
STANDARDIZATION OF CONTROLLED ENVIRONMENT RESEARCH

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The development of controlled environments in the early 1950’s with sufficient radiation intensity to obtain vigorous plant growth initiated a rapid explosion of environmental research. It was an explosion that provided a decade or a decade and one-half of real excitement in plant physiology. Many light, temperature and carbon dioxide interactions were unraveled, as it was possible to vary one factor and hold all other factors of the environment constant. The controlled environment was a must for plant physiologists if their work was to have real validity.

But the excitement has waned. Environmental research no longer enjoys this prestigious position. The National Science Foundation for the past five to ten years has funded very little environmental research with economic plants in controlled environments. For many researchers, the use of controlled environments has led to nothing but frustration. A comment by a well known physiologist at the recent Plant Physiology meetings in Madison emphasizes this frustration: “Growth chambers are a poor place to grow plants but the best place to grow plants under controlled environments.” This is characteristic of the feeling of many who work or have worked with controlled environments. The controlled environment of the 1950’s and 1960’s is slowly and painfully being recognized for what it really is: not a totally controlled environment but only a partially controlled environment in which some environmental parameters can be controlled but many other parameters are not being controlled. Certain parameters are under less control in a growth chamber than in the outside environment.

Thus, I would like first to emphasize, and maybe to over-emphasize, the problems in a controlled environment, and then discuss procedures to minimize and overcome certain major problems.

Light

The first and most significant aspect of controlled environment is the light – of an intensity that can duplicate the amount plants require in an outside environment. This the controlled environment does quite well but there are many problems in insuring that each plant receives the same amount of light. Actually, each square foot is usually at a different radiation level, with the maximum at the center and decreasing toward every side, with plants along the walls receiving 20% or less of the maximum. The problem is compounded when two different researchers agree to share a chamber with different specie of plants that are of different heights or when plants are planted on different dates so plants side by side are of different sizes. It’s particularly difficult to get uniform lighting when more than one type of lamp has to be used in the chamber or room. In most chambers there are hot spots under each incandescent lamp and the closer one’s plants have to be to the lamps, the greater the heating and the greater the variation between plants directly and not directly under the lamps.

Temperature is the other significant factor that is under control, but it is often controlled in the wall behind the plants, or sometimes on the wall of the cabinet. Unless one buys accurate sensing devices to monitor the air beside the plants, one usually cannot know the temperature of the air to which the plants are subjected. Even careful temperature setting can go amiss if the researcher squeezes too many plants in the chambers or places a tray under the plants.

Next, some comments on humidity. Many growth chambers are suitable for cacti, not tomatoes and lettuce. The refrigeration coils are the culprit. Each time the chamber cools (usually every 1–3 minutes), the cold coils take water out of the air of the chamber. Chambers without humidification operate at about 50% relative humidity ranging lower in the winter than in the summer. In addition, the air circulation system provides a steady wind over the plants creating essentially a xeric environment.

Humidification procedures to minimize the water stress in chambers are only partially successful. The controllers usually have hair operated or plastic strip humidistats, and these humidistats are not stable. Their calibration changes with wetting and drying cycles. They need to be checked daily to insure uniformity.

Use of plastic pots is a necessity to avoid excessive soil drying and excessive evaporative cooling from the pots. In order to keep enough water on the plants and to keep watering supply uniform, automated watering systems should be used. Try watering a chamber or room filled with pots by hand and bring each pot back to the same soil water content. It just doesn’t work.

Both plants and men cause variations in carbon dioxide. This is much greater in controlled environments than in the outside environment. In closed rooms, plants can exhaust or nearly exhaust CO₂ within an hour if rooms are sealed up. That is until the researcher walks in. After two breaths are taken in a chamber, CO₂ level is tripled or quadrupled. Carbon dioxide variations cannot be avoided if the level of CO₂ in the laboratory fluctuates with human activity. Chambers have significant leakage rates and exchange air with the surrounding room rapidly. Thus, fluctuations of CO₂ will be great if chambers are located in laboratories with a lot of research activity. CO₂ fluctuations may also be very significant even though the growing area is separated from laboratories, if the air supply for the chamber area is recirculated through a building in which people are working and breathing.

Sources of Contaminants

There are many contaminants in growth chambers, from people and from other sources. A smoking person contributes not only CO₂ but sulfur dioxide and ethylene. Chambers in closed areas can have problems from many materials used in chemical laboratories, or from paints and plastic emissions. One of the most troublesome problems is a broken mercury thermometer. This can contaminate a chamber
and will make it perform very differently from a similar chamber beside it. And how does one know there is mercury down in the cracks?

Because of this, a group of us that is concentrating research efforts on environmental physiology in controlled environments began working together in 1969, as a Growth Chamber Committee to develop ways to improve precision and repeatability of research in growth chambers.

The remainder of this paper will be devoted to discussion of those procedures that we have found to be most important in insuring repeatability within laboratories and among different laboratories.

The largest, most significant variable in growth chamber research is often the control of watering. Transpiration from plants and evaporation from pots and soils is not uniform in different areas of a growth chamber. It is also more rapid in growth chambers than in greenhouses. The continuous air movement in chambers, required for temperature control, accentuates water loss from plants and containers. We have found that adequate water in uniform applications can only be provided consistently through use of automatic watering and then only if pots are watered to excess. However, this eliminates the use of many field soils, for clay and loam soils cannot sustain good plant growth when watered to excess each day or several times each day. Therefore, the use of sand, peat, vermiculite, baked clay materials or mixtures of these very porous materials has been found to be essential.

W atering can be either with nutrient solution alone or with alternating applications of nutrient solution and distilled water. If only nutrient solution alone is utilized, a concentration of about half Hoagland’s is desirable, but it must be applied to excess to avoid concentration of salts in the medium. The amount of excess controls the degree of concentration. For example if half of the applied nutrient leaches through the containers, the salt concentration of the media can increase by a factor of 2. If only 1/10 of the applied nutrient leaches through the containers, the salt concentration of the medium can increase by a factor of 10 and will likely cause growth restrictions. On the other hand, if applications of nutrient are alternated with applications of distilled water, less liquid needs to be leached through the containers, but one should recognize that the plants are being subjected to fluctuating concentrations of nutrients in the root medium which may affect the physiological response of the plants.

Another significant problem in growth chambers is the gradient in conditions across the growing area: variations in light, temperature and air movement. This problem can be minimized by reducing the number of plants in each experiment so that they can be located in the center area of the chamber under similar conditions. It is better to have a small number of single plant replicates under similar light intensities than to double the replicates and have to place some plants close to the wall where light levels may be as much as 20% less.

Despite the desirability of utilizing only the center area of the chambers, we may have to undertake certain experiments that utilize most of the growing area of the chamber. If so, regular rotation of the plants in the room on a daily basis, or every 2–3 days, is a necessity to avoid large differences in growth of different plants.

We would also plead for single plants in each container so that each plant can be maintained as an individual with no overlapping of leaves with other plants in the chamber. Many researchers argue that they need more plants in each pot to increase the total number of plants so that effects of seed variability can be minimized. However, the variation caused by different seeds is usually much less than the variation caused by competition between adjacent plants in the same container. The other argument for maintaining several plants in a single container is that it is necessary to duplicate the planting distance in commercial production. However, growth in controlled environments does not duplicate field conditions and the disadvantages of close spacing far outweigh the advantages in growth chamber experiments.

Standardize measurement

Standardizing environmental measurement is a necessity. Environmental instrument standardization becomes quite complex. It involves the type of instruments to use, the location of the instruments and insurance that the instruments are calibrated accurately. The difficulty in meeting these different requirements of standardization increases in proportion to the order that I have presented them.

Agreement upon the types of instruments that provide accurate and comparable measurements has been encouraged by physiologists for many years. The availability in recent years of accurate instruments at much lower cost now makes development of standard instruments for use.

There are available high quality radiation sensors manufactured particularly for measuring light for plant growth. These are quantum meters that are filtered to insure sensitivity for only the quanta or photons of light that plants use for photosynthesis. Temperature probes are available as resistance elements or mixtures of thermocouples that can be easily positioned near plants, in soil, or attached to leaves to provide precise measurements. Wet and dry bulb psychrometers are available for humidity measurement in growth chambers, these can be remotely operated to provide measurements without opening the doors.

Hopefully, the efforts of an International Committee formed by Dr. Kramer at a Phytoconferences Conference, 4 years ago, or by a NCR 101 USDA Committee on Growth Chamber Use, will be effective in obtaining agreement on an outline of recommended instruments for use.

There is greater difficulty in standardizing positioning of sensors in the chamber at the start and during the course of the study than standardizing the type of instrument to use.

It is generally assumed at seeding that measurements should be just over the containers and as the plants develop that measurements should be just over the plant canopy. However at both the germination stage and later development stages, similar temperatures do not result in the same plant growth in different types of chambers. Direction of air flow is one of the significant variables. For example, in chambers where the air is moving upwards through the chamber air temperature will be cooler than in chambers where the air flow is down through the chamber even though similar air temperatures are monitored at the top of the containers. Vertical light gradients in chambers also vary significantly. With developing plants in chambers, the light gradient from the top of the plant to the base of the plant will be much greater in small room chambers than in walk-in chambers or rooms. Thus, equal light measurements at the top of the plant canopy do not represent equal illuminance or irradiance upon plants in different chambers. There is a significant need to develop protocol for standardizing the measurement position and it may have to be outlined for each different plant species separately.

Insuring that instruments are satisfactorily calibrated is also a difficult problem. In transit of instruments from the manufacturer or when shipped at intervals to a calibration laboratory, there is always a question of possible damage in transit that alters the calibration. And certainly there is the constant concern that in use the instrument has been bumped or the sensitivity of the instrument has been altered. Our growth chamber committee suggests the following procedure to insure adequate calibration of instruments. First, there is a need to have one instrument that is certified each laboratory facility with one instrument utilized regularly and the other instrument maintained only as a calibration instrument. This second instrument would only be utilized at necessary intervals to check the calibration of the regularly used instrument or instruments.

Calibrate the calibrator

Then, combined with this regular checking, must be a calibration at yearly intervals of the calibration instrument against a recognized standard that is traceable to National Bureau of Standards. This last step is the most difficult hurdle to cross. However, members of the USDA Growth Chamber Committee have made some progress in this area. They are working within the USDA NCR 101 Committee on Growth Chamber Use to hopefully make available a package of calibrated radiation, temperature and humidity instruments plus a calibrated cylinder of CO₂ gas for distribution to research institutions to be used as a primary calibration source. This package should provide the calibrations that are sorely needed by growth chamber scientists.

A very significant factor in uniformity between laboratories is the presence of air contaminants in the atmosphere of the chambers. This problem can usually be avoided if a large quantity of air from the outside is provided both for fresh air into the controlled environment chambers and to the outside of which the chambers are isolated. Chambers located in laboratories and basement areas that are supplied with building air are often subject to problems apparently from gaseous emissions of construction materials in the building or volatile compounds used in offices or laboratories. Usually problems occur only with certain species and certain cultivars of these species. Exper-
Growth chambers can be adapted in many ways to meet the special needs of plant stress studies. The purpose of this paper is to discuss special growth chambers and selected stress experiments requiring special techniques and extensive modification of growth chamber design. Use of growth chambers in air pollution, pesticide, radioisotope, and ultraviolet radiation research will be presented as examples.

Air pollution research

Growth chamber design for air pollution research should allow modification of light, temperature, humidity, air velocity, nutrition, and soil moisture status - factors that may markedly affect plant responses to air pollutants. Design features should also reflect consideration of the damage that air pollutants can do to growth chamber components as well as to the plants. Controlled and well-defined environments are recommended for studying pollutant uptake rates, visible and hidden injury, mechanisms of injury development, and amelioration of pollutant effects (7, 10, 27, 35, 60, 62). The pollutants used must be monitored and controlled to permit exposure of the plant material to known concentrations of the pollutant for specified periods of time, i.e., varying doses.

Chambers specifically designed for air pollutant studies have been described (1, 12, 17, 36, 37, 55, 85). In each case, the principal components are a pollutant generator and control system, a filter system, and a controlled environment exposure chamber.

In a single pass-through system, the air pollutant is injected into the air stream being blown into a glass- or plastic-walled growth chamber. The pollutant should not come in contact with any reactive metal surface. The system must be completely sealed to avoid contamination of the working area. The pollutant is distributed evenly in the air stream by turbulent mixing in front of a fan, or by using a large mixing chamber before entrance into the treatment chamber. To ensure precise control of pollutants independent of outside or laboratory air, designs for single pass-through systems should incorporate filters to remove pollutants of all kinds from incoming air before the desired pollutants are introduced.

Menser and Heggestad (55) have described the essential features of an ozone exposure facility. The principal components and accessory equipment are a large-capacity ozonizer, an activated charcoal air filter, and an exposure chamber. Ozone is generated by regulating the flow of tank oxygen through an ozonizer operated at high voltage. The filtered air and ozone pass into the chamber. A high level of humidity, at least 70%, is generally required to induce rapid and consistent stomatal opening, so additional humidifying equipment may be required.

As an alternative to the use of remote air conditioning units, the plastic or glass chambers used in a single-pass through system may be placed within a conventional growth chamber with filtered air drawn from the chamber, pollutant added in the incoming duct, and pollutant-containing air exhausted through ductwork to the outside such as the system described by Heck et al. (36). The alternative to the single-pass through system is a recirculating system. This type is particularly useful for experiments on the effects of pollutants on rates of carbon dioxide exchange, transpiration, and pollutant uptake. Such chambers have been described by Hill (38) and Taylor et al. (73).

Undesirable gases such as ethylene, terpenes and other volatiles may accumulate in the chamber atmosphere (22, 74, 75). Carbon dioxide may also be depleted and thus CO₂ control will be required (8, 25, 26, 43, 46). A system to control the entry and composition of make up air at a predetermined rate should also be incorporated in the design.

Commercial growth chambers of conventional design may be used for a recirculating system but many modifications will be required. Such a chamber system was developed by Wood et al. (85). One requirement is that leaks around doors be sealed in preparation for exposure. Leaks were sealed from the exterior with rubberized ducting tape and rubber-based caulking compound. An air exchange system, access ports, and a system for introducing pollutants were installed. The air exchange system consisted of an air inlet and an outlet, each of which was fitted with an activated charcoal filter. During the operation, air moves through a charcoal filter and through the inlet at the base of the chamber, laterally through refrigeration coils into the airways in the end walls, out of vents in the top of the chamber, and into the airway in the end wall near the base.

Literature Cited


PLANT STRESS STUDIES IN CONTROLLED ENVIRONMENTS

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HortScience, Vol. 13(4), August 1978