CONTROLLED ENVIRONMENT RESEARCH
GUIDELINES FOR RESEARCHERS AND REVIEWERS

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Controlled environment systems (CES), which include both growth chambers and greenhouses, offer great potential to speed scientific advances by providing well-defined environments in which plants grow. If not correctly managed and maintained, however, it is neither possible to interpret results of research conducted in CES nor to effectively duplicate the research. Researchers need to understand both the capabilities and limitations of CES.

The following is intended to provide recommendations to researchers and *Crop Science* reviewers for evaluating data generated from CES experiments. Reviewers should keep in mind that these are guidelines, not rigid rules. You must also consider the objectives of the experiment and intended use of the results as you apply these guidelines.

Experimental Design Issues

Limitations of CES research

It is important to recognize that container or pot size can restrict root growth and function. If possible, containers should provide a similar soil volume as would be available to plants under field conditions. If smaller containers must be used, then observations and conclusions drawn from observations should be restricted to seedlings or immature plants that are not yet affected by root growth restrictions. Furthermore, container-grown plants often differ from field-grown plants both in size and morphology. Researchers should avoid the temptation to extrapolate results from CES to compare them with performance of field-grown plants.

Repeating experiments and replicating treatments

The term 'replication' indicates that multiple experimental units received the same treatment. Replication makes it possible to estimate the experimental error directly using the deviation between the treatment mean and the observed value for each experimental unit. An experimental unit is the portion of experimental material to which a treatment is randomly applied (Steel and Torrie, 1980; Gomez and Gomez, 1984). This simple definition is often overlooked or misunderstood, but statistical analyses are predicated upon an accurate identification of the experimental unit.

Identification of the experimental unit in CES research can be particularly confusing. If the treatments do not involve varying the settings of the chamber, then an entire experiment may be contained within one chamber. Repeating this sort of experiment (analogous to multiple years for a field study) may be advisable, but it is not required in order to estimate an experimental error. On the other hand, if the settings of the chamber are varied to establish an environmental treatment, then the chamber is the experimental unit and additional chambers at the same setting, or a design such as a Latin Square is required to provide an estimate of experimental error. In this situation, the plants within chambers are subsamples rather than replicates.

Lee and Rawlings (1982) showed that the basic principles of experimental design could not be violated in CES research without losing the ability to accurately estimate experimental error. Poor design resulted in poor estimates of error. This may result in the declaration of a significant difference when there is none, and, of course, the estimates of probability and variance associated with the estimated differences are erroneous.

When subsamples are incorrectly represented as replications, an unknown level of bias is introduced in the estimation of treatment effects and the experimental error is underestimated. Even in a uniform environment, grouping of the experimental material for a given treatment and handling the group as a unit introduce positive correlations within the group, and the net result is that the experimental error is underestimated if subsamples are mistakenly identified as replications (Lee and Rawlings, 1982).

Proper monitoring and reporting of both experimental and environmental parameters used during the experiment is required. A brief discussion of the importance of understanding the limitations as well as proper monitoring of various environmental parameters in CES is presented below.

Quality assurance

Some federal agencies require research to prepare and follow a formal quality assurance (QA) plan before they will fund a project. Quality assurance plans document the experimental procedures, precision of apparatus used to make observations, frequency and methods for calibration, and handling of samples and data. Some controlled environment facilities have developed QA plans to guide their operations. If either the facility or project has developed a formal QA plan, then researchers should reference this plan as part of their methods. If not, they should report pertinent information regarding their monitoring and control procedures as described below.

Monitoring and Control Systems

Researchers often assume that the data displayed by CES sensors and monitors are correct. Improper calibration, installation, or other problems may result in significant discrepancies between the desired conditions indicated by the control sensors in the CES and the actual conditions inside the CES. Therefore, it is important to have separate monitoring instrumentation to ensure that the CES operates as intended.

Temperature

Considerable differences may exist between air temperature and plant temperature in a CES. This is particularly true rue under high radiation loads. Furthermore, older on-off systems can result in as much as 5 °C variation on either side of the set point temperature. Also, depending on airflow rates and other factors, there is usually a vertical temperature gradient in most CES.

Suggested measurement and reporting

Using an independent monitoring device, air temperature should be measured at the top of the plant canopy and various locations above and around the plant growing area. These temperatures should be compared to the control settings of the CES. Measurements should be taken daily during each light and dark period and at least 1 hour after light change. Temperature averages and standard deviations should be reported. The type of monitoring instrument used should be described and data reported in degrees Celsius.

Light

The output of all electric radiation sources decreases with hours of operation. A typical, very high output 1500 mA fluorescent lamp emits 70 percent or less of its original irradiance after 6000 hours (approximately 1 year at 16 hours per day). As much as 20 percent reduction in light emission can occur after 4000 hours of operation. In growth chambers, the horizontal pattern of radiation distribution varies significantly across the chamber and at different heights. Vertical radiation gradients occur in all growth chambers. The extent of the gradient depends on the size of the chamber, lamp type, lamp distribution, and shape of the luminaire.

Artificial light spectra do not generally match that of the sun. An unnatural ratio of red to far red light may affect morphogenesis in some plants. Researchers should consider possible photomorphogenic effects when interpreting their results.

Suggested measurement and reporting

Growth chambers. Irradiance should be measured at the beginning and end of all experiments or monthly for experiments longer than 30 days. Measurements should be made no sooner than 1 hour after beginning the photoperiod and at various positions at the top of the plant canopy. Averages of readings should be reported. The measuring instrument and sensor should be described. In addition, a complete description of the light source (e.g.

fluorescent and incandescent, high-pressure sodium, metal halide, etc.) should be provided. Radiation should be measured in the 400 to 700 nm waveband and reported as photosynthetically active radiation (PAR) as photosynthetic photon flux density (PPFD).

Greenhouses. Radiation should be measured periodically throughout the course of the experiment. Preferably, measurements should be taken continuously or integrated daily to account for the variable shading that occurs during a day. If supplemental lighting is used, the same procedures described for growth chambers should be used.

Humidity

Humidity affects plants in growth chambers both directly and indirectly. The influence of humidity on transpiration and other gas exchange is a direct effect, while modifying the plants energy balance and physical and biological environments would be considered an indirect effect. A single heating and cooling cycle of 1 to 3 minutes can change relative humidity by 10 to 20 percent. Relative humidity is the most difficult environmental parameter to monitor. Nonetheless, humidity is especially important and must be measured for research areas such as plant water relations, including plant transpiration, and foliar pathogens.

Suggested measurement and reporting

Growth Chambers and Greenhouses. For studies in which humidity may have significant effects, continuous measurement is recommended. In other studies, measurements should be made during each segment of the daily temperature and light regime at the beginning and end of each experiment. Measurements should be made at top of plant canopy near the center of the plant growing area. Averages and standard deviations of daily readings for both light and dark period plus standard deviation should be reported. The measuring instrument and sensor should be described. Relative humidity may be reported as a percent.

Carbon Dioxide

Carbon dioxide is probably the least controlled factor in growth chambers. And unfortunately, too little or too much carbon dioxide is hard to detect until the plant starts showing specific symptoms. Small variations in carbon dioxide can affect plant growth and development significantly. People working in or around growth chambers and even greenhouses many increase carbon dioxide concentrations, as may vehicles, some heating systems, and other sources of carbon dioxide generation. Few growth chambers manufactured today have carbon dioxide control or monitoring equipment installed as a standard feature. However, most chambers do have some degree of venting or air exchange. Good ventilation in the chamber helps to moderate extreme carbon dioxide buildup or depletion. Even if a growth chamber is well ventilated, it is important to remember that the surrounding area in which it is located should also be well ventilated.

Suggested measurement and reporting

For studies that do not include a carbon dioxide variable, the air exchange rate and location of the growth chamber should be reported. For experiments including a carbon dioxide variable, continuous measurement taken at the top of the plant canopy is desired. Minimum monitoring would include hourly carbon dioxide measurements throughout the study. Averages and standard deviations for daily readings of light and dark periods should be reported. Measuring instrument and sensor should be described.

References:

Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley, New York.

Lee, C., and J.O. Rawlings. 1982. Design of experiments in growth chambers - Uniformity trials in the North Carolina State University Phytotron. Crop Sci. 22:551-558.

Steel, R.G.D, and J.H. Torrie. 1980. Principals and Procedures of Statistics (2nd Ed.). McGraw-Hill, New York.