospheres. Subambient levels of CO_2, spanning concentrations that existed during the previous ice age 15,000 to 40,000 years before present, have been studied at Gainesville using soybean (Bisbal 1987; Allen *et al.* 1990a; 1991) and rice (Baker *et al.* 1990a; 1990b; 1990c). These systems, because they are closed, lend themselves to the study of water use and water-use efficiency by crop species as a function of temperature, humidity, and CO_2 concentration throughout diurnal cycles and throughout the season (Jones *et al.* 1985a; 1985b; 1985c; Allen *et al.* 1985b). The chambers are divided into two parts, an upper plant canopy chamber and a lower root zone compartment in which soil water content, 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.

Interactions of CO_2 concentration with other variables, especially temperature, vapor pressure deficit, and soil water content, have frequently been studied at Gainesville and at Mississippi State. *Rhizobium* nitrogen fixation of soybean as a function of CO_2 treatment was investigated at Clemson University (Reardon *et al.* 1990).

Greenhouses 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 since the beginning of the 20th century. Kimball (1983a, 1983b) reviewed over 140 reports and extracted more then 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. Kimball's analysis of these disparate data indicated an average 36% increase of the weight of crop yield for a doubling of CO_2, 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; Oechel and Strain 1985; Tissue and Oechel 1987), who designed small greenhouses capable of tracking ambient temperature and humidity while maintaining setpoint CO_2 concentration to study the effect of elevated CO_2 on Arctic tundra. The computer control feature of SPAR systems would easily permit them to track ambient CO_2 concentrations, air temperature, and humidity (as long as the ambient air humidity is well below saturation), although they have been used mainly to follow preprogrammed setpoints rather than setpoints of the changing atmospheric environment.

Open-top field chambers as described by Heagle *et al.* (1973, 1979; 1989), Buckenham *et al.* (1981), Ashenden *et al.* (1982), and Hogsett *et al.* (1985) have had extensive use as plant exposure units both in air pollution and in CO_2 effects studies in the field. These systems have been used to expose potted plants, annual crops, and trees to a variety of aerial pollutants and CO_2, and they are currently in use at a number of laboratories in North America and Europe. Hardy and Havelka (1975) first used a square-wall, 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.* (1983b) adapted the basic cylindrical open-top chamber system to generate large-scale CO_2 test atmospheres in the field. Kimball *et al.* (1983) and Nakayama and Kimball (1988) used a square-wall, open-top chamber with 0.2 m diameter perforated polyethylene ducts between rows of cotton. Drake *et al.* (1989) developed an open-top chamber system for CO_2 enrichment of saltmarsh vegetation that recirculated part of the input air. Computerized control and data acquisition systems have been developed (e.g., Nystrom *et al.* 1982; Weigel and Jäger 1988b; Mejer *et al.* 1988).

The need to study CO_2 effects on vegetation in a natural field environment has led to the con-

cept of artificially elevating CO_2 by release through an array of pipes. The history of this free-air CO_2 enrichment (FACE) approach can be traced to studies by crop scientists (Kretchman 1969; 1970; Baker *et al.* 1970; Allen 1973; Harper *et al.* 1973a; 1973b; 1973c; 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. A grid release system to study air pollutant effects in vineyards for the French Ministry of Agriculture was described by de Cormis *et al.* (1975). 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; 1990), 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 with air exclusion methods was designed by Shinn *et al.* (1977) and modified by Laurence *et al.* (1982) and by Reich *et al.* 1982). McLeod and Fackrell (1983) and McLeod and Baker (1988) reviewed methods of open-air fumigation.

The topic of "methods for exposure of plants and ecosystems to elevated CO_2 and other trace gases" was discussed thoroughly by Drake *et al.* (1985) and by Weigel and Jäger (1988a). This current chapter is an update of the earlier contribution to a U.S. Department of Energy report prepared by Drake *et al.* (1985). The main advances have been in the new, emerging technology of free-air CO_2 enrichment (FACE).

II. LEAF CUVETTES

There are so many cuvette 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 cuvette designs developed during the past three decades can be found in Musgrave and Moss (1961); Mooney *et al.* (1971); Šesták *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.* (1985a; 1985b).

The major design problem of leaf cuvettes is the same as for whole-plant growth chambers, namely, how to control the environment around the leaf. Thus, a leaf cuvette 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 for use outdoors (e.g., Sinclair *et al.* 1979; Field *et al.* 1982; and Valle *et al.* 1985a; 1985b) computers have been used in mobile field laboratories to integrate all subsystems as well as to manage data and provide either machine-readable or hard copy of results.

The simplest leaf cuvette 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 Šesták *et al.* (1971), and the interpretation of gas analysis data is discussed by Sharkey (1985).

Cuvette systems for measuring gas exchange between the leaf and its environment are either open or closed. In flow-through, open-exhaust systems, air of known composition passes once over the leaf, and the change in CO_2 and water vapor concentration caused by the leaf is determined. In closed-circulation systems, air is stirred continuously 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. Single flow-

through 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 a flow-through cuvette 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 3-1) described 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 it was not used for that purpose. This system was used in field studies to track environmental temperature, humidity, and solar radiation, as well as to measure continuously both water vapor and CO_2 exchange of up to 39 cuvettes concurrently.

The leaf cuvette 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 water jacket 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. Sinclair and Allen

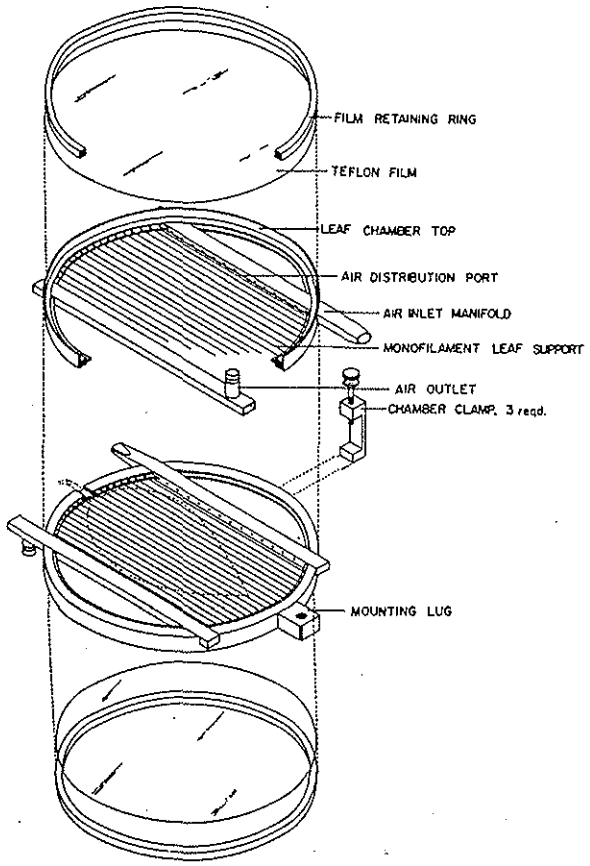

FIGURE 3-1. Leaf chamber for field measurement of photosynthesis. After Sinclair *et al.* (1979).

(1982) and Sinclair *et al.* (1983) used this system for studying response of citrus to environmental variables.

A system similar to the one employed by Sinclair *et al.* (1979) was used by Valle *et al.* (1985a; 1985b) and Allen *et al.* (1990b) for studying long-term responses of soybean (*Glycine max* [L.] Merr., cultivar Bragg) leaves to elevated CO_2. The cuvettes were used to measure photosynthesis and gas exchange in up to 12 leaves of plant canopies that were being grown in elevated CO_2 in outdoor, sunlit controlled-environment chambers. Managing a leaf gas exchange system with cuvettes mounted inside large (but not walk-in) outdoor plant growth chamber systems is much more difficult than managing cuvettes outdoors.

[1] Teflon is a registered trademark.

III. CONTROLLED ENVIRONMENT CHAMBERS FOR WHOLE-PLANT CANOPIES

A. 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 3-1. 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; Potvin and Strain 1985), sink strength (Clough *et al.* 1981; DeLucia *et al.* 1985), drought stress (Paez *et al.* 1983; Sionit and Patterson 1985; Wray and Strain 1986), light (Sionit and Patterson 1984), salinity (Bowman and Strain 1987), and mineral nutrition (Sionit *et al.* 1981; Cure *et al.* 1988).

In the past, the controlled environment methods used in phytotrons have usually involved small rooting volumes. These small rooting volumes may affect (restrict) the partitioning of carbohydrates to roots. This may impact CO_2 enrichment studies by restricting the sink for photoassimilates (Arp 1991; Thomas and Strain 1991).

B. Portable Greenhouse 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 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 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density.

[2] Formica is a registered trademark

TABLE 3-1
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	A range of 0-24 h of light continuously programmable in square or sine wave patterns. Outdoor chambers or field tracking chambers would receive natural photoperiods
Air Temperature	5° to 40°C
Thermoperiod	0-24 h phase-separated from photoperiod but in similar wave patterns
Air Water Vapor Pressure	Controllable to give a vapor pressure deficit of 0.2 to 5 MPa. Practically, this is highly dependent upon air temperature.
Air Velocity	0-3 m s^{-1} continuously adjustable
CO_2 Concentration	0-2500 μmol mol^{-1}. Practically, 100 to 1000 μmol mol^{-1}.

C. Continuous-Flow Stirred Tank Reactors

A widely used plant exposure chamber system in air quality and gas kinetics work (i.e., uptake by and release from plants) is the continuous stirred tank reactor or CSTR (Rogers *et al.* 1977). The unique feature of this chamber technique is that it provides direct kinetic data, as well as a uniform and readily characterizable environment. A CSTR system for continuously monitoring not only pollutant uptake, but also transpiration, photosynthesis, and respiration has been developed recently (Marshall and Ferman 1988). This system incorporated improved control technology compared to previous CSTR systems used for air pollutant studies (Heck *et al.* 1979). Although used mostly indoors, CSTR systems could be adapted for outdoor use.

D. Outdoor, Sunlit Controlled-Environment Chambers

Sunlit controlled-environment chambers based on "closed-loop" air circulation systems with computer-managed environmental controls were developed in the late 1950s and early 1960s. These chambers usually had 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 photosynthetic CO_2 exchange rates. Transpiration rates were 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 conditions (e.g., Musgrave and Moss 1961; Moss *et al.* 1961; Baker and Musgrave 1964, Egli *et al.* 1970). These systems were the predecessors to the units with large controlled root-zone containers as well as canopy-zone chambers (Phene *et al.* 1978; Parsons *et al.* 1980; Baker *et al.* 1982), which have been further modified for improved systems for CO_2-effect studies (Jones *et al.* 1984b; Acock *et al.* 1985b).

Details of the design, functioning, and use of these recently improved chambers have been reported by Jones *et al.* (1984a; 1984b; 1985a;

[3] Mylar is a registered trademark.

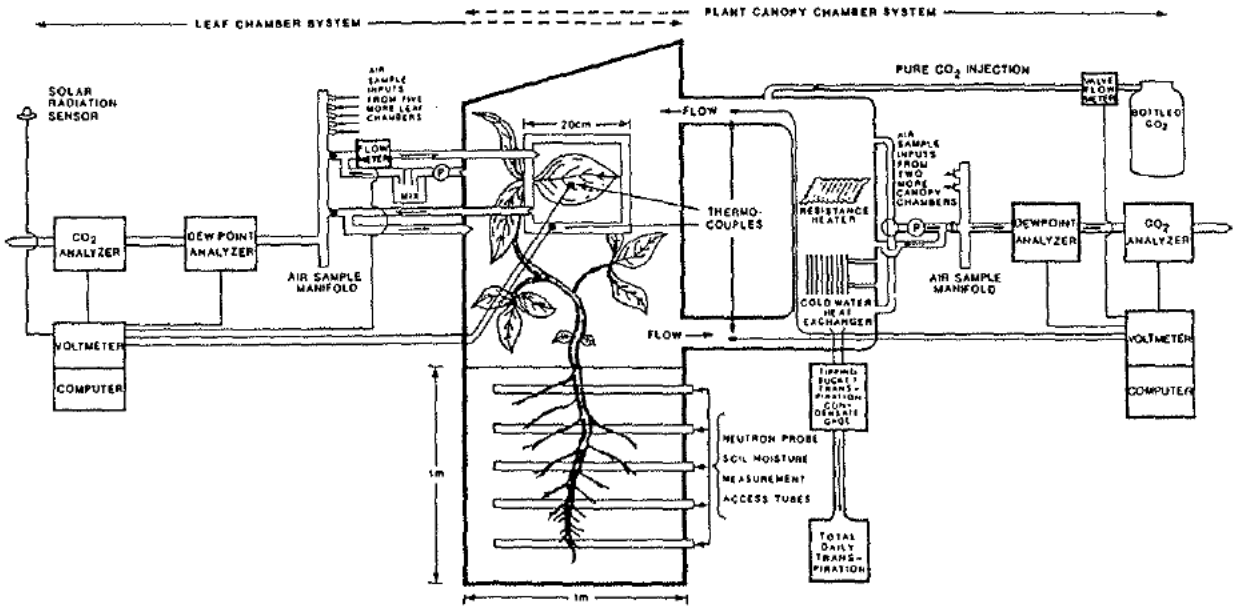

FIGURE 3-2. Closed system computer-controlled plant environments for CO_2 enrichment study. Plant canopy chamber system described by Jones *et al.* (1984a; 1984b; 1985a; 1985b; 1985c). Leaf chamber system described by Valle *et al.* (1985a; 1985b).

1985b; 1985c). A sketch showing the overall layout of this system is shown in Figure 3-2. 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 SPAR chambers were designed to provide accurate, flexible control of dry-bulb temperature, CO_2 concentration, and humidity of the canopy air, as well as control and measurement of soil water and root conditions. In contrast to open flow-through systems, the air in SPAR systems was 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., copper-constantan thermocouples, IRGAs, and dewpoint hygrometers, respectively), (2) feedback mechanisms such as thermostats or loops in computer logic that compared sensed with desired conditions; and which (3) actuated control devices such as heaters that were regulated to produce the desired temperature treatment conditions, CO_2 injection valves for replacing CO_2 as it was used in photosynthesis, and cooling-coil flow-valves for regulating dewpoint temperature.

Air in these SPAR chambers 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, air sampling ports, and control devices were located within the ducts so that the air circulated to the top of the canopy had the experimentally prescribed setpoints of 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, except during occasional operational problems such as excessive condensation in sampling lines or on chamber walls (during cold winter events) or failure of air sampling pumps, 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 SPAR 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 a fine sand soil. For single mainstem crops like soybean, the soil compartment could be sealed from the aerial compartment by growing the plants through slits in the floor, and filling those slits with closed-cell foam as soon as the plants were large enough. However, other similar systems may vary in size and in the rooting medium (e.g., Acock *et al.* 1985b). The SPAR systems were designed with deep rooting volumes to prevent the plants from becoming "root-bound". However, when these systems were used for studying paddy rice, the depth of the flooded vats were reduced to 0.6 m (Baker *et al.* 1990a; 1990b; 1990c). Because of the growth habit of rice (tillering), the soil compartment could not be sealed from the aerial compartment.

The SPAR chamber system described by Jones *et al.* (1984b) provided measurements of canopy photosynthetic CO_2 exchange rate and transpiration rate at 5-min intervals. Air samples from each chamber were analyzed for CO_2 on a 5-min cycle. Quantitative carbon balance was computed from the amount of CO_2 injected during each 5-min interval adjusted for the change in chamber CO_2 concentration from the beginning to the end of the 5-min period. If the concentration increased slightly, then the photosynthetic rate based on CO_2 injection was adjusted downward, and vice versa. The amount of CO_2 injected during each 20-s interval within the 5-min period could be adjusted based on measured incoming solar radiation. This frequent adjustment of CO_2 injection provided an accurate control of CO_2 to the setpoint. Transpiration was obtained from a tipping bucket raingauge measurement every 5 min. The design of SPAR chamber systems for continuous, ongoing measurements of photosynthetic rate and transpiration rate, coupled with their computer setpoint controls, provides unique research capabilities and opportunities for investigating interactions of CO_2 with temperature, humidity, and solar radiation effects on photosynthesis, water use, growth, and productivity of plants.

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; Allen *et al.* 1985b), as well as on interactions between elevated CO_2 concentrations and temperature (Jones *et al.* 1985a) and a moisture stress (Jones *et al.* 1985c). These are examples of the types of direct CO_2 effects and coupled climate or soil-water interactions that can be obtained in sunlit controlled environment chambers. These systems offer a high level of environmental control with a reasonable approximation to field sunlight and soil conditions. Yields of crops grown in SPAR systems (such as soybean and rice) have been very similar to crops grown outdoors in other experiments. Direct comparisons with outdoor crops are not usually possible, especially when the chambers are operated with fixed setpoints of temperature and humidity.

E. Field Tracking Chambers

The small, field tracking chamber used in studies of Arctic ecosystems (Prudhomme *et al.* 1984; Tissue 1984; Oechel and Strain 1985; Tissue and Oechel 1987) had a 127-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 3-3). The chamber enclosed a surface area of 1.65 m^2. Carbon dioxide concentrations inside the enclosure were continuously monitored and maintained at 340 $\mu mol\ mol^{-1}$ CO_2, representing ambient, or at an elevated level of 680 $\mu mol\ mol^{-1}$. Concentrations were maintained by either adding pure CO_2 gas or scrubbing the chamber 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 connected to a heat exchanger inside the chamber. The fans inside the chamber 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

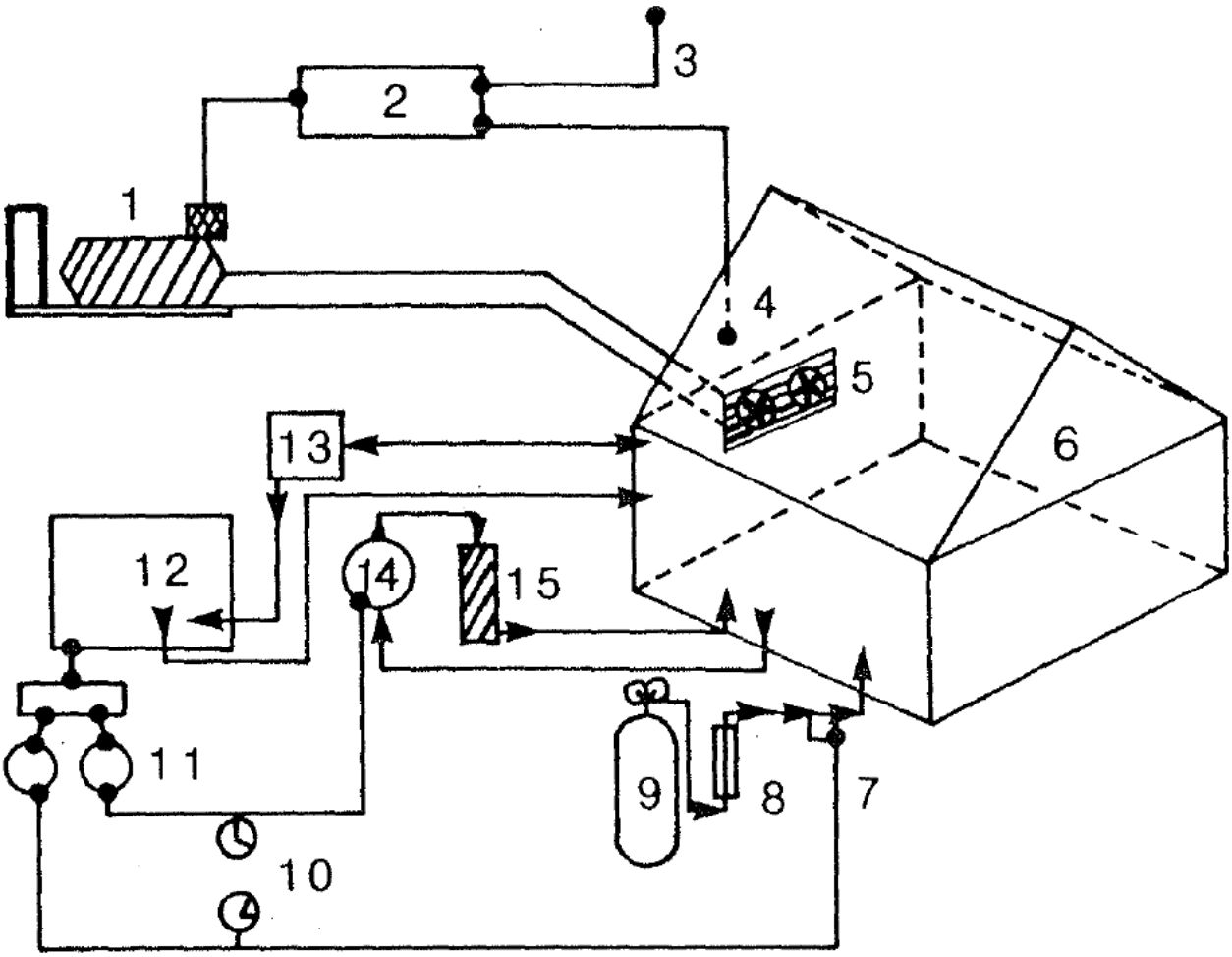

FIGURE 3-3. Schematic diagram of the field tracking chamber used to measure community CO_2 exchange of tussock tundra at Toolik Lake, Alaska; (1) Compressor, (2) YSI 71A controller, (3) external thermistor, (4) internal thermistor, (5) heat exchanger and fans, (6) chamber, (7) on/off solenoid, (8) flow 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. After Prudhomme *et al.* (1984).

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 setpoint 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.

The field tracking chambers are actually a subset of the outdoor, sunlit controlled environment chambers. They are very similar to SPAR chambers, except the field tracking chambers were used over natural vegetation whereas the SPAR chambers included a large contained, controllable soil volume.

IV. GREENHOUSES

Greenhouses are structured frames covered with nearly transparent glazing such as glass, fiber glass, polyvinyl chloride, acrylic, polycarbonate, 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) listed ways that greenhouse environments differ from the open field and Kimball (1986b) reviewed data available from the literature on the interactions of CO_2 enrichment effects with several environmental variables. The most obvious difference between greenhouse and open field environments is that temperatures can be modified in the former, usually with gas burners for heating or evaporators for cooling. 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 vegetative growth more at the higher ranges of temperatures over which plants are normally grown than at the lower ranges (Allen SG *et al.* 1990).

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 conducted during winter when light levels are only one-third to one-tenth of summer open-field intensities. From theoretical consideration of CO_2 effects on photosynthesis, Kimball (1986b) 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 (Kimball 1986b). 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 milder environment with respect to water relations, and they often appear more luxuriant than their shorter, thicker leaved, field-grown counterparts. In his review, Kimball (1986b) 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 as it was 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 regime 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 1986b).

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 1986b). Future farmers will need to adjust fertilizer rates to take advantage of the stimulation of yield by the increased atmospheric CO_2.

The economics of research are not the same as those of horticultural production. For research purposes, expensive refrigeration may be justified to control temperature (and even humidity) of closed, CO_2-enriched greenhouses and growth chambers. Alternatively, injecting large amounts of CO_2 during ventilation of greenhouses may also be justified. This approach uses much CO_2 but little electrical energy. Furthermore, Monteith

et al. (1983) controlled greenhouse temperatures easily in the United Kingdom as long as they were above outside temperatures, and maintained CO_2 by controlled injection.

V. OPEN-TOP VENTILATED CHAMBERS

Air flow properties around open-top ventilated chambers, particularly near the large opening at the top of the transparent walls, has been an ongoing concern in the development and use of this field technique for exposure of plants to CO_2 or gaseous air pollutants. Ordway (1969) provided an aerodynamicists qualitative analysis of the "Odum Cylinder" approach to measurement of CO_2 exchange in a forest (Odum and Jordan 1970). Ordway pointed out several actual and potential problems with a cylindrical chamber. First (from mean-flow hydrodynamics), a vortex would form near the base of the chamber on the upwind side and coil around both sides of the chamber, forming a horseshoe-shaped vortex when viewed from above. Second, a nonuniform penetration and entrainment of air into the opening at the top would be expected, which could lead to uneven mixing and a nonuniform concentration distribution of gases (e.g. CO_2). Third, Ordway pointed out that air flow through the vegetation would be unnatural. Ordway (1969) failed to point out the importance of atmospheric turbulence or gustiness on the penetration of eddies into the top of the chamber. The concerns about turbulent exchange in and out of the top of the cylinders, as opposed to strict mass flow incursions, led to several wind-tunnel evaluations of open-top chambers. These wind tunnel studies were conducted with and without baffles or constrictions at the top to reduce the incursion of ambient air (Davis and Rogers 1980; Davis *et al.* 1983). Baldocchi *et al.* (1989) designed a wind tunnel study to measure flow in and out of cylindrical chambers located in a scaled-down model forest. These wind tunnel scale chambers had various top size openings (25%, 50%, and 75%) with frusta having 30, 45, and 60° angles of elevation. In the wind tunnel conditions, little incursion of air occurred when the percentage opening was only 25%. Incursion of air from above increased with increasing angle and increasing opening of the frustum. Baldocchi *et al.* (1989) concluded that decreasing the size of the open top and decreasing the angle of the frustum would minimize turbulent incursion losses through the outlet of open-top chambers.

L. H. Allen (unpublished) found that air flow through an open-top chamber could be influenced greatly under high windspeed conditions. When high wind gusts were directed toward the intake of fans of an open-top chamber such as illustrated in Figure 3-4, mass flow into the open-top chamber would be increased. The variability of flow through the open-top chambers under windy conditions was greatest when the filter density (filter resistance) was lowest.

Open-top chambers have ranged in size from the very unwieldy Odum Cylinder (18 m diameter x 20 m height) to the very useful and practical chamber (0.8 m diameter x 1.0 m height) used in saltmarsh vegetation (Drake *et al.* 1989). Few measurements of actual performance in the field have been made, and those may vary considerably depending on the size and shape of the structure. Chapter 9 of this volume documents enriched CO_2 concentration and air flow measurements within three types of open-top chambers.

The open-top chamber used by Rogers *et al.* (1983a; 1983b; 1983c; 1984a; 1984b) was an open-ended, transparent cylinder roughly 3 m in diameter by 2.4 m high (Figure 3-4). 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 (Figure 3-4) 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 doubled-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.

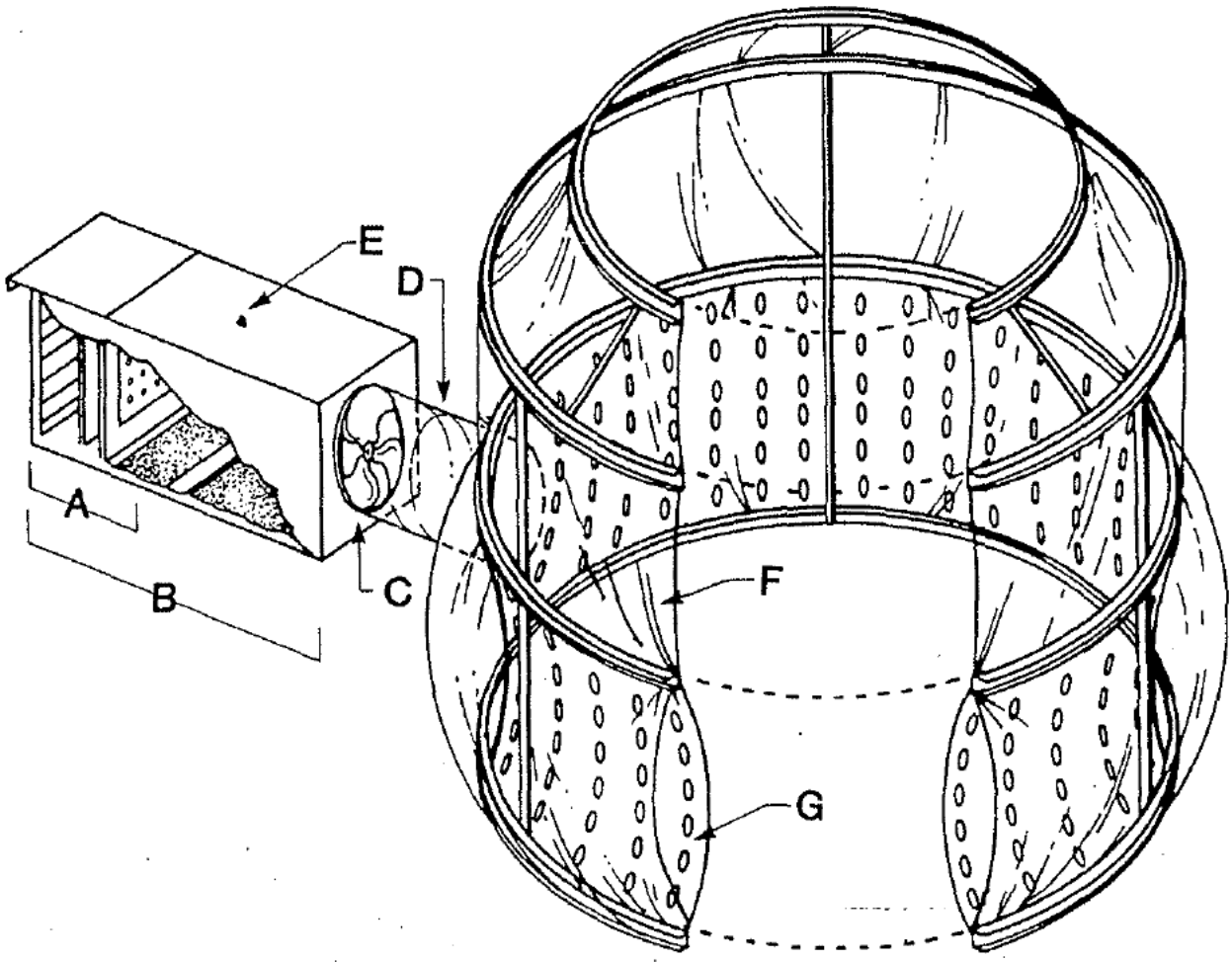

FIGURE 3-4. Open-top field chamber. (A) filters, (B) plenum, (C) blower, (D) air duct, (E) port of injection of CO_2, (F) single-layer wall, (G) double-layer wall with perforations for air entry. After Rogers *et al.* (1983b).

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. 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 µmol mol^{-1} above ambient. Ambient CO_2 levels and levels inside chambers with no added CO_2 were virtually the same.

A small open-top chamber system (1.0 m in height and 0.8 m in diameter) with a recirculation mixing blower has been used for measurement of CO_2 effects in salt-marsh vegetation since 1986 (Curtis *et al.* 1989a; 1989b; 1990; Drake *et al.* 1989; 1990; Long and Drake 1991; and Leadley and Drake 1992). These studies have shown that a C_3 sedge responded to elevated CO_2, 660 vs 350 µmol mol^{-1}, with increased growth, increased shoot densities, and reduced green shoot % N with unchanged % C (which resulted in a 20 to 40% increase in the shoot tissue C/N ratio). However, litter C/N was unaffected. Root dry mass increased 83% over a two-year period. No evidence for decreased acclimation in photosynthetic capacity that would counteract CO_2 stimulation was found. No effect of elevated CO_2 on *Spartina patens* or *Distichlis spicata* (C_4 grasses) was found. Chamber effects per se on plant response was not very noticeable. In contrast, soybean has been observed

to show chamber effect responses (Rogers *et al.* 1984a; 1986; Acock *et al.* 1985a).

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; 1989; Mandl *et al.* 1973; Kats *et al.* 1976; Shinn *et al.* 1976; Heck *et al.* 1979; Olszyk *et al.* 1980; Thompson 1981; Weinstock *et al.* 1982; Unsworth 1984a, 1984b; Last 1986; Fangmeier 1986; Unsworth 1986; Adaros *et al.* 1989a; Drake *et al.* 1989; Flesch and Grant 1992). 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 difference greater than 1°C over the range of 16 to 29°C; and Olszyk *et al.* (1980) found no difference greater than 2°C. Heagle (1989) reported mean chamber air temperatures to be 0.6, 2.2, and 2.8°C above ambient on cloudy, cold days, on partly cloudy, cool days, and on sunny, warm days, respectively. Drake *et al.* (1989) reported that night-time temperatures in their salt-marsh chambers were 2°C higher than outside because of heat from blowers. Air temperatures were generally from 1.2 to 2.7°C higher in chambered vegetation in the daytime, particularly in a *Scirpus olneyi* (C_3) community. Air speed was variable, ranging from 1.6 to 0.1 m s^{-1}. Average air speeds in other larger open-top chambers have been reported from 0.3 to 0.8 m s^{-1} (Heagle *et al.* 1979; Olszyk *et al.* 1980; Weinstock *et al.* 1982). Flesch and Grant (1992) reported that air speed ranged from 0.28 to 1.33 m s^{-1} and turbulent intensity ranged from 0.15 to 0.61 in their open-top chamber measurements. Musselman *et al.* (1986) reported average daytime chamber and ambient air temperatures of 26.2 ± 1.6 and 23.7 ± 1.8°C, respectively, for a closed-top ventilated field chamber, with temperatures at various locations being within 1 to 1.5°C of each other. Nighttime temperatures were usually 1 to 2°C warmer inside the chamber. Fangmeier *et al.* (1986) measured diurnal cycles of temperature and found the values to be about 1°C higher inside open-top chambers than outside near midday. Adaros *et al.* (1989a) found a maximum rise of air temperature in their open-top chambers to be 3.6 and 1.4°C under day and night conditions, respectively, but daily mean temperature increases were only 1°C. During the winter period, barley was observed to survive in open-top chambers, but not in the open air (Adaros *et al.* 1989b).

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; 1989) reported a reduction of total solar radiation of about 12 to 15%; and Adaros *et al.* (1989a) found irradiance to be decreased by 22 to 28%. 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). Fangmeier *et al.* (1986) measured diurnal cycles of relative humidity and found increases to range from nil to about 10%. Relative humidity increased by up to 12% by day and decreased by 15% by night in measurements by Adaros *et al.* (1989a).

Shinn *et al.* (1976) discussed problems associated with maintaining homogeneous pollutant gradients across the chambers, desiccation of some plants, and problems with adequate irrigation. Heagle (1989) found that the ozone concentration differences at various sampling positions within an open-top chamber were about 0.008 μmol mol^{-1} when the mean concentration was 0.15 μmol mol^{-1}, a 5% maximum difference.

Although solar radiation is attenuated by the largely transparent walls of open-top chambers, the flux density of net radiation inside the chambers is considerably higher than outside in the morning and midday hours. As pointed out by Unsworth (1986), net radiation (Rn) is given by the radiant energy balance equation, $Rn = St - r \times St + Ld - Lu$, where St is downwelling solar radiation, r is canopy reflectivity (assumed to be 0.2), Ld is downwelling long wavelength radiation, and Lu is upwelling long wavelength radiation. Unsworth (1986) provided typical values of these radiant flux densities inside and outside chambers at noon for a cloudless spring or summer day. The values of St, $r \times St$, Ld, Lu, and Rn were 800, 160, 350, 450, and 540 W m^{-2}, respectively, for outside conditions, and 760, 150, 500, 450, and 660 W m^{-2}, respectively, for chamber

conditions. The increase in Ld within the chamber thus more than compensates for the decrease of St within the chamber, and results in an increase of Rn of about 20% (Unsworth 1986). Thus, it is primarily the increase of downwelling long wavelength radiation from the walls of the chamber (emitting at near air temperature conditions) that causes increase of foliage temperatures inside open-top chambers. It seems probable that, under similar chamber ventilation rates, temperature increases in comparison to outside conditions would be greatest when emissivity of the wall material is large (transmissivity to Ld is low) and when sky conditions are clear with low water vapor content (which would give low values of Ld of sky radiation). To our knowledge, a detailed study of all these relevant factors has not been conducted for open-top chambers. However, this information may be available in greenhouse energy balance studies (e.g., Kimball 1986a).

Unsworth *et al.* (1984a; 1984b) found the boundary layer resistance of leaves and canopy (to water vapor and O_3 transfer, respectively) of soybean inside the open-top chamber to be similar to boundary layer resistance for soybean crop canopies obtained during the day using micrometeorological methods (Wesely *et al.* 1982). Flesch and Grant (1992) pointed out that greater differences in boundary layer resistances may exist between chamber and field conditions in low air speed spaces of the chambers, or when chamber conditions are compared with field windspeed conditions above 1.8 m s^{-1}. Dunin and Greenwood (1986) compared daily evapotranspiration from a lysimeter containing 6 eucalyptus trees surrounded by a ventilated chamber (open-top, tapered chamber, 11 m in height with 15 × 15 m square base and 2.5 m circular upper outlet). Evaporation determined by inflow-outflow gas analysis was 96% of the lysimeter measurements. During most measurement periods, evaporation was slightly lower (about 7%) than Bowen ratio-energy balance measurements of the surrounding forest. When windspeeds were low (outside), the ventilated chamber evaporation exceeded the Bowen ratio-energy balance measurements by 18%. Similar results were found for evaporation from a lupine crop (Dunin *et al.* 1989), although daily average vapor losses from the ventilated chambers were 5% higher than from the Bowen ratio-energy balance technique, with the largest value being 28% higher. The chambers used in this experiment had tapered entry and exit bays arranged to give a horizontal air flow rather than a vertical air flow. Leuning and Foster (1990) compared transpiration rates of a 7-year-old eucalyptus tree in their ventilated chamber with the Penman-Monteith equation applied to single leaves, and to a leaf energy budget method. Not surprisingly, the transpiration rates of the ventilated chamber were about 30% lower than that computed by the Penman-Monteith equation for single leaves, probably due to questions of scaling from leaf to whole tree canopy.

Although open-top ventilated chambers appear to provide quite reasonable evapotranspiration measurements, Dunin and Greenwood (1986) point out that their findings may not apply to chambers of different design nor to different vegetation. Also, Dunin *et al.* (1989) point out that inferences in plant control of evaporation can be misleading in chamber experiments. Nevertheless, relative evapotranspiration rates of canopies under different CO_2 treatments should be quite valid.

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. Plant height increases inside chambers may be due to the lack of sufficient mechanical stress due to wind gusts (Biddington 1986) or to other factors such as decreased solar radiation (particularly UV-B) or increased humidity. 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 of snap bean 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. Weinstock *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 microclimate parameters. Rogers *et al.* (1986) found large chamber effects for soybean. Acock *et al.* (1985a) were not able to successfully match GLYCIM model predictions to chamber-grown soybeans using external micrometeorological data. Olszyk *et al.* (1992) reported that canopy volume of Valencia orange trees (*Citrus sinensis* [L.] Osbeck) was 104% greater after growth for 51 months in nonfiltered air of open top chambers in comparison with ambient air. Total harvestable fruit for the last 3 years of the study was 98% greater in the chambered treatment than in the nonchambered control. Protection from damaging wind could have contributed to the beneficial effects of chambers on these citrus trees.

Olszyk *et al.* (1986) compared system exposure effects (air exclusion, open-top ventilated field chambers, and closed-top ventilated field chambers) with outside plots during an ozone exposure experiment with alfalfa across the growing season of two years. The air exclusion system itself had no effect on alfalfa response parameters. Open-top chambers caused lower weights, greater heights, and greater O_3 injury in nonfiltered open-top chambers compared with outside plots during three summer harvests in 1983. However, weights were increased by the open-top chamber for the October 1983 harvest. During 1984, the closed-top ventilated chambers generally increased dry weight and height with respect to both other systems.

Although direct comparisons with simultaneous field plantings of soybean were not made, the leaf size and plant height were generally larger, and mainstem strength generally lower ("viney") than outside plantings in SPAR chamber (Jones *et al.* 1984a; 1985a; 1985b; 1985c). However, morphological characteristics reported by Baker *et al.* (1990a; 1990b) for rice grown in SPAR chambers were similar to field plants reported elsewhere. Curtis *et al.* (1989a) reported a significant chamber effect on biomass of a C_3 saltmarsh sedge, *Scirpus olneyi*, but not on C_4 saltmarsh grasses or mixed communities. Probably the most extreme example of a chamber effect was reported by Kirkham *et al.* (1990) for closed-top ventilated chambers under rangeland conditions. They found that total aboveground dry weights of tall grass prairie plants grown in their chambers were more than 2-fold greater than weights of outside control plants.

Thus, the conclusion to be drawn is that the climatological differences imposed on plants by chambers can result in either insignificant differences or great differences in growth or yield. The extent of the observed differences are probably related to the natural outside environment under which the plants are being grown (e.g., arid vs. humid, windy vs. calm, hot vs. cold), as well as the species being studied and the chamber system being used.

VI. PORTABLE FIELD CHAMBERS

Rapid, short-term measurements of canopy evapotranspiration was developed by Reicosky and Peters (1977) using a portable field chamber technique. This technique has also been applied to canopy photosynthetic CO_2 exchange measurements in subsequent studies (Boote *et al.* 1980; Ingram *et al.* 1981; Jones *et al.* 1982; Boote and Bennett 1982; Daley *et al.* 1983; Zur *et al.* 1983; Boote *et al.* 1984; Garrity *et al.* 1984; Boote *et al.* 1985; Jones *et al.* 1986; Meyer *et al.* 1987; Albrecht *et al.* 1989). The portable field chamber developed at Gainesville, Florida (Jones *et al.* 1982; Zur *et al.* 1983; Boote *et al.* 1984) had a nondispersive infrared gas analyzer and a mirror-type dewpoint hygrometer for measuring carbon dioxide and humidity (dewpoint temperature), respectively. The portable chamber top was

1.033 m^3 in volume which was formed by an aluminum frame covered with Mylar film. During operation, the chamber was clamped onto a base for measurements over a duration of 1 to 2 min. A 30-cm fan was used to circulate air within the transparent chamber. Air was pumped through 0.6 cm diameter tubing at 8 ℓ min^{-1} for a short distance to the infrared gas analyzer and dewpoint hygrometer located in a small van. Part of the flow (0.8 ℓ min^{-1}) was diverted through the infrared gas analyzer. Output from the two analyzers, along with air temperature (from a copper-constantan thermocouple) and photosynthetically active radiation were recorded on strip-chart recorders. Photosynthetic CO_2 uptake rates and transpiration rates were measured from the slopes of the time rate of change of CO_2 concentration and humidity after the rates had stabilized (15 to 20 seconds after enclosure of the portable chamber). Temperature increases were about +1.5°C per min and CO_2 concentration decreases were about –60 μmol mol^{-1} per min in this chamber. This technique has also been used for measurements of CO_2 uptake rates and transpiration rates in FACE studies (D. R. Hileman, personal communication).

VII. AIR EXCLUSION SYSTEMS

Air exclusion systems were developed for exposure of crop plants to pollutant gases in the field. These systems were designed to replace the air within the plant canopy with air that had been filtered and enriched with a pollutant to a specified concentration. The essential features are large volume capacity blowers connected to large inflatable air exclusion tubes (0.15 to 0.32 m in diameter) with suitable exhaust ports that can be placed between rows of a crop. Hogsett *et al.* (1987a; 1987b) reviewed the performance and description of several of these facilities. The first system was described by Jones *et al.* (1977) for excluding ambient SO_2 from crops. This system was successful in excluding up to 85% of ambient SO_2. Another system was designed not only to exclude ambient air but also to inject O_3 or H_2S into the blower distribution system for exposure of plants in the field (Shinn *et al.* 1976; 1977; Shinn 1979; Bennett *et al.* 1980). Furthermore, this system could establish a linear gradient of gaseous pollutants along the air exclusion tubes. Also, the exposure plots were surrounded by a 0.6 m high barrier to help minimize the incursion of outside air into the plot. A similar system was designed to expose row crops to O_3, SO_2, and HF in multiple pollutant exposures (Laurence *et al.* 1982; Reich *et al.* 1982). Thompson and Olszyk (1985) designed another system for a variety of uses including ambient air exclusion, or for linear gradients of ambient air or SO_2. The response of plants with this system has been compared with open-top ventilated chambers and closed-top ventilated chambers (Olszyk *et al.* 1986).

Air exclusion systems have not been used for CO_2 enrichment studies, but in principle they would be very adaptable for this purpose, both with or without a linear gradient exposure. The open-top chamber system reported by Nakayama and Kimball (1988) comes very close to being an air exclusion system since they used large ducts for distributing CO_2 enriched air between rows of cotton in large rectangular open-top chambers with a large area-to-height ratio.

VIII. ENVIRONMENTAL GRADIENT

A small tunnel formed by a polyethylene cover on hoops was used by Kretchman (1969; 1970) to enrich strawberries with CO_2 (see Allen 1979). Carbon dioxide was injected into a blower air stream to provide enriched air at one end of the tunnel. Temperature and humidity was not measured along the length of the tunnel.

A temperature gradient tunnel was developed as an indoor plant growth cabinet facility (Grime *et al.* 1989). This system can quickly provide sets of temperature responses for small stature plants. The facility is computer controlled and can create a temperature gradient over the range of 10 to 35°C and also inject steam for humidification. However, the levels of photosynthetically active radiation were only about 125 μmol (photons) m^{-2} s^{-1}, far below typical summertime midday values of more than 2000 μmol (photon) m^2 s^{-1}. The small size and low light conditions limit the usefulness of such a facility for meaningful CO_2 effects research.

A controlled-environment tunnel for CO_2 gradient studies is under development by Mayeux

et al. (1992). Air is moved unidirectionally along five 7.6-m segments. Plants are grown under transparent covers in a soil volume that is 0.76 m deep and 0.45 m wide. Each soil container is partitioned into 0.6 m segments so that water and nutrients can be controlled along the direction of air flow. At the end of each 7.6 m segment, air passes through chilled-water heat exchangers and electrical resistance heaters for control of humidity and air temperature. The tunnel makes a 180° turn at the end of each 7.6 m working segment to allow the whole system to fit inside a 9.5 m by 8.5 m greenhouse that can itself be heated and cooled. The CO_2 of the air flow is depleted progressively by photosynthesis of plants grown in the tunnel chamber. The system produces a gradient of CO_2 from entry to exit of the whole tunnel system. Carbon dioxide depletion down to 150 to 200 μmol mol^{-1} can be achieved by adjusting the blower speed depending upon real-time measurements of CO_2 and light. A micrologger-controller system manages the blower speed and air conditioning equipment at the entry to each segment of the tunnel system in order to maintain desired levels of CO_2, air temperature, and air humidity. Although used so far only for investigating effects of subambient gradients of CO_2 on rangeland plants, the system of Mayeux *et al.* (1992) can easily be adapted for the study of superambient gradients of CO_2 by incorporating either fixed-rate or variable-rate CO_2 injection at the entry of the controlled-environment tunnel.

Greenhouse-size environmental-gradient tunnels have been under development in Japan (Horie *et al.* 1991). One such greenhouse tunnel is about 30 m long by about 3 m wide and about 2 m in height. Natural solar energy is used to create a temperature gradient in the greenhouse tunnel. Air flow through the greenhouse tunnel is controlled by three reversible-flow fans at one end of the structure. One, two, or all three of the fans can be operated to control the air flow rate and air residence time, and hence the air temperature gradient, from one end to the other of the greenhouse tunnel. At nighttime, the direction of the fans are reversed and heat is added to the system with a burner to maintain a similar gradient of air temperature with respect to the ambient air temperature. This system is used to track ambient air temperature conditions, with the warmer end of the tunnel maintained at a consistently higher temperature than the cooler end of the tunnel. Overhead fans were used over four segments along the greenhouse tunnel to prevent vertical gradients of temperature from developing. Air temperature was sensed throughout the tunnel.

Carbon dioxide by temperature interaction effects on rice were studied by injecting CO_2 at a controlled rate into the air flow of a companion greenhouse tunnel. Under the tunnel system and operating conditions established by Horie *et al.* (1991), a decrease of CO_2 concentration of about 10% was observed from the entry to the exit of the tunnels. Since both CO_2 uptake rate of the plants and the temperature rise in the tunnel system are driven by solar radiation, the increase in air flow required to maintain the desired temperature gradient along the tunnel also helps maintain the supply of CO_2 along the length of the tunnel. Horie *et al.* (1991) did indeed find CO_2 X temperature interaction effects on rice.

Both the controlled-environment tunnel systems of Mayeux *et al.* (1992) and the environment-tracking, greenhouse environmental-gradient tunnel system of Horie *et al.* (1991) provide additional methods for conducting CO_2 X temperature studies on crops and short ecosystems beyond those of the SPAR chamber systems discussed earlier. The greenhouse environmental-gradient tunnel has an unnatural solar and long wavelength radiation environment, but the air flow through and above the canopy should come closer to approximating outdoor wind profile conditions than any system other than free-airstream CO_2-enrichment systems.

IX. OPEN FIELD RELEASE INCLUDING RECENT FREE-AIR CO_2 ENRICHMENT TECHNIQUES

A. General Considerations

The free-air CO_2 enrichment (FACE) methodology is a "real-world" approach that may provide the best test for the effect of the impending global atmospheric CO_2 enrichment on natural and managed ecosystems (Allen *et al.* 1985a; Drake *et al.* 1985; Shinn and Allen, 1985; Hendrey

et al. 1988a; 1988b). A brief review of FACE-type methodology, or plume exposure systems, applied to air pollution studies in the field was given by Hogsett *et al.* (1987a; 1987b), and has been summarized in Chapter 4 of this book. The theoretical pros and cons of various FACE methodology (Shinn and Allen 1985; Allen *et al.* 1985a) are presented below. Recent advances in FACE technology by Brookhaven National Laboratory (BNL FACE) using closely controlled release of pre-diluted CO_2 through a circular array of vertical vent pipes represent a new approach in CO_2 vegetation effects research. These advances are also discussed in Chapters 1, 2, 5, 6, and 7 of this book.

The general FACE approach to CO_2 enrichment uses a distribution system of pipes or plenums designed to provide elevated CO_2 to the ambient air of the plant canopy. Gases emitted from the distribution system are diluted rapidly close to the release points primarily by horizontal advection and secondarily by vertical turbulent eddy diffusion. Entrainment of air by emission jets and continuous mixing by turbulence smooths out both temporal and spatial variations in CO_2 concentration. Because mean horizontal transport of CO_2 is much greater than vertical diffusion by eddy transport, an approximate "box budget" of mass can be used to make first-order estimates of horizontal mass transport of CO_2. The distribution system could be designed for either (1) area-source emissions (ground-level or elevated-height), (2) upwind vertical vent pipe emissions (circular or square array), or (3) a combination of area-source and upwind vertical vent pipe distribution systems. The objective is to avoid the need for an enclosure or chamber around the plant canopy.

The major differences between FACE-type techniques and either outdoor controlled-environment chambers or open-top field chambers are that FACE techniques in general eliminate the following chamber effects: (1) reduction of the solar radiation environment (and changes in the diffuse/direct beam ratio) by the transparent chamber walls and support structures (including further reduction of solar UV-B radiation by many glazing materials), (2) unnatural wind flow, turbulence, and micrometeorological patterns of temperature, humidity, and long wavelength energy exchange, and (3) disturbed soil water patterns, especially with open-top chambers, associated with diversion of natural rainfall by the chamber walls. FACE systems use real field soil for the rooting medium, which is sometimes not used for chambered CO_2 enrichment studies or even open top chambers. The direct chamber effects include slight reductions in photosynthetic rates due to lower light levels, and changes in rates of development and growth due to temperature and soil-plant-air water relationships. In many cases, plant morphology may be changed due to the absence or reduction of solar UV-B radiation, or to changes in thigmomorphogenesis or seismomorphogenesis factors due to insufficient movement or mechanically-induced stress (Biddington 1986) in the constant, but low wind and low turbulence, airflow environment of most chambers.

B. Scaling Considerations

For designing FACE-type experiments it would be very useful to have a set of scaling rules for adjusting release characteristics to the height and density of the plant canopy. Such rules will depend explicitly on the type of FACE system that is used. For example, based on the data of Allen (1975, 1979) Shinn and Allen (1985) showed that a **single line source** of pure CO_2 in a FACE-type release in a maize field required a downstream distance about 7 to 20 times the height of vegetation (H) before horizontal CO_2 concentration gradients became vanishingly small. Shinn and Allen (1985) and Drake *et al.* (1985) adapted 10 H as a minimum scaling factor for horizontal distances that should be considered for FACE-type CO_2 exposure systems. Air pollution exposure systems designed for short crops tend to exceed this minimum approximation. 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 and Fackrell 1983; McLeod *et al.* 1983; 1985; McLeod and Baker 1988).Based on both theoretical and experimental work, Shinn and Allen (1985) estimated that a plot would need to have a minimum area of magnitude 100 H^2, perhaps larger if wind direction changes are also considered using this type of control system. This approximation suggested that plot might be about 100 m^2 for a 1-m-height wheat field, 484 m^2 for a 2.2-m-height maize field, 48,400 m^2 (4.48 ha) for a 22-m-height forest, and 160,000 m^{-2} (16 ha) for a 40-m-height forest for a **ground-level area source** release of CO_2.

The plot area of the BNL circular FACE array of vertical vent pipes (VVP) is 380 m^2 for a cotton crop which grew to about 1.5 m in Yazoo City, Mississippi in 1987 and 1988, and to about 1 m in Maricopa, Arizona in 1989. These are the only FACE studies in which both temporal and spatial control of CO_2 concentrations are well documented. Also, the degree of control and shape of release from the BNL VVP FACE system is a significant advance over the ground-level area source release discussed above. The tight control over CO_2 concentrations achieved by this system, due to both the configuration of release equipment and the 1-s time step monitoring and feedback control subsystems, show that a new height scaling rule is needed. The earlier estimates of 100 H^2 seem to be excessively large for very tall vegetation and too small for very short vegetation.

A large study area is an advantage when part of the sampling problem is to obtain representative plant material from populations. Obtaining a CO_2 enrichment study area with sufficient size is especially a problem in forests or most natural ecosystems. Ecological studies of effects of elevated CO_2 on cycles of litter production, organic matter accumulations, soil respiration, nutrient cycling, aboveground 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. FACE techniques do provide much more plant material than open-top chambers that are typically 0.8 to 13 m^2 in plot area, depending upon vegetation type and height or SPAR chambers typically 2 m^2 in plot area.

The concentration of CO_2 in a large area supplied through a network of pipes **without feedback control** will depend inversely upon wind speed, directly upon the release rate (source) of CO_2 (Allen 1975; McLeod and Fackrell 1983; Shinn and Allen 1985), and inversely with vegetation height when mass continuity is taken into account (Hanna *et al.* 1982). To maintain CO_2 concentration constant on the average, however, the delivery rate must be increased at higher wind speeds and decreased at lower wind speeds, which requires a feedback mechanism based upon wind speed to be included in the FACE design (see Chapter 7, this book).

C. Early FACE System Experience

Experience has shown that in pipeline release systems including the U.K. CERL pollutant SO_2 release system, early FACE-type releases, and ZAPS, there were persistent vertical and horizontal gradients in the mean concentrations obtained by averaging measurements over periods of minutes to tens of minutes. Harper *et al.* (1973b) observed that when mean increases (ΔC) in CO_2 of about 100 $\mu mol\ mol^{-1}$ were obtained near the top of a cotton crop during **ground-level, area-source** releases, the vertical mean gradients near the release pipe (at ground level) were about 20 $\mu mol\ mol^{-1}\ cm^{-1}$. Although the observed vertical concentration variability is a drawback for **ground-level, area-source** releases, Drake *et al.* (1985) suggested that some reasonably constant mean CO_2 concentration could be maintained by clever design of distributed, multilayer, pipeline networks and vertical standpipe releases, coupled with a feedback system of detection and flow controls. Such a control system would increase the complexity of design, however, and it may also require a custom design for each experimental site to account for vegetation height and density. The BNL FACE systems seems to be a simpler solution to the problem, at least for crops.

Observations by air pollution ecologists **prior to 1983** showed that downstream concentrations resulting from SO_2 gas injection in essentially

uncontrolled open release systems tended to have a log-normal frequency distribution. McLeod and Fackrell (1983) compared the results of air pollutant 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 about **3 to 5 times greater than the median concentration** for any given location in the grid (Shinn and Allen 1985). On the basis of these earlier studies, if CO_2 enrichment (ΔC) of 300 μmol mol^{-1} were specified for a FACE-type system, then about 10% of the time the CO_2 concentration would be expected to exceed 900 to 1500 μmol mol^{-1}. 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, large variations in average CO_2 concentration from point to point might be anticipated, 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.* 1985a).

Such wide concentration variations could lead to problems of data interpretation in experiments where physiological mechanisms are the subject of investigation. Geometric fluctuations could possibly render certain in situ physiological measurements, such as stomatal diffusion resistance, photosynthetic rate, and water stress, questionable because they depend on quasi-steady-state conditions. Moreover, variation in long-term average concentration with height or horizontal space may make it difficult to specify the exact CO_2 enrichment level throughout the enriched volume, although modelling (Chapter 7 of this book; Allen and Beladi 1990) can be used to predict these distribution of CO_2 concentration. Natural variations in wind direction help to decrease the problem of horizontal CO_2 concentration gradients.

D. BNL FACE

Most of the problems with such large fluctuations in CO_2 concentrations in FACE systems appear to have been solved. Improvements in FACE technology that have been developed by BNL have greatly decreased the range of gas concentrations that were observed by others during CO_2 releases (see Chapter 7, Fig. 7-7) to smaller relative values than those observed in early SO_2 release experiments. The reasons for the smaller range of concentrations are: (1) predilution of gases before emission from the array to about 3% CO_2 (depending on windspeed), (2) vertical vent pipe injection jet design for entraining and mixing with the air stream, (3) rapid updating and correction of CO_2 release rates with a PID algorithm (Chapters 5, 6, and 7) based on rapid, direct measurements of wind speed, wind direction, and CO_2 concentration at the center of a vertical vent pipe distribution array, and (4) the fact that CO_2 is being released into a relatively stable background concentration of about 355 μmol mol^{-1}.

E. Scaling Requirements for Area-Source Releases

Shinn and Allen (1985) provided the earliest estimates of requirements for scaling up the CO_2 requirements and cost estimates for conducting FACE-type experiments in tall vegetation. Those estimates are based on FACE technologies prior to the improvements demonstrated by the BNL FACE system. The horizontal scale requirement for a **ground-level, area-source** FACE array is a symmetric plot with a minimum area of 100 H^2 where H is the height of the vegetation (Shinn and Allen 1985). Scaling up from a 2.2-m-tall maize field to a 22-m-tall forest would require about 100 times the plot area (48,400 m^2 compared with 484 m^2) assuming fumigation of the entire column from ground to crown. The source Q (mass of CO_2 per unit area per unit time) 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 μmol mol^{-1} the maize plot of 0.0484 ha requires 40 kg h^{-1}, but the forest plot of 4.84 ha would require 100 times more, 4000 kg h^{-1} (Drake *et al.* 1985; Shinn and Allen 1985).

If the 833 kg ha^{-1} h^{-1} rate of CO_2 were applied to a **ground-level, area-source** release on one 4.8-ha forest plot to attain a 100 μmol mol^{-1} increase in concentration above present ambient level, the consumption of CO_2 would be about 17.5×10^6 kg per year for a 12 h per day, 365 day per year release. A CO_2 treatment of 300 μmol mol^{-1} would require about 52.5×10^6 kg per year. A simple experimental design with one of each of the above treatments would require about 70×10^6 kg per year. Clearly, scale-up to forests using the earlier FACE technologies would become a logistics problem and the calculated daily consumption of 384 Mg would require large, liquid CO_2-holding reservoirs. About 30 CO_2 receivers, each the size of a tank truck (13 Mg), would be depleted each day in this scenario. Thus the need for improvement in FACE technology provided by the BNL FACE system is dramatically illustrated. Further study of the application of this new BNL FACE technology to forests is also needed to provide an assessment of whether or not such forest experiments are practical.

F. Real-World Dispersion: Forests

In real-world forests, CO_2 dispersion plumes from emitters are likely to meander considerably in comparison with plumes in shorter vegetation. Fluorescent particle releases from point sources as measured by Leo J. Fritschen *et al.* (1969; 1970a; 1970b) in a University of Washington experimental forest showed examples of extremely variable patterns of dispersion. Scaling up equipment for tall forests may make operation and maintenance much more difficult than for short vegetation.

Because larger plot areas may be required for tall forests, costs of CO_2 enrichment would be higher than for short crops, although the cost per unit ground area for a given level of enrichment may be similar. Costs obviously would vary linearly with wind speed. Since recent research suggests that elevated CO_2 may suppress dark respiration rates (Gifford *et al.* 1985; Reuveni and Gale 1985; Bunce 1990; Amthor 1991), 24 h per day releases may be required.

For 270 m × 270 m simulations in a 40-m forest (Allen and Beladi 1990) with CO_2 releases of 1,667 kg ha^{-1} h^{-1} from the upwind backup vertical vent pipe source and 1,667 kg ha^{-1} h^{-1} from the area-source, the amount of CO_2 released would be 24,300 kg h^{-1} and the cost of CO_2 (at $39 per ton) would be $948 per hour, or 291,600 kg d^{-1} of CO_2 at a cost of $11,372. For 365 days, this gives 1.064×10^8 kg of CO_2 at a cost of $4.15 million per year. If a decreased area (100 m × 100 m) were sufficient, the amounts and costs of CO_2 would be divided by 713. If smaller forest plot sizes are adequate say 213 m^2 equal to those used by Odum in his studies of a tropical forest in Puerto Rico (Odum and Jordan 1970) then 3.1×10^5 kg of CO_2 would be used per year at a cost of $12,000. Clearly, the range is determined by the size of the plot.

Likewise, for 13.5 m × 13.5 m grassland simulations (Allen and Beladi 1990) with CO_2 injections of 1,852 kg ha^{-1} h^{-1} from the upwind backup vertical vent pipe release and 1,852 kg ha^{-1} h^{-1} from the area-source release, the amount of CO_2 released would be 67.5 kg h^{-1} and the CO_2 cost would be $2.63 per hour, or 810 kg d^{-1} at a cost of $31.59. For 365 days, this gives 295,650 kg of CO_2 at a cost of $11,530 per year.

An elevated-height, area-source release used in conjunction with an upwind leading edge vertical vent pipe release should provide the maximum uniformity of mean CO_2 concentration over the canopy and within the canopy according to these simulation analyses. This was also the conclusion of McLeod and Fackrell (1983) and McLeod *et al.* (1983; 1985). However:

a. More emitters over the canopy volume targeted for enrichment would increase the short-term CO_2 fluctuations.
b. The CO_2 injection control technology would increase in complexity, although presumably a modified controller could command injection of both the vertical vent pipe array and a circular, elevated-height, uniform-emission, area-source array.
c. A controlled injection, vertical vent pipe circular array seems to be the best arrange-

ment for controlling CO_2 concentration and for economy in use of CO_2. At any given point in time, substantial gradients of CO_2 concentration along the mean wind path across the circular array may exist, but variable wind direction throughout a long-term period of enrichment may avoid a CO_2 gradient effect.

Scale up problems such as dispersion variability, larger equipment for injection of CO_2, and perhaps more complex control systems will make FACE studies in tall forest systems more difficult than in short vegetation systems.

Significant progress has been made since 1987 by Brookhaven National Laboratory on development of a circular array of vertical vent pipes for use as a FACE system in fields of cotton (see Chapters 5, 6, and 7). Most of the chapters of this book deal with the development and performance of this new BNL FACE system, and with the methodology and experimental findings of these studies of CO_2 enrichment effects on cotton obtained with this system. .

The new BNL FACE concepts for larger sized forest vegetation include injection of CO_2 into the crown space only with vertical vent pipe emission systems (personal communication by G.H. Hendrey, June 1992). This approach should reduce the area-to-height requirements very significantly for FACE experiments in tall vegetation, although the open trunk space at the bottom of the crown space will allow some CO_2 losses downward as well as upward. The area requirement proposed by Shinn and Allen (1985) of 100 H^2 is likely to be much too great for very tall vegetation, and much too small for very short vegetation, and new scaling criteria need to be developed.

X. SUMMARY

To learn how crop plants, native plants, and ecosystems will respond as atmospheric CO_2 continues to increase will require additional research using approaches described in this chapter. Advantages and disadvantages of the various methods discussed are summarized in Table 3-2. 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. The final confirmation of hypotheses generated from controlled environment studies may require field trials before prediction of field responses from laboratory experimentation can be validated.

Some of the problems of field vs. laboratory growth chamber responses of plants to aerial environments were discussed in phytotron studies by Van Volkenburgh and Davies (1977) at Duke University and by Raper and Downs (1976) at North Carolina State University. More recent studies show that limited rooting volume (small pot size) may be just as serious a problem for interpretation of responses to CO_2 as the unnatural aerial environment of chambers, due to small pot size effects on reduction of overall plant size (Bonzai Effect), root/shoot ratios, and relative growth response to CO_2 (Arp 1991; Thomas and Strain 1991). For example, elevated CO_2 caused enhanced branching of soybean roots (Del Castillo *et al.* 1989) and increased partitioning of dry weight to fine roots of trees, especially in poor soil conditions (Norby *et al.* 1985).

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. This uncertainty may increase, especially when restricted rooting volume containers are used for long-term studies.

Requirements for research in controlled environments have forced the realization that the light intensity provided in standard commercially available growth chambers is inadequate for many

TABLE 3-2
Advantages and Disadvantages of the Methods Described in This Chapter (from Allen 1979; Baker *et al.* 1982; Drake *et al.* 1985)

Method	Advantages	Disadvantages
Leaf Chamber	Single-leaf gas exchange kinetics obtainable. Can be used alone or in combination with long-term exposed plants.	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; interactions of CO_2 with temperature and vapor pressure.	Difficult to extrapolate to natural conditions; environmental factors usually constant; plant size limitations; plant root volume limitations; less than sunlight. Usually not set up for carbon uptake and transpiration.
Portable Greenhouse 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; provides for continuous integrated measurements of carbon and water balance; root zone similar to field; control to specified setpoints or track the ambient environment; temperature and humidity; same advantages as phytotron.	Complex control; chamber effects (humidity, temperature, wind gradients); limited replication usually; expensive per unit.
Greenhouse	Present data base on CO_2 large; natural sunlight; large area of plant material.	Difficult to maintain (CO_2) under some conditions; difficult to maintain temperature and humidity; 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 measurements of carbon and water balance.	Complexity of control functions in a remote setting; possible chamber effects; expensive per unit.
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.
Portable Field Chambers	Small, inexpensive to build; can be moved easily from plot to plot; can be adjunct with FACE system for snapshot of carbon and water exchange; good for relative measurements.	Not a long term exposure facility; modifies environment as soon as placed on crop.

TABLE 3-2 (continued)
Advantages and Disadvantages of the Methods Described in This Chapter (from Allen 1979; Baker *et al.* 1982; Drake *et al.* 1985)

Method	Advantages	Disadvantages
Air Exclusion System	Other than Face, closest to natural environmental conditions.	Complex feedback control; large air exclusion tubes interferes with access; only for row crops.
Tunnels	Can establish CO_2 and temperature gradients for interaction studies.	Possible chamber effects; gradients may be variable.
Free-Air CO_2 Enrichment (FACE)	Closest to natural environmental conditions; provides large area of plant material for measurements and sampling.	Complex feedback control; spatial gradients in CO_2; short-term variability in CO_2; large areas needed; cost may be high for tall vegetation.

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 at least 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 increased.

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 indoor 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. Furthermore, the reduction of windflow and turbulence inside chambers may influence water loss for both systems. Certain species may also respond to the sheltering effect of the chamber by changes in morphology.

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 previously) 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 some researchers these chambers remain the most convenient, if not 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, as well as providing for a natural below-ground environment. Its disadvantages include some spatial and temporal variations in CO_2 concentrations, and greater technical difficulty (except compared to the control technology of sunlit, outdoor controlled-environment systems). At present, it appears that costs of CO_2 might make FACE expensive for tall vegetation depending on plot size. It is practical for investigating the effects of elevated CO_2 on short crops, forages, pastures, or grasslands (Allen *et al.* 1985a).

Recent analysis of plot area vs costs of CO_2 and cost of total scientific commitment by Kimball (Chapter 17) shows a considerable advantage for FACE techniques. The FACE plot areas are much larger than those of open-top chambers or outdoor controlled environment chambers. Therefore, more experimental work can be conducted by a larger number of scientists. Considered as a total research program, the costs for CO_2 itself becomes a smaller component of the total costs of conducting research.

XI. CONCLUSIONS AND RESEARCH TECHNIQUE RECOMMENDATIONS

There are many technical difficulties in conducting research on CO_2 enhancement. Available facilities include greenhouses, phytotrons, outdoor controlled-environment chambers, leaf cuvettes, open-top chambers, air exclusion systems, free-air releases, and variations of these systems. 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 and offer the best available approaches to investigating plant responses to CO_2 under variable "real-world" conditions. The use of FACE techniques for validation studies of a cotton crop exposed to atmospheric CO_2 enhancement has been accomplished. Entire ecosystem responses to CO_2 enrichment should be further investigated using FACE. Each of the other techniques discussed has an appropriate place, summarized as follows, in CO_2 research:

1. Use controlled environments to study the effects of separate environmental factors (such as temperature, humidity, soil water, photoperiod, irradiance, etc.) and their interactions on the CO_2 response of organisms and ecosystems.
2. Use single-leaf cuvettes to study basic details of CO_2 and other environmental changes on photosynthesis and other physiological properties of leaves. This technique is important in quantifying upward regulation and downward regulation of photosynthesis over long time periods of exposure of the whole plant system. Branch chambers may offer some advantages (and disadvantages) of both leaf cuvettes and open-top chambers.
3. Use controlled-environment plant growth chambers to study long-term effects of continuous 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 obtain multiple factor controls and to gain space required for larger experiments. At northerly cool latitudes, use heated greenhouses for CO_2-effect interaction studies with temperature and soil-water using idealized diurnal cycles of temperature where the control temperatures are to be maintained above the outside temperatures.
5. Use portable field chambers for short-term measurement of canopy photosynthetic and transpiration rate, and for an inexpensive approach to the development of basic hypotheses.
6. Use sunlit, controlled-environment chambers and field tracking chambers to study canopy and ecosystem responses to a combination of variable and controlled-field environments. These responses include continuous CO_2 exchange and transpiration measurements.
7. Use open-top chambers to study vegetation and ecosystem responses under field conditions that approach those of the natural out-

doors. Evaporation and CO_2 uptake capabilities have been included in some of these chamber systems.

8. Continue the development of both controlled-environment chamber tunnels and field-tracking, greenhouse environmental-gradient tunnels for CO_2-effect interaction studies with temperature, soil moisture, and soil nutrients.
9. Continue to develop the unique capability of FACE techniques for validation studies of whole crop, forest, or entire ecosystem responses to atmospheric CO_2 enhancement. Use leaf cuvettes for documenting leaf level and stomatal responses, portable field chambers for short-term photosynthetic and transpiration measurements, and open-top chambers for growth and yield comparisons within FACE plots, and for quantifying the expression of the "chamber effect" by plants.

There is a need to integrate previous knowledge gained from experiments into whole ecosystem responses (Mooney *et al.* 1991). FACE techniques may allow such an approach without disturbing the natural system although "scaling up" to tall vegetation ecosystems may be complex. The greenhouse environmental-gradient tunnel concept could be used to investigate CO_2 X temperature interactions. Clever aerodynamic design with controlled-direction air entry scoops and air exit vents could both decrease the power requirements for ventilation of large greenhouse environmental-gradient tunnels and prevent the possibility of air flow reversals during windy conditions. A large facility such as this could be adapted for use in ecosystems research. Also, perhaps a large greenhouse tunnel on rails could be developed and operated to allow direct natural rainfall on the environmental-gradient site. Finally, many of these field exposure systems could also be used to investigate interactions of global CO_2 increases with regional or local air pollutant effects on vegetation (Allen 1990).

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