NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

# Killing Chloroplasts:
## Herbicides Targeting Photosynthesis

*by*
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## Part I – Illuminating Photosynthesis

Welcome to our plant sciences department here at TransBio! It's great to have you as an intern. I'm the lead scientist of a team developing new herbicides to use against invasive plant species. The team has been really swamped with work so it'll be great to have some help analyzing all the data we're collecting. Since it's your first day, a great way to get started with any research project is to brush up on the background knowledge you'll need to understand all the experimental work. Hopefully, you can put all that stuff you learned in biology class to good use!

Go ahead and start by identifying all the major steps and processes during photosynthesis. It'll help us later if we diagram this out and label each component with its basic function.

*See #1 in your worksheets!*

That looks great. Now you have a visual for how all the pieces are working together. Let's get a little bit more specific and decide if any pieces depend on any others.

Let's do an example by looking at the electron transport chain. Does the electron transport chain depend on any other component? It looks like it must depend on the Water-Splitting Photosystem to pass some electrons to it. See if you can do the rest of the components on your own.

Let's also start to think about how we can use this information in our herbicide experiments. We know that an herbicide should prevent plant growth and survival. What if the herbicide prevented plant growth by inhibiting some of the pieces in your diagram? What experimental variables would we need to measure to tell us if each part of photosynthesis is working? In other words what are the products of each major step? What do we expect to happen to each of those products if the herbicide is targeting that step or process?

*See #2 in your worksheets!*

You're off to a good start. Let's head to the lab and start looking at some data.

## Part II – Planting Yourself as a Great Intern

Here's our lab and some of the experiments we still have in progress. Instead of working with whole plants we can just work with isolated chloroplasts. Remember, each of the chloroplasts that we are working with contains all of the photosynthetic machinery that you are familiar with. So, we can see how these chloroplasts respond to the herbicides. Basically, we want to know if the herbicides prevent some or all of the major processes happening during photosynthesis. If photosynthesis is inhibited in the chloroplasts then that gives us a really good idea that the herbicide will also work against the whole plant. We have five herbicides that we are testing right now using a variety of experimental methods to look at the impact on photosynthesis.

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Here's an example of some of the data we've been collecting. Here we were looking at the effect of each herbicide on NADPH production. It would be great if you could analyze this data and draw up a graph to illustrate the findings.

*See #3 in your worksheets!*

## Part III – Digging into the Data like a Pro

Okay, the data you just analyzed is part of a larger set of data we have been collecting on these herbicides. I'm going to give you the rest of the data now and you can work on combining all the results together. Take a look at the data and see if you can figure out if any of the steps in photosynthesis are messed up by each herbicide. If so, then try to figure out which step is being targeted by each herbicide.

*See the data sheets and #4 in your worksheets!*

One of the lab technicians has done a lot of this work and she has come up with how she thinks each of these herbicides is inhibiting photosynthesis. Take a look at her list and see if you can match each one with what you came up with.

*See #5 in your worksheet!*

Thanks for your help today! I think after this round of testing we can be pretty confident that we know how these herbicides are working. Now, we can move on to some additional testing!

### *Reference*

Alberts, B, A. Johnson, J. Lewis et al. 2002. *Molecular Biology of the Cell*, 4th ed., Chloroplasts and Photosynthesis. Garland Science. <http://www.ncbi.nlm.nih.gov/books/NBK26819/>.

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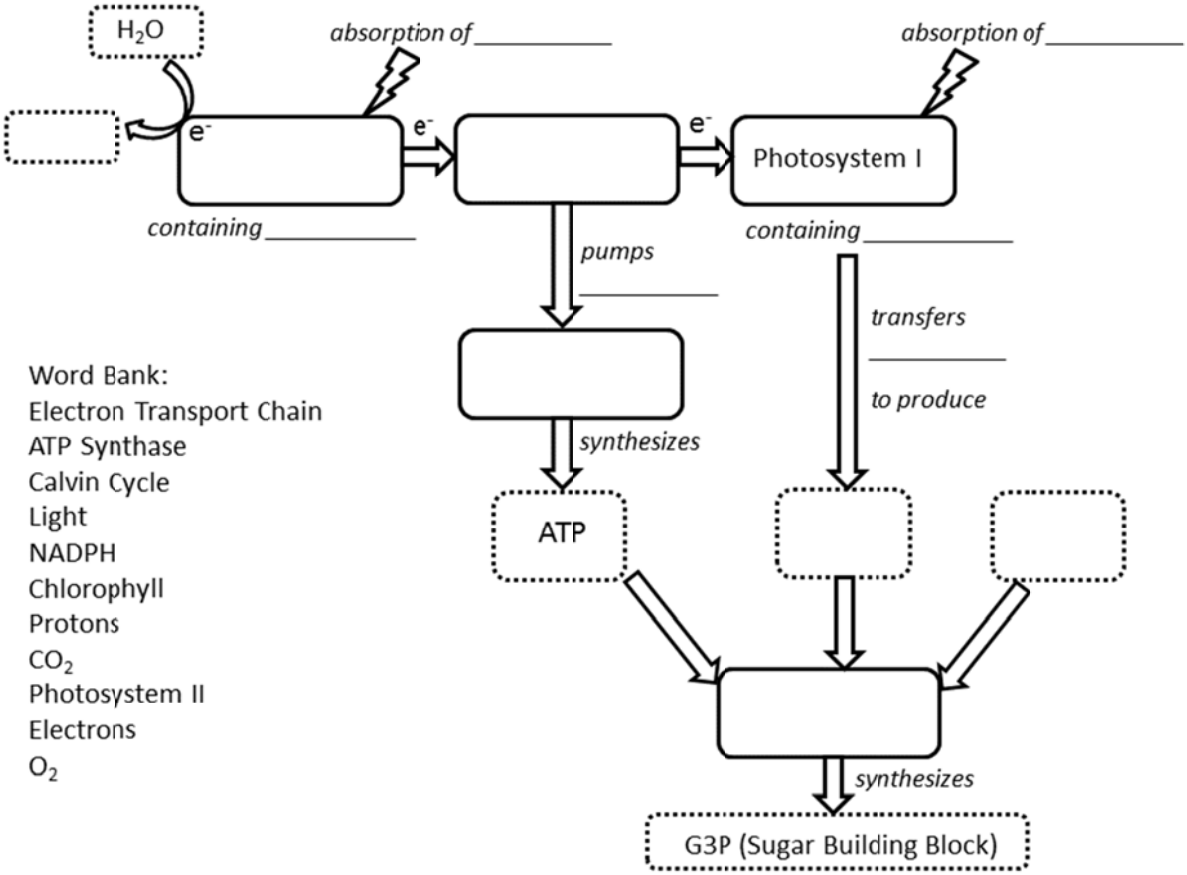
## Part I – Illuminating Photosynthesis

**#1:** Fill in this concept map depicting the major steps in photosynthesis in the chloroplast:




Word Bank:
Electron Transport Chain
ATP Synthase
Calvin Cycle
Light
NADPH
Chlorophyll
Protons
$CO_2$
Photosystem II
Electrons
$O_2$

**#2:** Fill in the table:

| Major Steps in Photosynthesis | Does this step depend on any other step? How? | Experimental variable to measure? | What would happen if an herbicide disrupted this step? |
|---|---|---|---|
| Photosystem II | | | |
| Photosystem I | | | |
| ATP Synthase | | | |
| Calvin Cycle | | | |

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## Part II – Planting Yourself as a Great Intern

**#3:** Here are five tables, one for each of the five herbicides being tested, showing data on NADPH production (nmol/g) in chloroplasts in the absence of herbicide (Before) and 10 minutes after addition of herbicide (After). Analyze the data and then draw a graph to represent the findings. The first herbicide (H-1) is done for you and there is a graph started. As appropriate, you will want to add additional bars for your experimental values, axis numbering and labels, and a legend. Remember, someone should be able to look at your graph and figure out what is being shown without anybody explaining it to them.

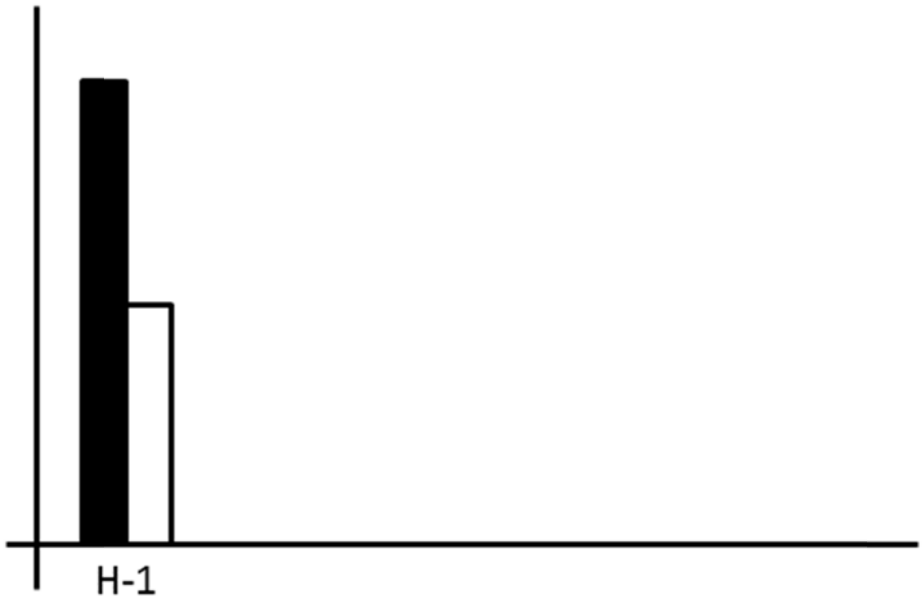
| Herbicide | Experimental Replicates | Before | After |
|---|---|---|---|
| H-1 | Replicate 1 | 14 | 8 |
| | Replicate 2 | 11 | 5 |
| | Replicate 3 | 13 | 5 |
| | Average | 12.7 | 6.0 |

| Herbicide | Experimental Replicates | Before | After |
|---|---|---|---|
| H-2 | Replicate 1 | 11 | 7 |
| | Replicate 2 | 14 | 4 |
| | Replicate 3 | 12 | 6 |
| | Average | | |

| Herbicide | Experimental Replicates | Before | After |
|---|---|---|---|
| H-3 | Replicate 1 | 15 | 6 |
| | Replicate 2 | 11 | 5 |
| | Replicate 3 | 12 | 5 |
| | Average | | |

| Herbicide | Experimental Replicates | Before | After |
|---|---|---|---|
| H-4 | Replicate 1 | 12 | 14 |
| | Replicate 2 | 11 | 11 |
| | Replicate 3 | 14 | 12 |
| | Average | | |

| Herbicide | Experimental Replicates | Before | After |
|---|---|---|---|
| H-5 | Replicate 1 | 10 | 12 |
| | Replicate 2 | 14 | 13 |
| | Replicate 3 | 11 | 12 |
| | Average | | |



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## Part III – Digging into the Data like a Pro

**#4:** Here is a table to help you organize your results. For each variable you can note if there is an increase, a decrease, or if it stays the same or is normal.

| Herbicide | Chlorophyll Absorption | Oxygen Production | NADPH Production | ATP Synthase Activity | G3P Production |
|---|---|---|---|---|---|
| H-1 | | | | | |
| H-2 | | | | | |
| H-3 | | | | | |
| H-4 | | | | | |
| H-5 | | | | | |

Based on your table of results, list the part/step of photosynthesis being inhibited by each herbicide:

H-1 _____

H-2 _____

H-3 _____

H-4 _____

H-5 _____

**#5:** Here is the list of herbicide functions that the lab technician came up with. Fill in which herbicide goes best with each proposed function.

_____ Disrupts proton flow and decreases ATP synthesis.

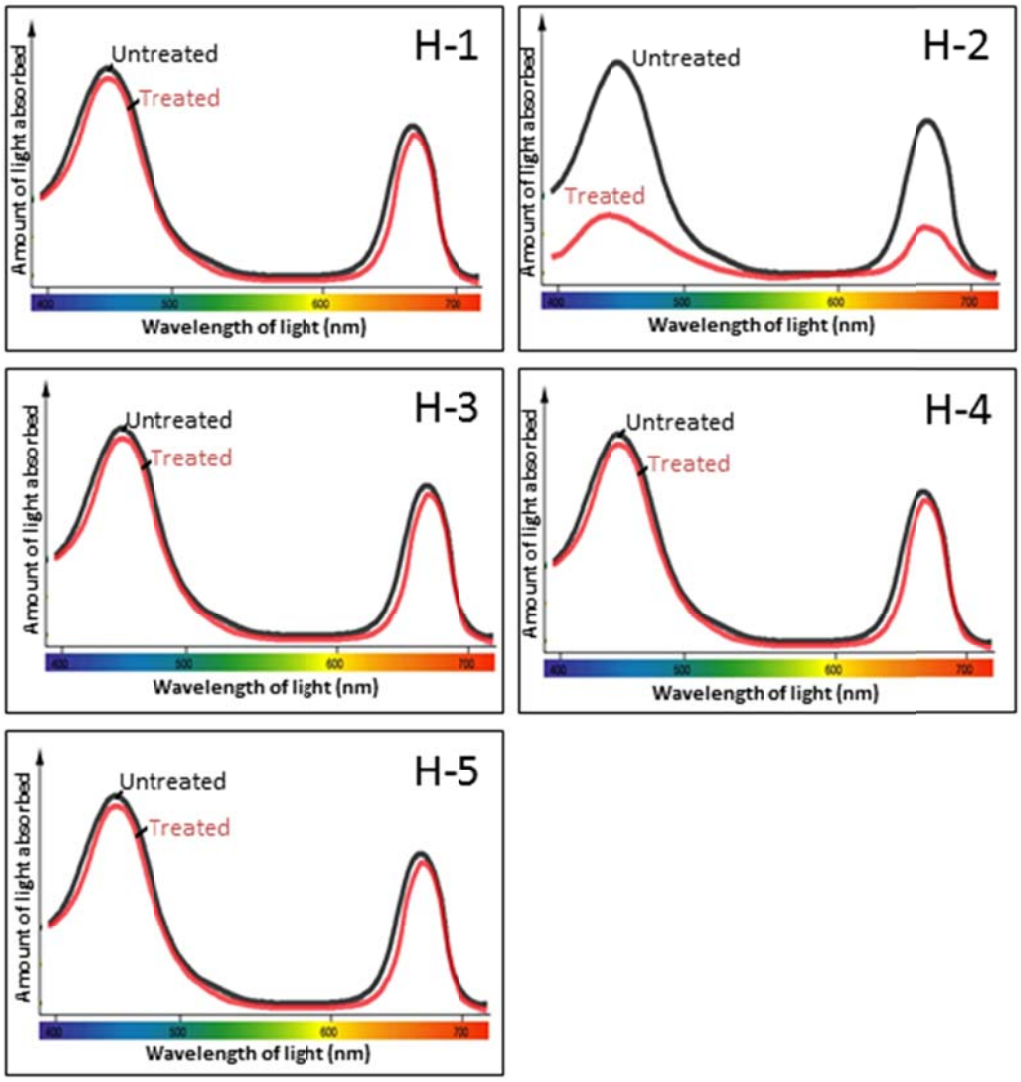
_____ Binds to a protein causing it to strip electrons from water and generate superoxide radicals.

_____ Binds to chlorophyll and shifts the absorption into the infrared range.

_____ Inactivates the enzyme that produces G3P, preventing sugars from being built.

_____ Takes electrons from the electron transport chain.

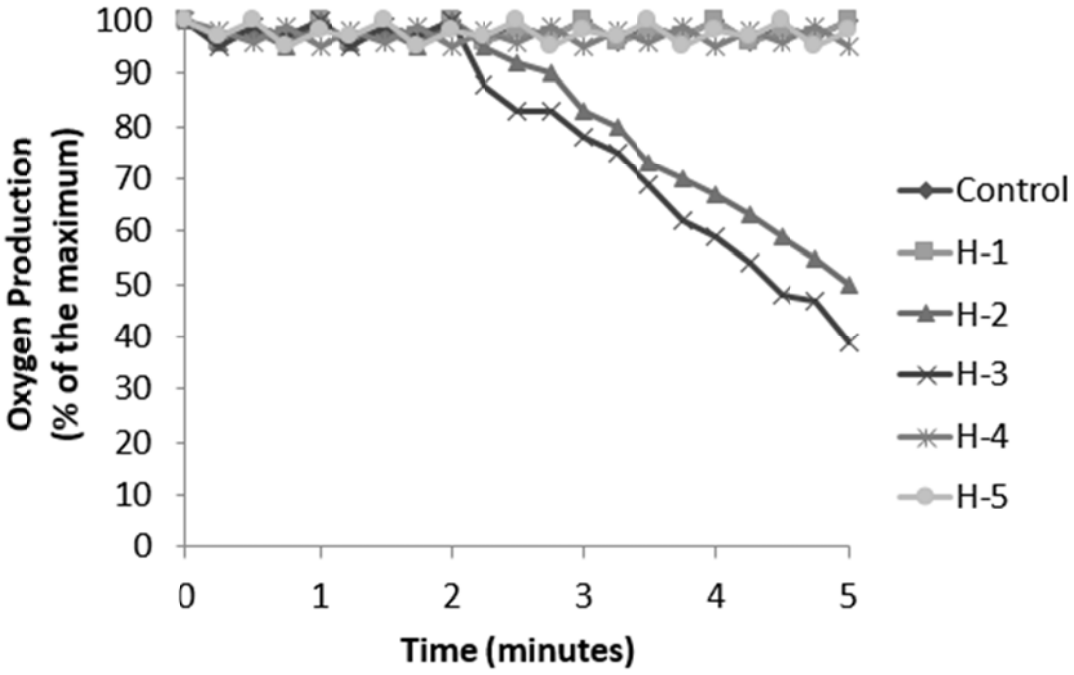
**Data Set 1.** Chlorophyll absorption in isolated chloroplasts. Black lines represent chloroplasts that are not treated with herbicide. Red lines represent chloroplasts treated with herbicide. Each panel is labeled with the herbicide tested (H-1 through H-5).




**Data Set 2.** Glyceraldehyde 3-phosphate (G3P) levels in chloroplasts before and after herbicide treatment. Values are reported in µmol/mg.

| Herbicide | Before Treatment | After Treatment |
|---|---|---|
| H-1 | 19.4±0.9 | 12.4±0.9 |
| H-2 | 19.3±0.6 | 7.3±1.1 |
| H-3 | 18.2±1.1 | 10.2±0.9 |
| H-4 | 18.9±0.8 | 10.3±1.1 |
| H-5 | 20.2±1.3 | 13.2±0.9 |

**Data Set 3.** Oxygen production in isolated chloroplasts. Oxygen production was measured over time. At 2 minutes, herbicide was added to each sample except the control.




**Data Set 4.** ATP levels (nmol/mg) in chloroplasts before (gray bars) and after (white bars) herbicide treatment. Error bars represent one standard deviation.