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DESIGN ISSUES FOR EVALUATING[†] SEEDLING EXPOSURE STUDIES

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SUMMARY

Tree seedling studies, covering a wide range of experimental conditions in pollutant treatment, species, facilities, and exposure regimes, have become commonplace in forestry research for assessing the actual and potential environmental effects of air pollutants on forest ecosystems. While assuring a wide breadth of scientific information, sufficient consideration has not been given to either the comparability of such population studies or to their appropriate inference. The populations of seedlings for which seedling experiments have inference, including the limitations in national or regional generalizations, should be made explicit in the results. Furthermore, the extent to which seedling results are applicable to mature trees and forests condition should not be left in doubt. Finally, the statistical power of any given analysis is almost never discussed, particularly when the outcomes are inconclusive. The approach for control of the exposure regime (i.e., Achieving treatment target levels) is often assumed, rather than assured through documentation.

Keywords: power, data quality, population inference, pollutants, seedlings

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INTRODUCTION

Seedling studies have become commonplace in forestry research for assessing environmental effects of stressors such as ozone and acid rain on forests (e.g., see Shriner et al., 1991). Most recently under the national acid precipitation assessment program (NAPAP). In this paper, the term “seedling” refers to trees small enough in size or stature (e.g. less than 1 meter height and one to three years old) to be used in standard open top chambers. Seedlings have been utilized in major research programs to provide early indications of relative sensitivity among important conifer and hardwood species (e.g., McLaughlin et al., 1988; Hogsett et al., 1989). The interest in forest health tends to focus on impacts to mature (i.e. reproductive stage) trees, with the hope that seedling studies might identify important physiological mechanisms or processes such as photosynthesis, carbon allocation, nutrient uptake, gas exchange, and water. Relations which, if affected by various air pollutant scenarios, might impact tree growth. However, because seedlings represent the future forest; studies of seedlings are useful in and of themselves, as a direct contribution to economic “risk” assessment for seedling populations in stand establishment and for regeneration. Therefore, the importance of these types of experiments cannot be overemphasized.

Although the long lived nature of trees requires changes in length of exposure (post exposure measurements and multi year exposures), and larger chambers (for a comprehensive review on the subject, see Hogsett, et al., 1987), Most of the exposure dynamics of seedling studies were patterned after crop studies (Heagle et al., 1973; Heck et al., 1988). Experimental designs are generally a variation of split plot or randomized blocks, with chambers rather than seedlings as the units of replication because chambers are the level at which the pollutant treatment can be applied. Most seedling studies incorporate repeated measurements of growth and growth process variables, and analyze data via analysis of variance and regression techniques, with the intention of statistically testing hypotheses. Though not unlike statistical issues found in other studies of population response, this paper focuses on (i) statistical power, (ii) data quality, and (iii) population inference, for their importance in evaluating outcomes from seedling exposure experiments.

STATISTICAL ISSUES

Power

The probability of detecting some consequential change in condition is fundamental to the success of controlled experiments. The consideration that should be given by policy and decision making managers to statistical power for testing research hypotheses, has received renewed emphasis (e.g., see Cohen, 1988; Mccaughan 1977; Millard, 1987; Barnthouse et al., 1983), Particularly for controlled exposure studies of annual crops (e.g., Rawlings, 1986) and tree seedlings (Peterson et al., 1989). In seedling exposure studies, the numbers of replicates (i.e. Chambers) usually influence statistical power moreso than the numbers of seedlings used per treatment combination. However, chamber replication is considerably more costly than seedling

Replication, and beyond a certain magnitude, may provide only a small increase in the power of a Statistical test. The amount of information varies with species and response variable, such as Diameter, height, bud elongation, and biomass, and is a function of the variance components (e.g., coefficient of variation) and sample size. Since differences in biomass due to treatment are commonly tested, an example of power curves for detecting relative differences in biomass components of ponderosa pine (figure 1) has been computed from a well-replicated study¹ using data from two and three chambers (treatment replicates) with over 30 seedlings per chamber. As might be expected; there is a higher probability ($p > 0.70$) of detecting treatment effects when the differences in biomass are quite large (e.g., greater than 40%). Conversely, the power of the experiment to detect differences of less than 20% was quite low, particularly when limited to only two replicates.

It is our opinion that most scientists or administrators do not have sufficient familiarity with statistical power, or some similar expectation for detecting a response to treatment, to appreciate its usefulness in aiding decisions with regard to the funding of ecological research. That is, will this experiment provide adequate replication and precision? However, even when the *a priori* coefficients of variation and percent differences point to a reasonable experiment, larger-than-planned experimental variations may result in low power of statistical tests. Nonetheless, even with the absence of formal significance due to low power, evidence of treatment effects may still be present in the form of trends or patterns that should not be overlooked. As seedling studies evolve to experiments on larger (e.g., sapling) trees, where the interest tends to be on changes to processes or mechanisms instead of linear growth changes or differences among means of biomass, it is likely that the documenting and interpreting of trends and patterns will become increasingly important in determining treatment effects.

Data quality

The goal for data quality assessment in biological research is to provide procedures that document and reduce random and systematic error in treatment application and response measurements within defined (statistically supported) limits. These measures traditionally have been applied to analytical measurements and have generally been limited to one plant response variable, namely final crop yield. For the NAPAP tree seedling studies, procedures were developed to apply statistical quality control to dozens of biological variables and physical variables, including measurement and monitoring of microclimate factors such as temperature and humidity (Mickler and Medlars, 1987; Cline and Burkman, 1988). Of particular interest is the data quality in treatment applications, in terms of accuracy, precision, and comparability. Although the assessments of all data from NAPAP seedling studies are not yet completed, preliminary results such as those in figure 2, where treatment targets are consistently missed or overlap with other treatment levels, raise concerns as to the appropriateness of the analysis of variance and post anova procedures that assume treatment targets were met. Given a consistency in treatments, measured variables, protocols, and experimental conditions, separate studies of a similar problem do not require the same treatment levels, in order to be comparable. However, the question is whether the analyses will take into account the missed target levels.

¹ Data on file with the EPA, Environmental Research Lab, 200 S.W. 35th St., Corvallis, Oregon.

Scope of inference

The ambient air quality and species at risk in nature (i.e., natural or artificial stands) should be the major determinants for designing and characterizing controlled seedling experiments. Clearly, the usefulness of seedling studies is to establish a baseline for inference to seedling populations of the particular species. Insofar that seedlings represent the future forest, treatment impacts to seedlings may give some indication as to how those same seedlings might later develop into mature trees. Although seedling studies could indicate where to look for impacts to mature trees and how to design appropriate studies, they should not be used to infer impacts to existing forests of mature trees or be extrapolated to regional generalizations.

Combining results across studies has the potential to increase the power of conclusions drawn from the individual experiments and consequently, an element of regionalization can be brought into the evaluation of results. Although the methodology may take the form of combining probabilities or test statistics (Fisher, 1932), it will more than likely involve a response surface or regression approach in seedling studies (e.g. Rawlings et al., 1988). However, an issue in inference of seedling studies is not the statistical procedure for a combined analysis but the extrapolation of results to a more general population. There are definite limitations to regional or national generalizations from seedling studies. For example, chambered studies throughout a region might be combined to better characterize dose/response patterns. However, the inference is still to the subpopulation of seedlings in experimental studies, since the air and soil growing conditions and exposure dynamics in the chamber will not likely be the same as those for seedlings in the field, particularly those seedlings which exist under the canopy of other trees or shrubs. Furthermore, it is the pollutant dosage (i.e., uptake by the plant), rather than ambient exposure level, which must be used to properly characterize the plant response to air pollutants.

In a paper by Wang et al. (1986), the authors found no foliar injury on seedlings that exhibited growth decreases, and vice-versa. From this, they concluded that since foliar injury to seedlings did not correlate well, if at all, with seedling growth changes, then widespread or regional growth decreases are likely to be present in mature trees for which there is no visible injury (see the discussion of Brennan and Harkov 1987, with Wang et al. 1987, on Wang et al. 1986). Not only would their conclusions (Wang et al., 1986), based on unassociated response between the growth and foliar injury, be spurious for other seedlings, but the added inference from seedling to mature tree and from experimental study to regional generalization illustrates a lack of understanding or appreciation for the inference from such studies. Regional generalizations of potential air pollutant impacts on forests should more appropriately come from a unified analysis that integrates related studies on seedlings and mature trees with forest stand dynamics and processes. Thus, the issue of generalizing from controlled seedling experiments to regional impacts on seedlings, let alone impacts on mature trees and forests, is our primary concern surrounding seedling exposure studies.

RECOMMENDATIONS

By the very nature that seedlings represent the future forests, they are an important population in and of themselves. They are readily used in a variety of growing facilities and lend themselves to robust designs of controlled experiments. In order to realize the potential strengths

of these designs, and further enhance the intrinsic value of seedling research, we offer the following recommendations:

* *Population Inference.* Results from seedling exposure studies can indicate potentially adverse impacts of pollutants on mature trees. However, those experimental results are sometimes directly, and wrongly, inferred to mature trees, or misused in making regional generalizations to seedling populations. Therefore, the relevant population of interest, the limits within which the experiment will mirror that population, and the environment (soil and air) in which that population exists, should be explicitly defined in both biological and spatial scale, in order to reduce, the likelihood of inappropriate inference from the experimental outcomes.

* *Power.* Given the significant investment of scarce resources required for exposure studies, particularly for long-lived plants such as tree species, workplans for initial or continuing research should address the probability of detecting a consequential change in tree condition specific to the variable or process being measured however, even with the absence of formal significance due to low power, evidence of treatment effects may still be present in the form of trends or patterns that. Should be reported.

* *Data Quality.* The magnitude and direction of the treatment applications and microclimate changes need to be monitored and documented throughout the duration of the experiment. Although it is not necessary that treatment levels be the same among studies, or that target levels always be achieved, evaluating the experimental outcomes can only be fulfilled when these fluctuations, as well as the error associated with the measurement of response variables, are known and documented.

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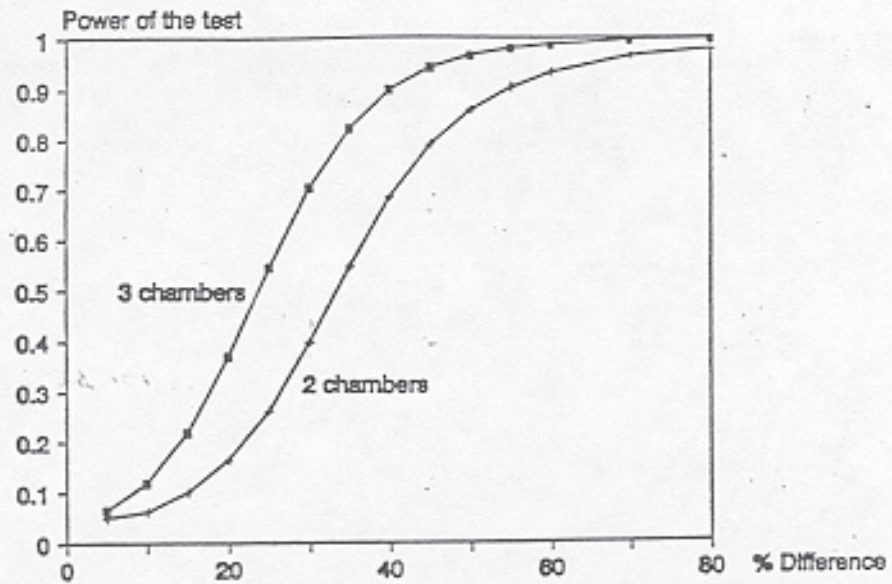


Figure 1. Increasing replication from 2 chambers per treatment (+), to 3 chambers per treatment (■), dramatically improved the probability of detecting relatively small differences in biomass due to treatment (i.e., increases power of the experiment).

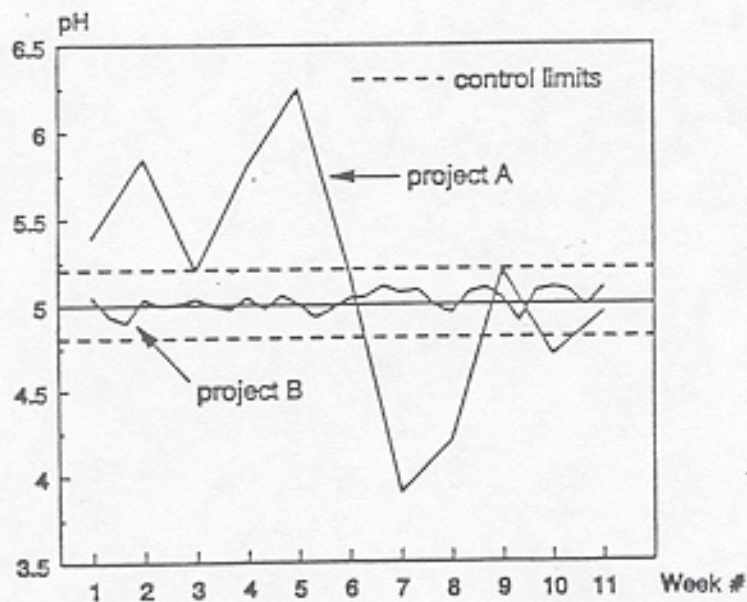


Figure 2. Failure to maintain target levels in a project (A) could result in overlapping treatment levels for that project, thereby confounding plant responses, or limit planned comparisons with results from another project (B).

