

Randomization and Decision Making

Name _____

AM or PM (circle one)

INTRODUCTION

As you may recall the basic steps in a scientific approach to problem solving includes: a) observe, b) ask questions, c) formulate a testable hypothesis, d) design and execute a test of your hypothesis, e) analyze the data generated by the test, e) interpret the results, and f) refine the hypothesis.

Palomar College's San Marcos campus is found in the middle of an ecosystem described as Coastal Sage Scrub. Some plants of the coastal scrub practice allelopathy, in which they produce allelotoxins that inhibit the growth of plant seedlings. In a habitat short on water and soil nutrients, allelopathy limits competition for these important resources from germinating plants. The fire cycle of the ecosystem is important because these toxins tend to be heat sensitive. When fire rages through the coastal scrub it destroys allelotoxins in the soil or leaf litter, creating an opportunity for seeds to germinate.

The question we are going to consider in the laboratory exercise is this: Does tree tobacco (a common plant in our local community), *Nicotina glauca*, possess allelotoxins? To answer this question an aqueous extract of tree tobacco leaves was prepared by mixing tree tobacco leaves with a volume of water in a blender and filtering the resulting slurry through cheesecloth. This solution will be used to test a more specific hypothesis: **An aqueous extract of tree tobacco leaves will inhibit the growth of radish seedlings.** This hypothesis will be referred to as the "initial hypothesis" later in the laboratory exercise.

EXPERIMENTAL DESIGN

Each pair of students will receive two petri plates. About a week ago ten radish seedlings were placed in each petri plate, and saturated with fluid. One petri plate was saturated with deionized water, and the other with tree tobacco extract. They have been allowed to grow under artificial light for the last week.

1. Which ten seedlings constitute the control group in this experiment, how is the control group different from the experimental group?

2. What is the independent or manipulated variable in this experiment?

3. What is the dependent variable in this experiment?

4. What variables should be held constant for both the experimental and control groups? List as many as possible.

PROCEDURE

1. As mentioned above, each pair of students will receive two petri dishes of ten radish seedlings--an experimental group and a control group.
2. Lay each seedling flat on the lab bench and measure its length from root tip to shoot tip in millimeters (mm). Record the lengths in the table below. Return each seedling to its petri dish. **Be consistent in your technique.**

Seedling	Experimental Group	Control Group
Seedling 1		
Seedling 2		
Seedling 3		
Seedling 4		
Seedling 5		
Seedling 6		
Seedling 7		
Seedling 8		
Seedling 9		
Seedling 10		
SUM OF SEEDLING LENGTHS		
MEAN SEEDLING LENGTH (SUM/10)		

Original Test Statistic (OTS) = |Control mean - Experimental mean| = _____.

Table 1. A comparison of the experimental and control group seedling lengths.

3. After recording the lengths of the seedlings in Table 1, also record the seedling lengths on 3 x 5 cards. Record the experimental seedling lengths on cards of one color, and the control seedling lengths on cards of another color. Each pair of students will then have ten 3 x 5 cards of one color, and another set of ten 3 x 5 cards of another color, with each card representing the length of a particular seedling.
4. Return to Table 1. Add the sum of the lengths of the experimental seedlings, and then divide that sum by ten to determine the mean experimental seedling length. Repeat for the control group.

5. Next determine the absolute value of the difference between the Control mean, and Experimental Mean. This value will be called the **Original Test Statistic (OTS)**. Record in the space above.

ANALYSIS OF DATA

The OTS describes the difference between the experimental and control groups. If the difference was zero, for example, one might conclude tree tobacco extract has no effect on seedling growth. Likewise, a number greater than zero suggests tree tobacco extract has an effect. One must be careful not to jump to conclusions, however. When ANY two sets of data are compared there is (almost) always a difference. For example if the mean height of one class were compared to another class there would be a difference. It might be a small, but there would be a difference. The question then becomes, is there a cause for the difference, or is the difference due to chance, merely a sampling artifact.

Hypothesis formulation.

To help answer the question above, statisticians work with standard hypotheses: the **null hypothesis**, and the **alternate hypothesis**. The null hypothesis (H_0) is always the same regardless of the data being analyzed and can be stated as follows: “The difference between the experimental and control groups is the product of chance”. Acceptance of the null hypothesis means one is taking the position that any difference in two data sets is due to chance, a sampling artifact, and is not due to a cause. The alternate hypothesis (H_A) is also always the same and can be stated as: “The difference between the experimental and control groups is the product of something other than chance.” The “something other than chance” of the alternate hypothesis means there is a cause for the difference.

In this test, seedlings grown in distilled water were separated from those grown in tree tobacco extract. There is a difference in the mean length of the two groups, the OTS. What must be considered is this: What if one had randomly picked ten seedlings from the twenty comprising the experimental and control groups, and compared their mean length to the remaining ten. Would the absolute value of the difference be equal to or greater than the OTS calculated by comparing the experimental and control groups? If this value, known as a Randomized Test Statistic (RTS) was greater than or equal to the OTS, it demonstrates that it is possible to get differences as great or greater than the OTS due to chance alone. An RTS smaller than the OTS would indicate that chance, at least in that instance, did not duplicate the OTS lending credibility to the position that something other than chance (i.e. a cause, the tree tobacco extract) is responsible for the OTS.

It is important, then, to determine the frequency, or probability, that chance duplicates the OTS. If this probability is high it is reasonable (defined as 5 or more times per 100) to conclude that the OTS is due to chance, i.e. that the null hypothesis is true, and that the difference between the control and experimental groups was not due to the tree tobacco extract, but is merely a sampling artifact. Conversely, if this probability is low, say less than 5 times per 100, one could reasonably conclude that the OTS was due to something other than chance, i.e. that the alternate hypothesis is true, and that the difference between the control and experimental groups was due to the tree tobacco extract.

Randomized Test Statistics

1. Take the twenty cards you created previously and shuffle them several times so that the experimental and control seedling lengths are thoroughly mixed together.
2. Deal the cards into two piles of ten cards each. There are now two sets of ten cards of mixed colors, randomly generated.

- Determine the mean length of the seedlings from each pile.
- Subtract the smaller mean from the larger mean to generate a Randomized Test Statistic (**RTS**). Record this value, RTS 1, in Table 2.

RTS 1 =	RTS 2 =	RTS 3 =	RTS 4 =	RTS 5 =
RTS 6 =	RTS 7 =	RTS 8 =	RTS 9 =	RTS 10 =

Table 2. Test statistics of randomization test.

- Repeat steps 1-4 nine more times, generating a total of 10 RTS's. These can be compared to the **Original Test Statistic (OTS)** generated from the experimental and control groups.
- How many times (out of ten) was the randomized test statistic equal to or greater than, the original test statistic?
- Report the results from question 6 to the instructor who will put **class totals** for following values on the board. Record those values below.

Class totals

Total number of times the RTS was equal to or greater than, the OTS= _____

Total number of randomized tests performed by the class (10 x number of pairs in class) = _____

- The overall probability that an RTS equaled or exceeded the OTS is calculated as follows (p = probability):

$$p = \frac{\text{Total number of times randomized test statistic equaled or exceeded the original test statistic}}{\text{Total number of randomized tests performed (#RTS generated)}}$$

- Multiply p times 100 to convert the probability to percent, making it easier to interpret. $p \times 100 = \underline{\quad\quad\quad}\%$. This value is the frequency or probability that your data is the result of chance, a sampling artifact.
- Subtract p from 100 to get the percent confidence that your data is due to a cause, i.e. the tree tobacco extract. Percent confidence = $\underline{\quad\quad\quad}\%$.

Let us return to analysis of the standard hypotheses. At what point should one “draw the line,” so to speak, and decide whether an experimental result is due to chance or something other than chance. Biologists generally require a “confidence” **of greater than 95%** to accept the alternate hypothesis. This means that to accept the alternate hypothesis randomized results (RTS) must exceed the original results (OTS) fewer than 5 times per 100 tests ($p < .05$). This translates to a confidence of greater than

95% and is considered “significant” by the scientific community. When scientist use the phrase “there is a significant difference...” it means chance duplicates the experimental results less than 5 times per 100 trials ($p < .05$).

In our specific case, if less than 5 out of 100 ($p < .05$) RTS’s equaled or exceeded the OTS, one would reject the null hypothesis, and accept the alternate hypothesis. If 5 or more out of 100 RTS’s equaled or exceeded the OTS, the confidence would be 95% or less, and one would accept the null hypothesis and reject the alternate hypothesis. As one can see, this is a very conservative standard. If the RTS equals or exceeds the OTS even 5% of the time, then the experimental results are **not** considered “significant,” and therefore suspect or unreliable.

STUDY QUESTIONS

- 1) What is the null hypothesis –quote directly from the laboratory exercise?

- 2) Do you accept or reject the null hypothesis? *Explain your answer.*

- 3) What is the alternate hypothesis--quote directly from the laboratory exercise?

- 4) Do you accept or reject the alternate hypothesis? *Explain your answer.*

- 5) What is the initial hypothesis in this laboratory exercise?

- 6) Do you accept or reject the initial hypothesis? *Explain your answer.*

- 7) If randomization analysis showed that tree tobacco extract inhibited seedling germination, would that prove that *Nicotina glauca* leaves possess allelotoxins? *Explain your answer.*