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FREQUENTLY ASKED QUESTIONS

with Dr. Patrick Friesen

FAQ QUESTION #11

What should I consider when designing experiments using growth chambers or rooms?

Consistent growth and development is a priority for many growth chamber users, regardless of your plant growth or insect rearing goals. Growth chambers and rooms generally provide more consistent plant growth and development compared to greenhouses or the field, and this growth consistency is a strength for conducting experiments. Sources of environmental variability do exist inside growth chambers, and it is prudent to be aware of these when designing experiments (Hammer & Hopper 1997, Poorter et al 2012). Many of the same experimental design principles used by ecologists and agronomists in the field and greenhouses can apply to experiments in growth chambers (Cottenie & De Meester 2003, Hurlbert 1984, Lee & Rawlings 1982, Oksanen 2001, Potvin & Tardif 1988). Think about how environmental variability inside growth chambers may affect your response (dependent) variable(s) in the context of experimental design. Most, if not all environmental conditions can affect response variables related growth and development (e.g. total dry biomass after 30 days of growth). On the other hand, only one or two environmental conditions may marginally affect your response variable if it relates to flower petal color. Understanding how environmental variability within growth chambers may affect your response variable(s) can guide how you block or rotate plants or insect colonies. Reconciling the environmental variability of your growth chamber with the inherent biological variation in your response variable(s) helps determine sample sizes and how much to replicate treatments. Here I will discuss the kinds of experiments performed in growth chambers, how to replicate through space and/or time, common sources of environmental and biological variability, and how to put it all together to match statistical and biological significance.

A) Categories of growth chamber experiments and how to replicate through space and/or time

Investigators perform three general categories of experiments in growth chambers (Lee & Rawlings 1982, Potvin & Tardif 1988). In category I or within-chamber experiments you apply an exogenous treatment (e.g. reduced nitrogen fertilizer) to independent replicates or experimental units (plants or groups of plants) within a given chamber. For category I experiments, ensure that your experimental units adequately cover the environmental variability of your growth area(s) and that both control and treatment plants are interspersed within the same chamber. Adequately covering the environmental variability of your growth area(s) with both treatment and control plants gives independence to your experimental units and allows you to calculate experimental variance and error in category I experiments (Hammer & Hopper 1997, Hurlbert 1984, Lee & Rawlings 1982). After one round of category I experimentation however, the conclusions you draw are limited to that chamber, at that time, in that place. Repeating the experiment across multiple chambers and/or over time will capture more variability and allow your conclusions to be more robust and universal (Lee & Rawlings 1982, Potvin & Tardif 1988). Category II experiments are those in which the growth chamber's environmental conditions are the treatment itself. In category II experiments, the treatment condition affects all plants; plants or groups of plants are samples and not independent replicates or experimental units. Here measurements between plants or groups of plants reveals sampling error, not experimental error. To determine experimental error it is imperative to replicate the environmental treatment over time with new cohorts of plants, or over space with other growth chambers. Category III experiments are a combination of category I and category II where you apply different treatments within the chamber in conjunction with an environmental treatment from the chamber itself (Potvin & Tardif 1988).

Whether you replicate through space or time depends on the conclusions you wish to draw, your sources of variability, and of course the number of growth chambers available to you. As in category I experiments, pairing control and treatment groups together within a given chamber whenever possible will allow you to assess variation across space (multiple chambers) and time (new cohorts) in category II and III experiments. Often in category II or III experiments where an environmental condition is the treatment itself, treatment and control conditions cannot be achieved simultaneously within a given chamber. In situations where simultaneous treatment and control environmental conditions are required, multiple chambers are necessary. With access to two chambers, the experiment can be replicated through time by swapping and alternating treatment and control conditions between the chambers. Replicating through space across two or more chambers allows you to assess chamber effects, some bias or effect one chamber has compared to another (Porter et al 2015, Potvin & Tardif 1988). Having access to two or more chambers of the same make and model (and lighting system),

installed in the same area of a given building, is generally the best scenario for isolating biological variation while reducing chamber effects. Having access to two or more chambers that differ in their make, model, lighting system, and/or location inside a facility, may introduce unavoidable environmental variation. If the treatment effect can still be resolved however, the response is more environmentally universal, which may be desirable for some research questions. If you have access to only one growth chamber, you will have to replicate through time. Replicating through time can be an effective solution, however more time is required to complete experiments compared to having multiple chambers. Here, instead of chamber effects you may introduce time effects, which are factors that can change over time. Changes in seed quality (germination rate and time to emergence), potting mix, or fertilizer solution may cause unwanted variability for your experiment. The environment within a single chamber could also change through time. For example, humidity differences of the air entering your facility due to seasonal changes, CO_2 concentration differences due to changes in building occupancy (e.g. holidays vs classes at a university), or light intensity decline from aging lights (Hartung et al 2019). Both chamber and time effects can be incorporated as random factors into your statistical model to test for significance of their respective effects (Potvin & Tardif 1988, Quinn & Keough 2002a). For more information on how to build models and select the one that best fits your data, please read Zuur et al., (2009).

In all categories of experiments, moving or re-arranging your potted plants daily or bi-weekly across the growth area is one approach to minimize the effects of environmental variability and reduce the variance (Hammer & Hopper 1997, Hartung et al 2019). Grouping plants together in clusters or blocks (blocking) over the growth area is another approach to detect if and how environmental variability affects your response variable(s) in both control and treated plants. Spatially blocking plants over the growth area is a good choice if you know or suspect beforehand that environmental variability may affect your response variable. Once blocked, a fixed blocking factor can be incorporated into your statistical models and used to justifiably remove outlying blocks from your analysis and conclusions (Quinn & Keough 2002b, Zuur et al 2009). For example, lower light intensity slows the growth of plants in the corner block of a given chamber, significantly reducing their growth (Hartung et al 2019, Potvin et al 1990).

Randomly assigning placement of sample or replicate plants within a chamber or allocating replicate plants to different chambers can often reduce experimenter bias (Hammer & Hopper 1997). Randomization becomes more effective at eliminating bias as the number of samples or replicates increases; with smaller numbers there is a chance they could still be randomly assigned in a biased fashion. To randomize placement of samples or replicates, one method is to sequentially number the locations over a growth area, or the number of chambers you have available. Samples or replicates (plants) are then randomly assigned to a growth area location or chamber using a random number source within the desired range. One approach is to draw out of a hat (Hammer & Hopper 1997). Another way is to use this resource that generates random numbers from atmospheric noise, which they argue is superior to random number algorithms: https://www.random.org/.

Details of experimental designs commonly used inside growth chambers such as completely randomized, Latin square, or Latin rectangle are beyond the scope of this article. For more information on these and other designs, and their advantges/drawbacks, please read Hammer & Hopper (1997) and Quinn & Keough (2002b).

B) Sources of inherent biological variability in response (dependent) variables

A common goal of plant biologists is to understand the inherent biological variation of their response variables in control and treated plants. Understanding the inherent biological variation of your response variables will help determine the sample sizes and replication required to have enough statistical power to reveal significant effects or differences from treatments if they exist. Often the biological variation for a measured response variable is tied to the genetic variability of the plant material you start with. For example, often the genetic variability of wild collected seed > certified seed of a given cultivar > clonally propagated material. Determining the level of biological variation in your response variable and whether it changes in control vs treated plants helps determine sample sizes; more biological variation requires greater sample sizes to detect treatment effects.

C) Common sources of environmental variability in growth chambers and rooms

The environmental variability within growth chambers is usually somewhat predictable, and re-arranging your plants or blocking often mitigates its effects on your experiment. If you suspect that variability of one or more environmental factors will affect your response variable(s), it is best practice to measure these environmental factors across your growth area before you start your experiment.

Light intensity (PPFD): Photosynthetic photon flux density (PPFD) is generally lower at the edges and corners compared to the center of the growth area, and this discrepancy is often the biggest source of environmental variability affecting plant growth and development inside growth chambers and rooms (Poorter et al 2012, Potvin et al 1990). PPFD changes dramatically with distance from the lights, and is often measured at the top of plants using a quantum sensor. For more information on how PPFD affects plant growth, please read Friesen (2021a).

Plant tissue (leaf) temperatures: Air movement, PPFD, and the radiative heat load from your light source work together to determine how much leaf and other plant tissue temperatures may deviate from the air temperature set point. Some air movement is critical to reduce the boundary air layer around plant tissues. A smaller boundary layer of air couples plant tissue temperature to chamber air temperature (set point) through convective heat transfer and drives evaporative cooling through transpiration to offset the radiative heat load from the lights. In chambers and rooms with HID or fluorescent/halogen lighting, the radiative heat load can be substantial. Significant temperature gradients can occur across plant tissues as they grow closer to fluorescent/halogen or HID lights, even with adequate air movement. In newer

chambers and rooms with LED lighting, this vertical temperature gradient is often less, as LEDs generally emit a lower radiative heat load compared to older lighting systems (Nelson & Bugbee 2015). Increasing PPFD helps drive evaporative cooling through stomatal opening and transpiration, in tandem with increasing air movement that helps to offset the radiative heat load from your lights. For more information on how plant tissue temperatures affect growth and development, please read Friesen (2021b).

Relative humidity (%): In growth chambers without humidity control, the relative humidity (%RH) inside your growth chamber is a product of the fresh air intake humidity, fresh air flow rate, chamber temperature, and how many plants are inside (sources of water vapor). Although the chamber temperature and number of plants can be effectively held constant and replicated through time, the humidity of the fresh air coming in can vary seasonally with time. If chamber temperature must be held constant, adjusting the fresh air flow rate may help mitigate seasonal variability in the humidity of the air inside your building. Keep in mind that your response variable of interest may not be measurably affected by changes in humidity. Although in some plants the rate of photosynthesis and growth is acutely affected by changes in %RH, in other plants these same changes in humidity have little effect. With ample air mixing, the %RH should be homogenous within the chamber space (see Friesen (2021c)). For more information on how %RH affects plant growth and how to control relative humidity inside growth chambers, please read: Friesen (2021d, 2020a https://www.biochambers.com/pdfs/vapour_pressure.pdf).

CO_2 concentration (ppm): In growth chambers without CO_2 control, the CO_2 concentration inside your growth chamber (ppm) is the product of the CO_2 concentration of your chamber fresh air intake, the fresh air flow rate, and the net CO_2 assimilation (uptake) and respiration (output) of the plants and soil inside your chamber. Although the chamber fresh air flow rate, and the amount of plants and soil can be effectively held constant and replicated through time, the CO_2 concentration of the fresh air coming in can vary with time due to changes in building occupancy or changes in industrial activity near your facility. During the daytime when most plants are assimilating CO_2 through photosynthesis, the CO_2 concentration of the fresh air can be drawn down, in some cases well below ambient atmospheric, especially in large growth rooms filled with large plants. Growth CO_2 concentrations below ambient atmospheric (~423ppm) can significantly reduce the growth of most plants. To mitigate CO_2 drawdown, your chamber should be set to full fresh air flow. With ample air mixing, the CO_2 concentration should be homogenous within the chamber space (see Friesen 2021c). For more information on whether you need CO_2 control and CO_2 drawdown, please read: Friesen (2017, 2021e https://www.biochambers.com/pdfs/fresh_air.pdf).

Air homogeneity: Air homogeneity can be defined as how homogenous the air is within a growth chamber or room regarding its movement, temperature, CO_2 concentration, and water vapor content (to determine %RH). More air movement results in more mixing, which will act to create a more homogeneous air parcel within your growth chamber and vice versa. Two factors affect the degree of air movement (mixing) within your growth chamber: the fan-speed and the placement, size, and number of potted plants, trays, or other materials. The fan-speed setting directly affects the air speed and degree of air mixing within your chamber. Our default setting of 85% is suitable for most applications and provides sufficiently homogeneous mixing while mitigating most effects caused by too much air movement that may be undesirable. When placing your potted plants or trays, think about where the air movement is coming from. Airflow inside most growth chambers and rooms is either up from the floor (eg. TPC series), downward from the ceiling (eg. TPR series), or horizontally through plenums in the sidewalls in equipment with shelves for shorter plants (eg. SPR, FXR series). As more and more potted plants are placed adjacent to the source of air movement, the pattern of air movement changes, and at some point air movement can become obstructed enough to create significant temperature, CO_2 concentration, and %RH gradients. In tightly packed chambers with dense plant canopies and still air pockets, %RH tends to increase whereas CO_2 concentration tends to decrease underneath compared to outside the plant canopy. Allowing channels of airflow between plants creates more mixing and air homogeneity. For more information on the effects of air movement on plant growth, please read: Friesen (2021c).

D) Considerations when using environmental conditions as treatments

When changes in environmental conditions themselves are the experimental treatment (category II and III), blocking or rotating control and treatment plants in the same manner should continue to avoid potentially confounding the control and treatment conditions. In some cases changing one environmental condition will indirectly affect another which may be immediately evident; relative humidity will increase at lower temperatures without humidity control, leaf temperatures will generally increase above chamber set point with higher PPFD under HID or fluorescent lighting. Perhaps a less widely known or intuitive change is decreasing PPFD with air temperatures below 25°C under fluorescent lighting, if the fluorescent tubes lack sleeves (exposed to air temperature) to keep their temperature constant.

E) Putting it all together

After determining which category (I, II, or III) of growth chamber experiment you will perform, and the number of chambers you have to work with, you will need to make a plan of how to replicate your treatment(s). In doing so, think about your sources of environmental, between chamber, temporal, and biological variability and how they may affect the variance of your response variable(s). Design your experiment to isolate and identify environmental, between chamber, and temporal variability through blocking, randomization, and incorporating chamber (chamber A, B, C) and time (round 1, 2, 3…) into your model. Aim to reduce sources of variability by maintaining consistent materials and methods, and the effects of environmental variability by re-arranging and rotating plants. In practice, there will inevitably be sources of variability, but we must hold the premise that the same plant or insect material will show the same variance in their response variable(s) when grown or reared under the same conditions.

The next step is to match statistical significance with biological

significance. For example, the mean difference of your response variable between treatment and control plants or between genotypes or cultivars. In statistics, this is one example of effect size, and ideally your experimental design is able to detect a statistical effect size that matches the effect size of biological significance (Quinn & Keough 2002c). Many real life systems show a gradient of biological significance. Here, your experimental design and significance testing should ideally be set-up to detect the smallest effect size that is biologically meaningful. To accomplish this, you will have to balance the type I (α) and type II (β) error rates to your experimental goals. The type I error rate (α) is the false positive error rate, or the chance you will detect an effect that is not actually real. The type I error rate (α) is often pre-determined, and is the threshold where you reject the null hypothesis and conclude significance of your p-values, that there is a significant effect or difference from your treatment or between groups of plants. Often α is set to 5% (0.05), which means we accept that 5% of the time our p-value could be due to chance alone. Reducing α from 5% to 1% or 0.5% when interpreting p values is a common way to decrease the chance of interpreting a false positive. The type II error rate (β) is the chance you will miss detecting an effect that actually exists (Quinn & Keough 2002c). Its difference from 1 (1 - β) is the power of your test, the power to detect an effect of a given size if it exists. Reducing α will increase β, and vice versa, as they are inversely related to each other when significance testing. If you achieve significance after your first round of experimentation (being diligent to try and reduce variability, set α to an acceptable level), then you may not need to probe deeper into your methods and design. If however, you do not achieve significance, it may be due to a lack of power. In many situations, the acceptable β is 0.2 or lower, giving you a power of 0.8 or greater (Quinn & Keough 2002d).

Increasing sample size is a way to reduce β and increase the power of your experiment after due diligence to reduce and account for variability (Quinn & Keough 2002d). In growth chambers, caution is required when increasing sample sizes, which are often the number of potted plants inside a given chamber. When chambers are filled with as many plants as can physically fit inside, neighboring plants may shade each other (reduced and variable PPFD, light quality changes) and air homogeneity can be reduced (airflow obstruction, gradients/microenvironments). If your response variable is related to photosynthesis or growth rate, the increased variability and reduced plant quality from cramming your chamber full of plants may outweigh any benefits from increased sample size. Another idea to increase sample size may be to decrease pot size (root volume) to increase the number of plants you can comfortably fit inside a given chamber. Reducing root volume can substantially reduce plant growth (see Friesen 2021f), so if your response variable is related to growth, reducing pot size, especially for larger plants at later growth stages, may reduce the quality of your data enough to exclude it as a viable option.

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