Chapter 11

Special Use Chambers

J. Craig McFarlane, Robert C. Morrow, Douglas P. Ormrod, and Steven H. Schwartzkoff

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Introduction

Special chambers create environments otherwise unattainable. Generally, they are modifications of existing equipment or chambers built within standard growth chambers. In some cases, new chambers have been built because needed conditions could not be obtained by simpler means, or because it was cheaper to start from scratch.

This chapter is not an exhaustive survey of all special chambers, but a compilation of examples demonstrating the considerations that create the special conditions, while preserving the basic features discussed in the rest of this handbook. Modifying an existing chamber often results in a compromise, which reduces some aspect of the designed use. This discussion identifies those compromises and the limitations they may impose on environment, space, or cost of research.

A large variety of small chambers, typically called cuvettes, accommodate a single leaf or a small plant and are used to make measurements, such as photosynthesis, transpiration, respiration, or volatile gas releases. These small chambers will not be included in the discussion, although in using and constructing them, one must attend to the same considerations as for the larger chambers.

The short bibliographies provide sources of information on construction and applications of the different kinds of special chambers.

Closed Chambers

The environmental isolation provided by closed chambers offers a number of advantages. It allows the conduct of experiments involving toxic or radioactive materials under atmospheric

conditions that would be difficult or impossible to conduct in other chambers. It also enables accurate measurement of the changes in concentration that occur in the constituents of the medium (air, soil, nutrient solution) sealed inside the chamber. And, it provides a means for precisely controlling and monitoring the amounts of materials added to, or subtracted from, the sealed environment. Although the reasons for closure will generally dictate a peculiar size, geometry, and material for each chamber, several considerations are common to all.

Carbon Dioxide

Plants growing in closed environments absorb CO_2 from the air until the CO_2 compensation concentration is reached and the plants cease to grow. The speed of this process depends on the size and activity of the plants, the starting CO_2 concentration, and the chamber volume. With eight, 30-cm tall soybeans in a 0.4 m^3 chamber, it took about 20 minutes for CO_2 to decrease to the compensation concentration when starting at the ambient level. Several methods exist for controlling CO_2, each having certain advantages and disadvantages.

The easiest system is managed by a dedicated, continuous CO_2 analyzer connected to a switch that opens a valve and injects CO_2 into the chamber when the concentration drops below a preset limit. This injection system creates a sawtooth concentration pattern with the magnitude of the serration dependent on the chamber size and injection rate. To keep the concentration more stable, the injection should be greater than, but close to, the rate of plant assimilation.

Other systems depend on matching the injection and assimilation rates. Typically, a computer calculates the assimilation rate from the change in CO_2 concentration and the CO_2 injection rate. It then sends a signal to an electronic gas flow controller to reset the injection rate at the new value. Thus, the CO_2 concentration is maintained at a desired level, and the net photosynthetic rate is measured by the gas flow controller. This system has the advantage of allowing more than one closed chamber to share the same CO_2 analyzer because CO_2 injection is continuous and altered periodically as needed.

Another option is to take advantage of the CO_2 compensation concept, i.e., that the rate of CO_2 assimilation is dependent on the CO_2 concentration. Thus, if the rate of CO_2 injection into a closed chamber is less than the plants' photosynthetic capacity, the CO_2 concentration will adjust to the level that balances the injection rate with the photosynthetic rate. This technique can be used to manage the net photosynthetic rate of plants from zero to the CO_2 saturation level. It also allows the investigation of environmental effects (i.e., temperature, air turbulence, PAR, etc.) on gas exchange and photosynthesis by monitoring the change in CO_2 concentration as those conditions are manipulated. The easiest way to control CO_2 injection is by using an electronic gas flow controller, but it may also be controlled with a precision valve or a pressure regulator and a length of capillary tubing.

When the lights are off or the plants are damaged, more CO_2 is respired than is assimilated and in a closed chamber, CO_2 concentrations increase. A benefit can be obtained from this condition by stopping CO_2 injection and measuring the rate of CO_2 evolution (respiration).

It is sometimes argued that, to mimic ambient conditions, excess CO_2 should be removed during the dark cycle. Because high CO_2 concentrations cause stomatal closure, a physiological perturbation may be imposed. In the dark, however, the stomata are normally closed and this argument is moot. There is no compelling evidence that high CO_2 concentration in the dark causes any important effects; thus, CO_2 generally is allowed to increase in the dark and natu-

rally decrease after the lights come on. Nevertheless, some investigators have removed CO_2 from their closed chambers during dark cycles. Carbon dioxide scrubbers typically depend on base absorption, such as with NaOH. Bubbling sufficient air through a solution is difficult except in small chambers, and none of the solution must be aspirated into the chamber air. Passing the air over a solid base also can reduce the amount of CO_2 in the air, but the base is generally hydrophobic and tends to create a gooey mass in the container.

Oxygen

Oxygen is a photosynthetic product; thus, the air concentration increases in proportion to the amount of CO_2 assimilated (molar equivalence). Because of the different ambient concentrations, a change in the relative O_2 concentration occurs about 300 times more slowly than a relative change in CO_2. To calculate this value on a daily basis, the amount of O_2 used during dark respiration must be subtracted. In long-term experiments, concentrations may build up to levels significantly above ambient. If this is thought to be an experimental problem, some removal procedure is needed. Possible techniques include moving part of the air past an oxygen-trapping material such as is used to condition carrier gasses in gas chromatography. In very large chambers, H_2 could be burned to conserve O_2, producing water and heat as the only products. In this case, some safety device is needed to ensure that H_2 is fed to the burner only when there is a flame. If not, explosive concentrations of hydrogen could accumulate. The Occupational Safety and Health Administration (OSHA) considers levels of O_2 greater than 25% in a closed chamber a safety hazard. Higher levels could become a fire hazard, especially when in contact with some of the plastics used for chamber construction or with dried plant tissues or debris.

Some closed chambers separate roots from shoots. In this application, oxygen for root respiration must also be supplied, generally by adding air or O_2 to a hydroponic system or by moving oxygenated air over soil or other rooting media.

Water

Without a removal system, water vapor from transpiring plants quickly saturates the environment of a closed chamber. When the air saturated, net transpiration ceases, and any surface colder than the air (typically chamber walls) serves as a condenser where water collects and causes problems inside the chamber. Commonly, transpired water is removed by providing a temperature-controlled condensing surface and moving the air past it. If the heat exchange is sufficient to reduce the air temperature to that of the condenser, humidity is controlled at the dew point of the condenser. This level usually is not achieved, however, and chamber humidity remains somewhat higher than dew point. Different kinds of heat exchangers have been used, usually consisting of finned metal tubes over which the air is blown. The surface area of the condenser, and the amount and speed of circulated air (dwell time) determine the efficiency.

The condensed water must be removed from the chamber or returned to some component of the system. If water is returned to the hydroponic system or used as an irrigant, the material of the condenser becomes important because toxic concentrations of copper, nickel, or cobalt may be released from the condenser surface and increase to toxic concentrations. If water is removed from the chamber, a simple "J" trap allows the water to leave without changing the chamber volume. Removal of water obviously modifies the concept of closure for water and water soluble gasses. Because CO_2 is water soluble, a correction to the photosynthetic rate

could be considered; normally, however, the loss is justifiably ignored because it accounts for an insignificant amount of CO_2 removal.

Contaminant Gasses

One potential problem associated with closure is the accumulation of volatile compounds in the chamber atmosphere. Most chamber materials out-gas solvents and plasticizers to some degree. Some plastic materials and sealants are particularly problematic; others, such as Teflon and "ultra-high molecular weight, high density, linear polyethylene" are much better. Plants also produce volatile compounds, which accumulate in the atmosphere. In extreme cases, volatile compounds produced by either of these sources can inhibit plant development or may even be lethal (see Chapter 5, Air Contaminants). Consequently, a chamber should be heated and aerated for several days before first use. This treatment helps cure the materials and can dramatically decrease the amounts of volatile compounds emitted from the chamber itself. Little can be done to control plant volatile emissions; however, with suitable oxidation hardware (Lang and Tibbitts, 1983), both plant- and chamber-derived volatile compounds can be removed.

Temperature Control

The greenhouse effect is evident in closed chambers, but is more problematic in some types than in others. Chambers made of thin films, such as those made of Teflon by Mc Farlane and Pfleeger (1987), are less affected by heat build-up because heat is transferred rapidly through the film. Chambers made of thicker materials may heat up in the light and require a cooling mechanism to manage temperature. If high-irradiance lighting is used as an outside radiation source, thermal wave bands of energy may be removed by a filter of several cm of water.

Air circulation in a closed chamber has the same importance as in any other plant environment; enclosure, however, imposes some restraints. If a fan or impeller is used, one must either mount the motor outside and drive the implement with a magnet or a drive shaft through the container wall or locate the motor within the container. An outside mounting presents the problem of sealing around the shaft, a difficult task because the action of a fan creates positive or negative pressure (relative to outside the chamber) at the shaft. If the motor is mounted within the chamber, an electrical arc at the brushes may cause ozone formation, and the seals may release traces of oils into the air. Explosion-proof motors prevent the ozone problem, and traces of lubricants typically are nontoxic.

Testing Closure

Degree of closure may be tested in several ways with equipment at hand in a plant growth laboratory. A CO_2 analyzer can be used to determine the presence and location of leaks. Elevate the chamber CO_2 concentration (relative to ambient) and observe the rate of change or fill the chamber with air devoid of CO_2 and observe any increase. If leaks occur, add CO_2 to the chamber until the concentration is very high (far off-scale for the analyzer), and use an air sampling wand to draw air into the

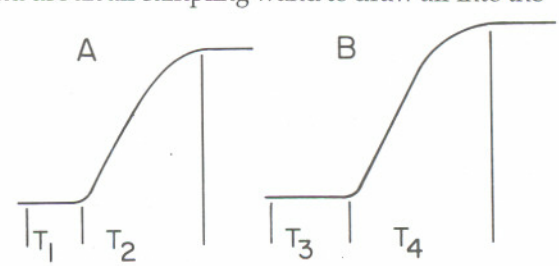

Figure 1. Chamber mixing rate. (A) The CO_2 concentration changed in response to instantaneously changing the air source at the chamber sampling inlet. The time (T_1 = 4.2 s) represents the period required to purge the sample plumbing, manifolds, and pump. The time (T_2 = 9.0 s) represents the IRGA purging time and delay in the electronic response.

(B) The CO_2 concentration changed in response to injecting 50 cm^3 of CO_2 into a stable (empty) plant exposure chamber. The time ($T_3 - T_1$ = 1.5 s) represents the period from injection until some of the injected gas reached the IRGA. The time $(T_3 + T_4) - (T_1 + T_2)$ represents the chamber mixing time.

analyzer. Moving the wand around the seals will indicate where CO_2 is leaking.

The example in Fig.1 shows a method of determining the chamber mixing time by using the same equipment. This was done by measuring the time required for a chamber to reach a stable CO_2 concentration after an instantaneous injection of CO_2. The air-sampling system consisted of tubes, valves, a pump, and the volume of the infrared gas analyzer (IRGA), all of which must be considered in determining the chamber mixing rate. In this case, by using a medium impeller speed (500 rpm), the time required to completely mix an introduced gas in the chamber was about 2 seconds. This rate is rapid compared with any changes expected from treatments; thus, ideal mixing was assumed in determining all gaseous rate constants.

Materials

Some closed chambers are constructed of transparent acrylic (Plexiglass) or polycarbonate (Lexan) plastics. Because these plastics absorb water vapor, and consequently CO_2, it may be advisable to coat the internal surfaces with transparent Teflon tape. If the radiant energy source is external to the chamber, construction materials may modify the spectral quality of the incident radiation. Although the materials appear transparent, they will attenuate all wavelengths to some degree, and some materials absorb or reflect long wave and ultraviolet radiation in a peculiar manner. UV-transmitting acrylics are available.

Glass is one of the best construction materials because it is nonreactive and impermeable to most gasses and transmits the wavelengths of PAR almost equally. Working with glass is difficult, however, and typically requires the use of adhesives in the construction. Most adhesives release solvents and plasticizers that may affect the experiment. Glass construction may also require a metal structure to support the weight of the glass. That may limit the shapes and sizes possible.

Thin plastic films make good chambers because they allow heat transfer and offer numerous size and shape advantages (i.e., blow-up chambers). Most films can be heat-sealed into various shapes or stretched over a frame. They suffer from being permeable to various gasses, however. Specifications suggest that Teflon is one of the least reactive with various gasses and that polyvinilidene is one of the least permeable to numerous gasses. Although these materials allow the loss of CO2 and H_2O from the chamber, rates of loss are low enough to be insignificant in most photosynthetic and transpiration rate calculations.

Steel and stainless steel chambers are sturdy but are not transparent, thus requiring the lights to be inside or mounted behind a glass plate. Paints and solvents have the same problems listed for sealants, and welding fluxes also may pollute chamber air.

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Air Ion Chambers

Air ions can be defined as atmospheric particles that have become electrically charged by the gain or loss of an electron. These charged particles have been reported to be capable of affecting a variety of physiological processes in plants (Kotaka, 1978), although these responses have not been well defined. Investigating the effects of air ions on plant development requires rigorous control of the physical environment because factors such as humidity and air quality directly affect air ion concentration and distribution. In addition, minimizing environmental variability is necessary to ensure that observed effects are indeed related to differences in air ion concentrations. Conventional growth chambers can provide adequate control of environmental factors such as light, temperature, and humidity, but modifications are required to maintain proper conditions for the generation and uniform distribution of biologically active air ions. These modifications involve changes to the air handling system and the addition of ion generation and electrical shielding equipment.

Discussion

To maintain the proper conditions for ion generation while providing the environmental and cultural needs of plants, we recommend a "chamber within a chamber" design. Because few of these have been built, the unique aspects of an air ion exposure system can best be illustrated by referring to a system designed and constructed for moderate-sized plants (Morrow and Tibbitts, 1987). The main component is a clear Plexiglass chamber, open at both ends (Fig.2). A woven fiberglass air filter at the intake end of the chamber removes water droplets and

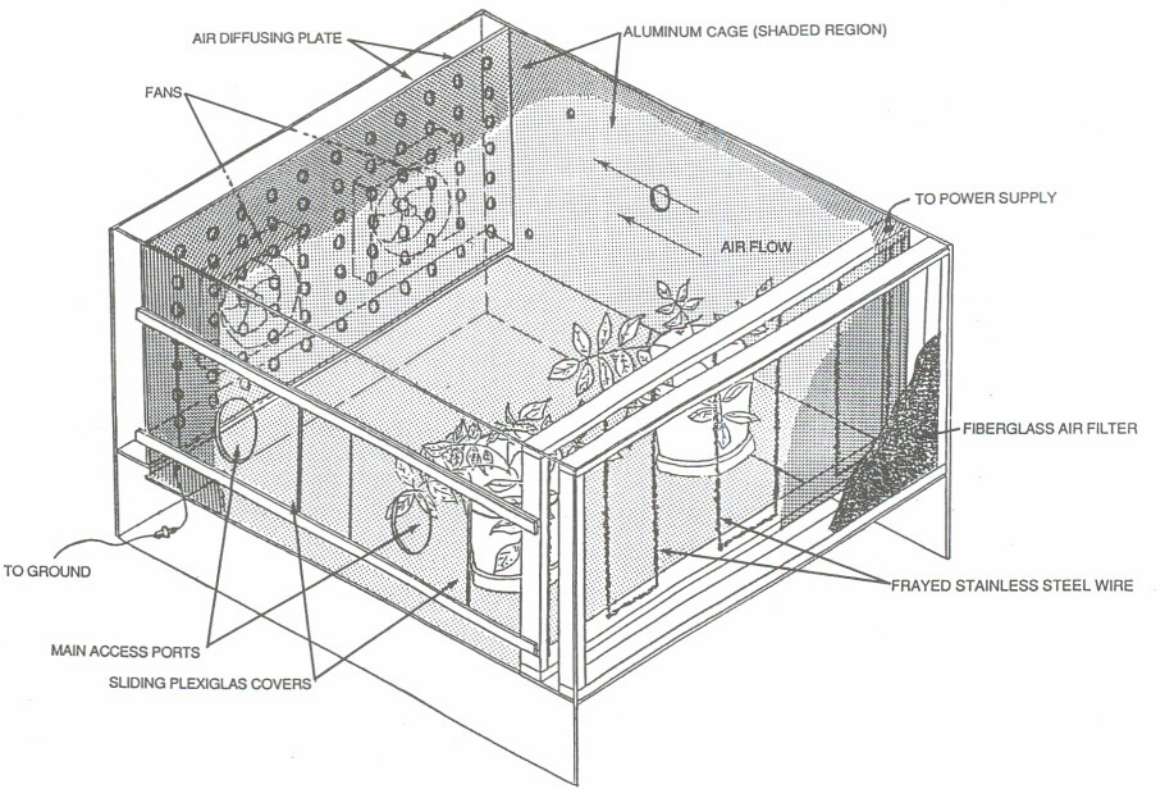

Figure 2. Air ion "chamber within a chamber" designed and constructed for moderate-sized plants (Morrow and Tibbitts, 1987).

particles. Fans and an air diffuser at the exhaust end create a uniform air flow across the chamber. The floor is slanted, with a modified elbow joint at the low end to allow water drainage while preventing unfiltered air from entering. Additional ports through the side walls provide access for drip tubes for plant watering and the insertion of instruments. The chamber is lined with a grounded metal cage to shield the system from outside electrical interference, thus maintaining a uniform electrical environment required for good ion distribution. Wires are also attached to electrically ground each test plant, otherwise they become charged and repel additional ions during exposure. An ion generator, consisting of frayed steel wires through which a high voltage is passed, is located near the intake end. During operation, conditioned air from the parent chamber is drawn through the particulate filter, past the ion generator, and is exhausted from the chamber after passing the plants.

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Wind Tunnels

Air in motion can be considered a transport mechanism. The molecules making up "normal" air, as well as the polluting gasses or particulates and condensed or crystallized moisture, are carried in the stream of air. The impact of that combination of molecules and particles upon plants may be altered by the velocity of the air. A wind tunnel, therefore, may be a valuable tool for research on pollution problems, heat transfer, stem strength or other wind damage, pollen transfer, insect attraction, and bacteria or particulate dispersion.

Air circulation in growth chambers is necessary to transfer heat from the chamber to the cooling coils. Normally, this is a "light breeze" sufficient to provide some turbulence around the leaves, but it is seldom controllable or uniform in direction or magnitude. For simple studies, it may be possible to arrange an enclosure within the growth chamber (or outside it if connected with flexible ducts to a temporary door) through which the conditioned air of the chamber is forced by a centrifugal or propeller fan. More demanding situations usually require a wind tunnel designed to meet specific needs of size, wind velocity, flow pattern control, safety, filtration, noise reduction, etc.

Care must be taken in designing research projects using a wind tunnel because different effects may result from turbulent versus laminar flow. Consider also the effect that a dense canopy will have on the flow within a confined test section, as well as the vastly different conditions that will exist on the windward and the leeward side of the canopy. Also, plants alter the environmental conditions, and this must be considered in the design of the systems.

Wind tunnel design is beyond the scope of this manual. However, if a project is to be done in an existing wind tunnel, all the conditions described in the other chapters must be consid-

ered because such a device likely will be temperature controlled but not have control of radiant energy, humidity, or CO_2 concentration.

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Air Pollution

Many air pollution studies on plants are best done in controlled environments. However, introduced reactive gasses may corrode the chamber materials and eventually make the chamber useless. Chamber surfaces also may absorb the pollutants, removing them from the atmosphere and making it impossible to obtain the desired exposure conditions. Several solutions are possible: **closed chambers** made of inert materials (discussed earlier); **single air-pass chambers** designed to allow conditioned, polluted air to pass the plant area only once before being exhausted; and **semi-closed chambers** in which polluted air is added, mixed, recirculated, and a portion continually exhausted.

Single air-pass chambers are expensive to build and operate because they require large volumes of clean, temperature- and humidity-conditioned air for mixing with the desired pollutant. To ensure precise environmental control, incoming air should be treated to remove ambient pollutants before the test compounds are introduced. Filtration typically consists of the following sequence: dust filters (furnace-type), high efficiency particulate filters to retain particles above 5 microns, and activated charcoal filters to remove reactive gasses. Additionally, air may be drawn initially through a water scrubber to cool and humidify it and reduce the pollutant content. Because the air makes only one pass, this equipment is often large and expensive; however, it produces conditions that assure experimental exposure as desired and planned. Care must be taken to safely exhaust the polluted air from the chamber, or the air must be cleaned by removing pollutants before discharging.

Control of incoming air temperature and humidity is often too expensive; therefore, ambient conditions are accepted. The open-top field chambers currently being used in many air pollution studies are operated in this way.

Semi-closed chambers also are useful in air pollution studies. If they meet certain criteria for mixing, they can be used as continuously stirred tank reactors (CSTR), and reaction rates can be determined by measuring the difference in concentration of reactive gasses entering and leaving the chamber. Clean air is important in these studies, but because much less volume is used in these chambers than in the single air-pass chambers, the cleaning and pollutant mixing systems can be much less expensive. Chamber air must be mixed thoroughly and rapidly so the exhausted air is an accurate representation of the exposure concentration. Typically, this is accomplished with a flat-blade impeller, which creates turbulent flow, rather than with a propeller that creates a convective vortex. These chambers allow the calculation of pollutant/plant reaction kinetics as well as determinations of net CO_2 assimilation and transpiration rates.

Chamber materials that don't react with the test compounds should be chosen. This is less important than for closed chambers, however, because the reactive gasses are continually being introduced and exhausted.

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Pesticide Chamber

Work with pesticides can be successfully done in various types of chambers. As with all research, however, the physical and chemical properties of the test chemicals must be taken into account when they are used in controlled environments. Many pesticides and their metabolites are sufficiently volatile to be lost to the growth chamber atmosphere in significant quantities from treated soil, nutrient solution, or plant surfaces within a few hours or days. Once released, these compounds will recirculate within the chamber or be carried into adjoining spaces with the exhaust air stream. They may adsorb on initially untreated plant surfaces, on plant containers, or on chamber components, with subsequent release to the experimental environment. Thus, the pesticide treatment may differ from that intended; the results may be ambiguous; the apparatus and neighboring experiments may be contaminated; and hazardous materials may be released into the work environment. Therefore, provision should be made for scrubbing the air entering the growth chambers and venting, selectively filtering, or scrubbing the exhaust gasses. If a common venting system is used for several chambers, care should be taken to ensure that exhaust gasses from one cannot backflow into another.

Many pesticide studies are done with radiolabeled chemicals. This adds the dimension of radiation safety to the study, and additional attention must be paid to preventing contamination of materials and exposure to personnel. As a benefit, radioactive tracers simplify the task of finding contamination and may be used to trace pesticides that are fugitive from controlled studies.

Investigators should familiarize themselves thoroughly with the physical and toxicological properties of any pesticides used in experimentation, especially in enclosed spaces. The following manuals are among the most comprehensive available.

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