Seed Germination Worksheet

Name ____________________________

This project will involve setting up an experiment with a counted number of seeds, observing the number of seeds which sprout in the experiment, statistically analyzing the class results, and reporting the results in a properly executed figure. The writing assignment includes both a materials and methods section and a results section.

**The effect of light on seed germination**

**A. Observation.** Seeds of some species germinate in the light but do not do so well in the dark. The pigment phytochrome has two forms, Pr and Pfr, which absorb red and far-red light, respectively. Perhaps this pigment helps seeds detect the light.

**B. Question.** Do species requiring light to germinate use the phytochrome pigment to determine if they are in the light or darkness?

**C. Hypothesis.** Light-sensitive seeds do use the phytochrome pigment.

**D. Prediction.** If the hypothesis is true, then lettuce seeds placed in red light will germinate much better than seeds placed in darkness or far-red light. Moreover, seeds in far-red light should be particularly inhibited compared to seeds in darkness.

**E. Experiment.** Obtain four 10 cm Petri Dishes from the supply and put one 9 cm disc of filter paper in the bottom of each dish. Label the dish covers (and bottoms!) with the marking pen. The labels should include: **White Light**  **Red Light**  **Far-red Light**  **Dark Control**

Put 5 mL of distilled water in each dish, and obtain a square of aluminum foil so that you are ready to wrap the "dark" dish immediately. **BE READY!**

With your partner, carefully count out four groups of 50 lettuce (*Lactuca sativa* 'Tango') seeds. Of course the counts must be accurate, but **WORK QUICKLY!** Place one group of 50 seeds into each dish on top of the moist paper.

**IMMEDIATELY** wrap the "dark" dish in aluminum foil so that it is completely enshrouded. Place the "red light" dish under red filter with fluorescent lighting. Place the "far-red light" and the "dark control" dishes under red and blue filter with incandescent lighting. Note: dishes are not dark nor made of light nor produce light…this lab jargon should be avoided in your writing assignment!

After the initial treatment of ___ days and after the follow-up treatment of ___ more days, count out the number of seeds germinated and calculate the percent germination. We will count a seed as germinated if the radicle penetrates the seed coat and extends 1 mm beyond the seed coat; look closely! **After the initial treatment, add 2.5 mL dH2O and move all the dishes of seeds into white light for the follow-up treatment.**

<table>
<thead>
<tr>
<th>Initial Treatment</th>
<th>After Initial Treatment</th>
<th>After White Follow-up Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Germinated (%)</td>
<td>Newly Germinated</td>
</tr>
<tr>
<td>White light</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Red light</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Far-red light</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Darkness</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

**F. Analysis.** The most seeds germinated during the initial treatment in:

<table>
<thead>
<tr>
<th>red light</th>
<th>far-red light</th>
<th>darkness</th>
</tr>
</thead>
</table>

The fewest seeds germinated during the initial treatment in:

<table>
<thead>
<tr>
<th>red light</th>
<th>far-red light</th>
<th>darkness</th>
</tr>
</thead>
</table>

The germination-stimulating form of phytochrome is: [Pr Pfr]

**G. Decision.** The hypothesis: "Light-sensitive seeds do use the phytochrome pigment"

is: rejected not rejected

**Questions to Answer At Home**

Color of initial light treatment stimulating chlorophyll synthesis: [red far-red darkness].

Color of initial light treatment only stimulating carotenoid synthesis: [red far-red darkness].

Defend the statement: "Lettuce seeds need light to germinate" using the data you obtained in laboratory.

Lettuce seeds do need light to germinate, because germination of seeds was greater less in the dark than in white light. Moreover, after the initial treatment of darkness, the ungerminated seeds were exposed to white light and more no more germinated.

Condemn the statement: "Lettuce seeds need light to germinate" using the data you obtained in laboratory.

Lettuce seeds do not need light to germinate, because germination of seeds in the darkness was more than 0 5% in white light control.

Explain how you can tell whether far-red light had any effect.

Germination in the initial treatment of far-red light was greater equal to less than in the control seeds, which were in white light red light darkness.

The failure of a seed to sprout could be due to the fact that its germination is indeed inhibited. But there is also a second possibility: perhaps the treatment does not inhibit seed germination directly, but simply kills the embryo. Such a situation would certainly not produce many germinating seeds either. In this exercise you exposed the seeds treated with far-red light to white light for an additional time and observed germination again. Present arguments for both possibilities.

**Far-red light inhibits germination.**

My evidence is that germination after white light follow-up exposure among seeds initially exposed to far-red light increased by ____%.

**Far-red light kills the embryo.**

My evidence for this topic is that germination after white light follow-up exposure among seeds initially exposed to far-red light was greater less than among seeds initially exposed to darkness. This does does not argue that far-red light kills the embryo.
**Statistical Analysis of Percent Germination in Experiments**

In addition to learning how to write a materials and methods section, we will also be learning how to carry out a Z-test for the difference in two proportions in Excel™. We will create a spreadsheet tool that will compare the percent germination of seeds in any treatment to its corresponding control. By saving the resulting file, it can be used in the future to compare any lab experiments that generate percentage or proportion data. Apply this analysis tool to the 10 experiments in your project!

**Setting up the areas on the spreadsheet:**

- In A1, type: Z Test for the Difference in Two Proportions
- In A3, type: Data –and make it bold
- In A4, type: Hypothesized Difference
- In A5, type: Chosen level of $\alpha$ –to do this, type a, select a, and select Symbol font
- In A6, type: Control –and make it bold
- In A7 type: Seeds Germinated
- In A8 type: Total Sample Size
- In A9 type: Treatment –and make it bold
- In A10 type: Seeds Germinated
- In A11 type: Total Sample Size
- In A13 type: Intermediate Calculations –and make it bold
- In A14 type: Control Germination
- In A15 type: Treatment Germination
- In A16 type: Difference in Proportions
- In A17 type: Average Proportion
- In A18 type: Z-Test Statistic –and make it bold
- In A20 type: Two-Tailed Test –and make it bold
- In A21 type: Lower Critical Value
- In A22 type: Upper Critical Value
- In A23 type: $p$ –make it bold –double-click the column separator

**Inserting the formulas into the spreadsheet:**

- In B4 type: 0
- In B5 type: 0.05
- In B7 type the number of seeds germinating in your control (for now enter 40)
- In B8 type the number of seeds sown in the control (50)
- In B10 type the number of seeds germinating in your treatment (for now enter 20)
- In B11 type the number of seeds sown in the treatment (50)
- In B14 type: =$B7/B8$ yes, you have to type = sign or it will not calculate!
- In B15 type: =$B10/B11$ did you type the = sign?
- In B16 type: =$B14-B15$ yes, all formulas start with the = sign!
- In B17 type: =(B7+B10)/(B8+B11)
- In B18 type: =(B16-B4)/SQRT(B17*(1-B17)*(1/B8+1/B11)) Note: if all is well: =4.082482905
- In B21 type: =NORMSINV(B5/2)
- In B22 type: =NORMSINV(1-B5/2)
- In B23 type: =2*(1-NORMSDIST(ABS(B18))) then set to 3 decimal places, make bold!
- In A24 type: =IF(B23<$B$5,"Reject the null hypothesis","Do not reject the null hypothesis")
- In A25 type: =IF(B23<$B$5,"Treatment Effective","Treatment Ineffective")

Save your spreadsheet for repeated use…enter data in B7 and B10 for each similar experiment.
### Analysis Worksheet

**Testing Hypotheses on Germination of *Lactuca sativa* '____________' seeds (1)**

From page 1 of your Seed Germination worksheet, transfer your data here: (8)

<table>
<thead>
<tr>
<th>Initial treatment</th>
<th>Initial germinated</th>
<th>Total germinated (after follow-up white exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>white light</td>
<td>A= ___ of 50</td>
<td>E= ___ of 50</td>
</tr>
<tr>
<td>red light</td>
<td>B= ___ of 50</td>
<td>F= ___ of 50</td>
</tr>
<tr>
<td>far-red light</td>
<td>C= ___ of 50</td>
<td>G= ___ of 50</td>
</tr>
<tr>
<td>darkness</td>
<td>D= ___ of 50</td>
<td>H= ___ of 50</td>
</tr>
</tbody>
</table>

#### Using your Z-test spreadsheet

Did the initial treatment *(A→C)* have a significant effect? Compare to darkness control *(D)*. (9)

<table>
<thead>
<tr>
<th>Initial Treatment</th>
<th>p-value</th>
<th>Decision on null hypothesis</th>
<th>Treatment effect on seed germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>white light</td>
<td></td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>red light</td>
<td></td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>far-red light</td>
<td></td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
</tbody>
</table>

Did the darkness treatment *(D)* have a significant effect? Compare with 0 germination control. (3)

<table>
<thead>
<tr>
<th>Initial Treatment</th>
<th>p-value</th>
<th>Decision on null hypothesis</th>
<th>Treatment effect on seed germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>darkness</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
</tbody>
</table>

Did the white-followup treatment *(E→H)* overcome any inhibiting effect of the corresponding initial treatment *(A→D)*? Use the initial value *(A→D)* as the control and E→H as treatment. (12)

<table>
<thead>
<tr>
<th>Initial Treatment</th>
<th>p-value</th>
<th>Decision on null hypothesis</th>
<th>Treatment effect on seed germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>white light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>red light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>far-red light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>darkness</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
</tbody>
</table>

Was there any significant long-term effect of the initial treatment on total germination? Compare the data after the follow-up white light treatment *(E→G)* to the data for seeds initially exposed to darkness control *(H)*. (9)

<table>
<thead>
<tr>
<th>Initial Treatment</th>
<th>p-value</th>
<th>Decision on null hypothesis</th>
<th>Treatment effect on seed germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>white light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>red light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
<tr>
<td>far-red light</td>
<td>___ ___</td>
<td>reject cannot reject</td>
<td>stimulates ineffective inhibits</td>
</tr>
</tbody>
</table>

Note: This page must accompany your writing assignment!
Organizing Your Seed Germination Writing Assignment

You will write about your work with seed germination of lettuce, Lactuca sativa. Your assignment should follow the model of your previous one on double-y plots. It should be double-spaced from beginning to end, written in past tense, have a proper title on the first line, an author line, a contact information line, at the top of a sheet. You continue on that sheet with a major heading of Materials and Methods, with sufficient details under subheadings. You follow this with a Results heading and text that indicates any other observations and that makes observations of the figure. The Results section continues with the figure itself. For this project, you need to provide a proper column graph with overlapped columns clearly marked to show significant differences (in % germination between treatment and control) based on your Z-test analyses.

See the Double-Y Plots assignment for general information, and you may want to read these two laboratory report sections in the departmental style manual (Pechenik, J. A. 2012. A short guide to writing about biology. 8th ed. Addison Wesley Longman Publisher).

Some critical details were omitted from the worksheet:

- Lactuca sativa 'Tango' from Veseys Seeds, PO Box 9000, Calais, Maine 04619-6102.
- Light sources were positioned 25 cm above the seeds.
- Filtration was used to supply monochromatic light treatments.
- For white light, the source was a 23 watt compact fluorescent bulb.
- For red light, the source was a 23 watt compact fluorescent bulb filtered through a deep red Plexiglas™ sheet.
- For far-red light, the source was a 25 watt incandescent bulb filtered through a combination of a deep red and a deep blue Plexiglas™ sheet.
- For the dark control, the dish was immediately wrapped in aluminum foil and placed in the far-red light treatment to control for temperature.

In writing your materials and methods section, please remember that you want to be concise (avoid redundant words and phrases), you want to avoid writing about things that a scientist knows to do anyway (labeling dishes, keeping good records, etc.). Remember you are not giving directions to someone else (do not use imperative voice!) but are telling what you did (use past tense). You should remember that this writing is about seed germination. It is NOT about the lab (room). It is NOT about Petri dishes. So be sure to focus your writing on the seeds! The writing sequence is the sequence...you do not need to use "then," "before," "after," etc. If you need to use these sequence words, it probably means that your text sequence is out of order and needs revising! Subheadings might include germination methods, light treatments, and data collection and analysis.

In proofreading your writing, be sure you are not putting the light source inside the box with the Plexiglas filter, or that the distance is somehow a space inside the box. Your dishes are not white, red, far-red, or dark; all the dishes are colorless plastic! Remember that the monochromatic light is a treatment for the seeds (not for the dishes!). The subject of each sentence must be capable of doing the verb!

In preparing your graph for the figure, remember to use Arial font at proper sizes and boldness. The graph is a column graph, and the followup treatment was white, so the corresponding bars might need a white fill and a black border of 2 points. You know how to make the axes and plot area bold, and how to limit tick marks to 4-8 on each axis. You will want the initial treatment results in front of, and to the left of, the white followup results and the usual 60% overlap of the bars.
One critical trick in this project is needed if you got 0% germination in any treatment. Excel will just eliminate a bar for a value of 0 rather than showing a bar with the rectangular borders and no fill. To make sure that the 0 datum is shown by Excel, you will likely need to enter a very small value rather than 0. You are striving to show two points of top and bottom border for this bar, but not so tall as to show any fill. This will seem like "fudging" data…but we are simply working around an Excel problem. These data are still showing a 0 in our notes and worksheet, but the Excel spreadsheet for the graph will have to show some small percentage to force Excel to show the 0 bar on the graph. Your graph needs to show all 8 bars.

Another critical fix for this graph is to show the results of your statistical analyses in the figure. After you have pasted your graph into Word, you will need to insert text boxes that float in front of the regular text. These will hold some symbol (maybe * or ^ in 24 point Arial font) that you can move up into the Excel graph next to a bar, or place at the top of a bar to indicate that this percentage is statistically different from a dark control (*) or an initial control (^). The way text boxes are created and formatted varies among different versions and the two platforms for Word. If in doubt, ask. There will be substantial penalties for NOT showing the outcomes of your z-test analysis in your Figure. Do not forget to explain your symbols in the Figure caption at the end, as part of indicating your statistical treatment of the data. Obviously this means you need to understand what you did for statistical analysis and what the analysis means!

In writing your Results text, you need to describe what you observed in the outcomes. You need to provide a callout to the graphic in the first sentence referring to it (Fig. 1) for each paragraph. This draws the reader to look at your figure. But you tell what you observed in that figure too. However, remember NOT to give numerical values…that is the purpose of the figure. Rather focus your writing on what happened. You describe the relationships you observed. Be sure to use powerful words such as "statistically more," "stimulated," "inhibited," or "no significant effect" that tie the seed germination to the statistical analysis of your data. Of course you need to understand the outcomes of those statistical tests before you can effectively write about them.

Other important problems to avoid in Results include the following. Dishes do not sprout or germinate. Neither does light of any color. The light may induce a seed to germinate and this is what you need to write about. Also, light does not cause a seed to appear magically…but a seed clearly exposed to that light may germinate. Your writing must communicate clear thinking.

The results section does not interpret the meaning of the outcomes or draw conclusions about the outcomes. There is another section of a formal report that will do this…the discussion section…and you will learn about writing that in the sophomore courses in our biology major.

Assembling Your Writing Assignment

Put the pages of your paper in the correct sequence!!

Pages (as needed) showing these parts: Title, Authors, Materials and Methods, Results (text), Figure 1 and its caption. You will separately hand in the Analysis worksheet (maybe on the same due date, maybe not; see the syllabus).

Staple these sheets together in the upper left corner of the first page.

Yes, YOU must staple the stack of papers together. If you do not have a stapler, please visit just about any office on campus before the due date. Biology is on the third floor in the faculty wing of the Science building. Then, at your next birthday, you know what to tell Aunt Bessie you need for college.