Name___________________

DATA Nugget

Tiny, but mighty: leaf miners take on aspen trees

Featured scientists: Jenifer Wheeler (she/her) and Diane Wagner (she/her) from the University of Alaska Fairbanks. Written with Denise Kind (she/her).

Research Background:

Caterpillars might look small, but can they actually be harmful? Yes, if there are enough of them! **Aspen leaf miners** are moths as adults, but before that, aspen leaf miner caterpillars are incredible herbivores. In the spring, they lay their eggs on the surface of leaves, and they sink into the outer cell layer. When they hatch, the caterpillars quickly get to work. Using their blade-like mouth parts, they slash through the surface cell layer and drink the contents that are released. They leave a trail of silver, damaged cells in their path.

In recent years, aspen leaf miners have been increasing in numbers in Alaska and Canada. Aspen trees are an important component of the boreal forest, providing nutritious food for wildlife, supporting a diverse ecosystem, and capturing carbon dioxide (CO_2) from the atmosphere at a high rate. Jenifer and Diane are two plant biologists who want to know if the aspen leaf miners are affecting leaves' ability to do their job. Trees rely on their leaves to capture light energy for photosynthesis. During this process, cells convert CO_2 gas from the atmosphere into sugar. Gases enter and leave through pores called **stomata** - CO_2 comes in, and water vapor leaves.

Left and middle: Diane and Jenifer taking measurements in the field; Right: a mined aspen leaf. Photo credits left to right: D. Wagner, D. Kind, D. Wagner.

Name___________________

To avoid excessive water loss, leaves carefully control when the stomata are open. Stomata are surrounded by two sausage-shaped cells, called **guard cells**. The default position keeps the stomata closed. Different signals can cue the guard cells to open, but if too much water escapes, the stomata will close up again.

In aspen trees, the guard cells are only on the bottom of the leaf. Jenifer and Diane suspected that leaf miners on the bottom side of the leaf would be destroying guard cells as they feed. They thought that if guard cells are no longer able to function properly, they would get stuck in the default closed position. This would limit photosynthesis because they would no longer be able to take in CO_2 or let out water vapor.

To test their idea, Jenifer and Diane set up an experiment on the University of Alaska campus in Fairbanks. Jenifer and Diane let leaf miners lay eggs on leaves, like normal. Each caterpillar is so small that it stays on a single leaf side to eat. Jenifer and Diane removed eggs and marked about 10 leaves on each of 14 aspen trees. They randomly assigned each leaf to one of three treatments by removing the leaf miner eggs from either the top, bottom, or both sides of the leaves. To remove eggs from leaves, they wore magnifying headsets, just as a jeweler might wear, and carefully scraped each tiny egg off the leaf using a sharp probe. By doing so, they had leaves that were 1) mined on the bottom surface only, 2) mined on the top surface only, and 3) unmined (control) leaves.

Leaf miner trails on the front (right) and back (left) of aspen leaves.

After the leaf miner caterpillars were finished feeding for the season, Jenifer and Diane came back to assess the damage. To understand the effect of the leaf mining damage on the leaves, they measured the **photosynthesis** and **stomatal conductance of water vapor** in the different treatments. To do this, they used a special piece of equipment called a portable infrared gas analyzer. The analyzer has two chambers - one that can be clamped onto a single leaf and one that is a control with no leaf. Gas is pumped through both chambers, and the gas concentrations from the leaf and the control are recorded and compared. The difference in CO_2 concentration between the chambers is used to calculate the rate that CO_2 is taken in by the leaf from photosynthesis. The difference in water vapor is used to measure how easily water vapor is passing out of the leaf through the stomata.

Jenifer and Diane predicted that if the stomata are stuck closed, less CO_2 would be taken from the atmosphere and less water vapor would be lost from the stomata. In other words, leaves with leaf miners on the bottom would have lower photosynthesis rates and decreased stomatal conductance of water vapor compared to the other treatments.

Name___________________

Scientific Question: How does herbivory by aspen leaf miners affect photosynthesis?

What is the hypothesis? Find the hypothesis in the Research Background and underline it. A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies.

Scientific Data:

Use the data below to fill in the summary table and answer the scientific question:

Photosynthesis rate			
Tree number	Leaf Mining Treatment		
	Bottom only	Top only	None
1	11.7	12.1	15.1
2	5.2	9.5	10.5
3	10.0	11.9	15.2
4	9.5	11.1	14.3
5	13.9	14.7	15.5
6	14.8	13.1	16.9
7	11.1	13.2	15.3
8	8.9	11.3	12.4
9	9.5	11.1	13.7
10	10.5	11.2	10.0
11	9.4	11.4	13.6
12	7.2	8.4	7.8
13	8.4	10.5	12.2
14	8.5	10.1	12.9

Note: the units for photosynthesis rate are micromoles per square meter per second

Stomatal conductance of water vapor			
Tree number	Leaf Mining Treatment		
	Bottom only	Top only	None
1	0.26	0.34	0.31
2	0.08	0.22	0.22
3	0.17	0.29	0.22
4	0.20	0.31	0.24
5	0.33	0.35	0.48
6	0.32	0.38	0.31
7	0.25	0.32	0.34
8	0.18	0.24	0.25
9	0.20	0.28	0.26
10	0.16	0.17	0.19
11	0.23	0.37	0.29
12	0.10	0.09	0.12
13	0.16	0.28	0.24
14	0.16	0.22	0.22

Note: the units for stomatal conductance are moles per square meter per second

Name___________________

	Photosynthesis rate		
	Leaf Mining Treatment		
	Bottom only	Top only	None
Mean			
Standard deviation	2.5	1.6	2.5
Standard error	0.7	0.4	0.7

	Stomatal conductance of water vapor		
	Leaf Mining Treatment		
	Bottom only	Top only	None
Mean			
Standard deviation	0.07	0.08	0.08
Standard error	0.02	0.02	0.02

*Standard deviation (SD) tells us about the amount of variation in the data. A large SD means there is a lot of variation around the mean, while a small SD means the data points all fall very close to the mean. Standard error (SE) is SD divided by the square root of the sample size (N) and tells us how confident we are in our estimate of the mean. A large SE means we are not very confident, while a small SE means we are more confident.

What data will you graph to answer the question?

Independent variable: ____

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Dependent variables: ____

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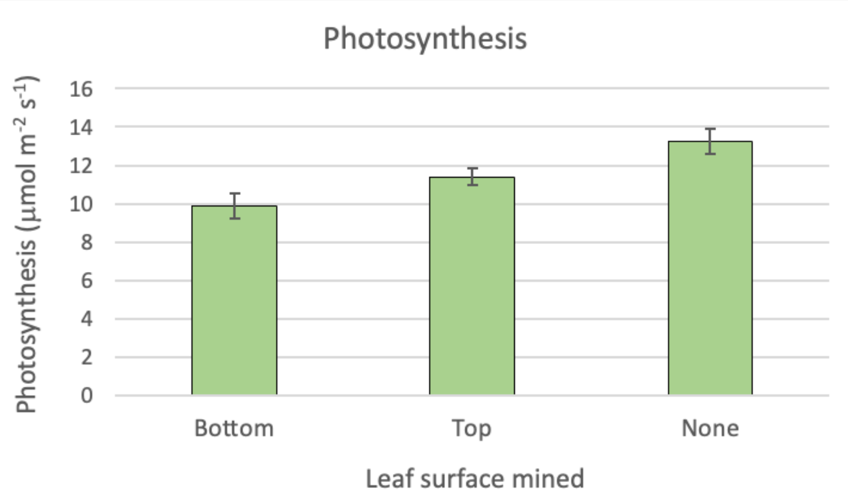
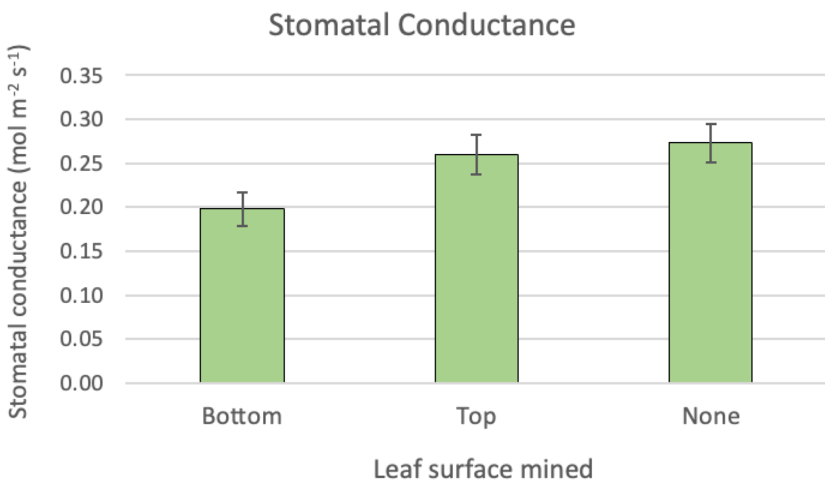
<u>Below are graphs of the data</u>: Identify any changes, trends, or differences that you see in your graph(s). Draw arrows pointing out what you see, and write one sentence describing what you see next to each arrow.



Name___________________



Interpret the data:

Make a claim that answers the scientific question - How does herbivory by aspen leaf miners affect photosynthesis?

What evidence was used to write your claim? Reference specific parts of the table or graph(s).

Name___________________

Explain your reasoning and why the evidence supports your claim. Connect the data back to what you learned about how guard cells determine the flow of gases, such as CO_2 and water vapor.

Did the data support Jenifer and Diane's hypothesis? Use evidence to explain why or why not. If you feel the data are inconclusive, explain why.

Your next steps as a scientist: Science is an ongoing process. What new question(s) should be investigated to build on Jenifer and Diane's research? How do your questions build on the research that has already been done?