Plant Leaves: Holly Spines vs. Height

SC Academic Standards: 4.L.5A; 5.L.4A-B; 6.L.4A; 6.L.5B; 7.L.3B; 7.EC.5B; H.B.2B; H.B.6A

NGSS DCI: 4-LS1.A; 5-LS2.A-B; MS-LS1-A-C; MS-LS2.A-C; MS-PS3.D; HS-LS1.A; HS.LS2.A-C

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7; S.1A.8

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Structure and Function; Scale, Proportion, and Quantity; and Systems Models.

Focus Question(s): What type of protective adaptations do plants have to deter herbivores?

Conceptual Understanding: The Plant Kingdom consists of organisms that primarily make their own food (autotrophs) and are commonly classified based on internal structures that function in the transport of food and water. Plants have structural and behavioral adaptations that increase the chances of reproduction and survival in changing environments.

Ecosystems have carrying capacities, which are limits to the numbers of organisms and populations they can support. Limiting factors include the availability of biotic and abiotic resources and challenges such as predation, competition, and disease.

<u>Background</u>: The **shoots** (stems and leaves) of a plant are used for photosynthesis and, unlike roots, are covered by a waxy **cuticle** which helps to prevent transpiration of water. However, this protective cuticle can limit gas exchange (and plants need to take in CO_2 from the air and respire O_2 out. So, the cuticle has tiny chlorophyll containing cells called **guard cells** that together in pairs can create an opening between them – and when open, gases can be exchanged (CO_2 in, O_2 out). The opening is called a stomate (**stomata**, pl.) and is generally found on the underside of the leaf. The other cells you will find on the leaf's surface are the **epidermal** cells, which secrete the waxy cuticle. The epidermal cells are clear (they do not contain chloroplasts or chlorophyll) and act to focus light towards the mesophyll cells. Cells between the top and bottom epidermal layers are mesophyll cells, and contain chlorophyll and do photosynthesis. Leaves of some species, like the holly and magnolia, are thick and leathery, mainly as a way to keep critters from eating them. In addition some plants have evolved teeth or spines as an adaptive feature to reduce predation.

Spiny leaves are widely believed to function as a deterrent against browsing (herbivory). In fact, holly shrubs with very spiny leaves were browsed less often than less spiny shrubs. And, in the absence of browsing ungulates, the spinescence of holly leaves decreased. Browsed shrubs exhibited reduced annual shoot growth, increased branching, and produced smaller leaves with high spinescence.

While the spines of holly leaves are set too far apart to deter insect herbivores, they have been found to interfere with browsing by ungulates. Spines are more effective against the larger vertebrates than smaller invertebrates because the spines are too large and far apart to deter small invertebrates, such as caterpillars, and so vertebrate herbivores exert a stronger and more consistent selection on leaf spines than invertebrate herbivores.

Holly is just one example of the fluctuations in shape and form of an organism; the variation that any species can exhibit. It is important to recognize that variation, or plasticity, is normal, and often relates to different environmental conditions (including competition and predation). The ability of an organism to change its characteristics in response to environmental variations is known as phenotypic plasticity and it is a key driving factor in the evolution of a species. Leaf plasticity in plants can be considerable – thinking about holly, their leaves may vary in:

- The number of spines on a leaf
- The number of spines on each side of the leaf
- The length of the leaf (do longer leaves have more spines?)
- The shape of the leaf
- The 'greenness' of the leaf – depending on whether the leaf develops in shade or full sunlight
- The shininess of the leaf – the thickness of the waxy cuticle
- The holly tree / shrub itself can vary in sex (it comes in male or female trees).

In this lab we will answer the questions: Are the leaves and number of spines the same on male and female holly plants? Is the number of spines the same on leaves found at all heights on the holly tree?

Materials: Access to a tall, unpruned, holly tree, plant loppers or shears, and a ladder (or, the every-handy saw on a pole from Lowes). If necessary you can remove the leaves ahead of time and bag them (in a paper bag to let them dry and reduce mold / rot) – you will want a minimum of 20 holly leaves of about the same size, cut from a range of heights above the ground (e.g. 0.5m, 1.5m, 2.5m, 3.5m and 4.5m). You will also need a computer with excel to run a t-test, and, if measuring the height of a tree that you won't be climbing, a ruler and calculator.

Previous Knowledge: (plant biology): Many flowering plants are monoecious and produce both male and female flowers on the same plant (hermaphroditic plants have “complete” flowers with male (stamen) and female (carpel) parts in the same flower). Examples of monoecious plants include the oaks, beeches, walnuts, and many gymnosperms like pines and cedars, which produce both male and female cones (not flowers) on the same tree. However, there are some plants that are dioecious, and produce male flowers on the male plant and female flowers on the female plant. These plants include holly, ash trees, and persimmon plants. Since the pollen from the male flower travels to and pollinates / fertilizes the female flower, and the seed develops in the ovary of the female flower, with the ovary swelling into the fruit, it is easy to see which plant is the female: the plant that produces fruits is always female. Female holly trees develop green berries that ripen into red berries in autumn.

Procedure:

1. Find one tall, un-pruned, holly tree. Point out that this means the branches are genetically the same, environmentally the same (light, minerals, water, temperature, sunlight etc.)

2. Choose leaves randomly (don’t just pick ones at the tip of a branch, or have a color you like). Be careful to control for leaves in shade or sunlight – Discuss why this is important.

3. Select 10 leaves from each of 5 height levels: 0.5m, 1.5m, 2.5m, 3.5m and 4.5m. You may want to let students collect the low leaves, and have some branches, labeled, that you picked earlier, with the aid of a ladder, available.

4. If using the saw on a pole method to cut down branches (rather than climbing a ladder) it may be necessary to calculate the height of a tree. You can do this using the shadow method, or, on a cloudy day, using a ruler:

    a. Shadow Method: On a sunny day you can determine the height of a tree by comparing the length of the shadow of a friend and the shadow of the tree. First measure the length of your friend's shadow and then measure the length of the tree's shadow. The trick is to figure out how many of your friend's shadows it would take to fill the tree's shadow. To do this simply divide the length of the tree's shadow by the length of your friends's shadow. Now all that remains it to multiply your friend's actual height by how many friend shadows you needed to fill the tree's shadow.

    b. “Ruler” method: First, have a friend stand with their back to the tree. Then you hold a ruler at arms length from your eye and walk backwards until the entire tree fits in 10" of the ruler. Measure where the top of your friend’s head (standing by the tree) is on the ruler. Your friend will only appear to be a few inches tall. Divide the 10" of the ruler by the apparent height of your friend. Now measure the actual height of your friend by the tree. Multiply this number by the previous measurement. That will be the height of the tree! Hint: it is easier to make all measurements in inches, then divide by 12 to determine feet. A little harder, but more accurate than the previous method.

c. "Indian" method: While bending over and looking between your legs, walk away from the tree until the top comes into view. Stop! At this point the height of the tree will be equal to your distance from the tree. Not so accurate, but fun!

5. Count the number of spines per leaf, and calculate and average number of spines for each height.

Height above ground (m)	**# of spines on leaf**					**Average number of spines**
0.5						
1.5						
2.5						
3.5						
4.5						

Table 1. Number of spines on holly leaves versus height of leaf on tree.

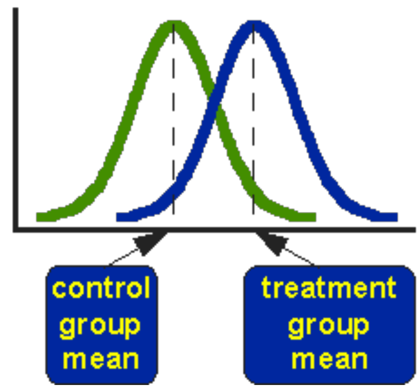
<u>**Data Analysis**</u>:

- First, you want to graph the number of spines on the Y axis versus height from which leaf was taken on the X axis. Note that each group has ten leaves, and the class as a whole has many more per height level.
- Once you have graphed this data, do a t-test to determine if the difference is indeed significant. Use Excel to do the preliminary calculations, and

compare two groups: leaves taken at the lowest level (0.5 m from ground) versus leaves taken from the highest level (4.5 m from ground). Use 25 or 30 as 'degrees of freedom' (leaves cannot have an infinite number of spines; but they could have more than the maximum found in our sample); plot suitable graphs to show each set of results (there is almost **no** overlap).

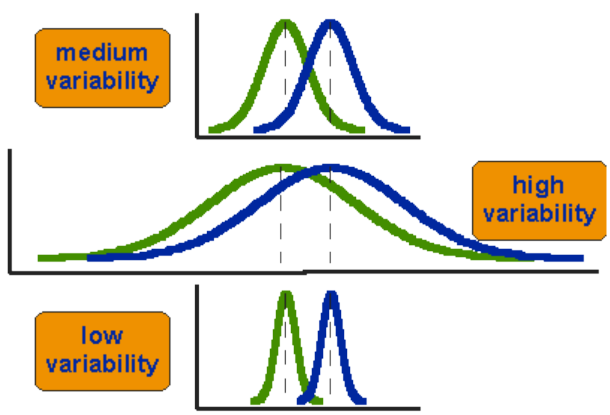
- Calculations sometimes give a t-value of $p > 0.05$ for **personal** (with ten leaves) results (meaning it is not significant), but $p < 0.05$ for **class** results (60 leaves per group) . This shows the vital importance **of sample size and replication.**

- Run a **t-test** to compare the number of leaves on the lowest branches (0.5 m off ground) with number of spines from leaves on the highest branches (4.5 m above the ground).



The t-test assesses whether the means of two groups are ***statistically*** different from each other. This analysis is appropriate whenever you want to compare the means of two groups.

What does it mean to say that the averages for two groups are <u>statistically different</u>? Consider the three situations shown (right). The first thing to notice about the three situations is that ***the difference between the means is the same in all three***. But, you should also notice that the three situations don't look the same -- they tell very different stories. The top example shows a case with moderate variability of scores within each group. The second situation shows the high variability case. The third shows the case with low variability. Clearly, we would conclude that the two groups appear most different or distinct in the bottom or low-variability case. Why? Because there is relatively little **overlap** between the two bell-shaped curves.



In the high variability case, the group difference appears least striking because the two bell-shaped distributions overlap so much. This leads us to a very important conclusion: when we are looking at the differences between scores for two groups, we have to judge the difference between their means ***relative to the spread or variability*** of their scores. The t-test does just this.

The bottom line is that if your t test value is <u>less than 0.05</u> then you are 95% certain that the two groups **are** statistically significantly different – your null (no difference) hypothesis is rejected. Generally, when means are graphed, you will see error bars. If the error bars *Don't Overlap* then usually $p<0.05$ and the means *ARE* significantly different.

<u>Extensions:</u>

1. Do unshaded leaves produce the same number of spines as shaded ones?
2. Are the number of spines dependent on the time of year the leaf was formed?
3. Do longer leaves have more spines?
4. Are the number of spines on each side of a leaf the same on average?
5. Are the length of spines related to the amount of browsing or pruning a stem receives?
6. Is the number of spines related to the sex of the tree? (Holly is either male or female; female Holly trees have red berries, males do not).
7. Use math / proportions to measure tree height.

<u>Reflection Questions:</u>

- **Do holly trees show any pattern with number of leaf spines versus height from which the leaf was taken?** (European holly, *Ilex aquifolium*, like American holly, exhibits higher spinescence on lower branches, and leaves with almost smooth edges occur in the upper canopy (Crawley 1983; Supnick, 1983) Although it is generally accepted that the higher up the leaves the less spines they will have, the relationship is not always straightforward. The number of spines produced varies from one variety to another. Also, constant pruning e.g. to produce a hedge, appears to increase spine production).

- **Can you think of other factors that might cause a difference in number of spines per leaf?**
  1. Do unshaded leaves produce the same number of spines as shaded ones?
  2. Are the number of spines dependent on the time of year the leaf was formed?
  3. Do longer leaves have more spines?
  4. Are the number of spines on each side of a leaf the same on average?
  5. Are the length of spines related to the amount of browsing or pruning a stem receives?
  6. Is the number of spines related to the sex of the tree? (Holly is either male or female).

- **Forming spines takes energy - it is an adaptation. But to what? Why would spiny leaves be an advantage to holly trees?** (Spiny leaves are widely believed to function as a deterrent against browsing (herbivory) by ungulates (rather than insects)).

Models and Explanations: In this lab we explored relationships between number of spines on a holly leaf and the height of the holly leaf on the tree. **A student who demonstrates understanding** of this concept can first explain why some plants have adaptations such as spines (they must be beneficial, else why would a plant waste resources / energy to make them?) and secondly walk the observer through the experimental design, clearly identifying independent, dependent and controlled variables, clearly stating results and conclusions (including mathematical significance).

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Student Worksheet:

Spiny leaves are widely believed to function as a deterrent against browsing (herbivory). In fact, studies show that holly shrubs with very spiny leaves were browsed less often than less spiny shrubs. And, in the absence of browsing ungulates, the spinescence of holly leaves decreased. Browsed shrubs exhibited reduced annual shoot growth, increased branching, and produced smaller leaves with high spinescence. Holly is just one example of the fluctuations in shape and form of an organism; the variation that any species can exhibit. It is important to recognize that variation, or plasticity, is normal, and often relates to different environmental conditions (including competition and predation). Leaf plasticity in plants can be considerable – thinking about holly, their leaves may vary in the number of spines on a leaf, the number of spines on each side of the leaf, the length of the leaf (do longer leaves have more spines?), the shape of the leaf, etc.

This investigation is being carried out to see if there is a significant difference between the number of spines on a leaf and its height above the ground.

Question:

Hypothesis:

Prediction:

Experimental Design:

Results: **t-test result: p < or > 0.05 ?**

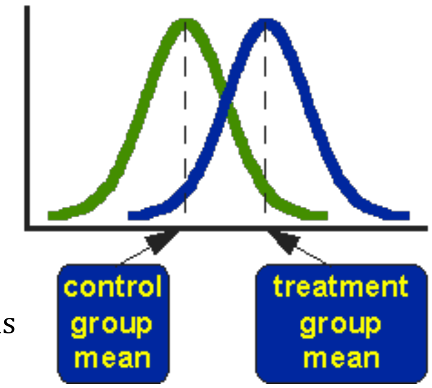
Is the number of spines on low branches significantly different from the number of spines on high branches? ___________________

Height above ground (m)	# of spines on leaf					Average number of spines
0.5						
1.5						
2.5						
3.5						
4.5						

Table 1. Number of spines on holly leaves versus height of leaf on tree.

Conclusion:

Design your own experiment: Does the number of spines on holly leaves differ between male and female trees?



Run a **t-test** using a computer stats program (such as excel) to compare the number of leaves on the lowest branches (0.5 m off ground) with number of spines from leaves on the highest branches (4.5 m above the ground).

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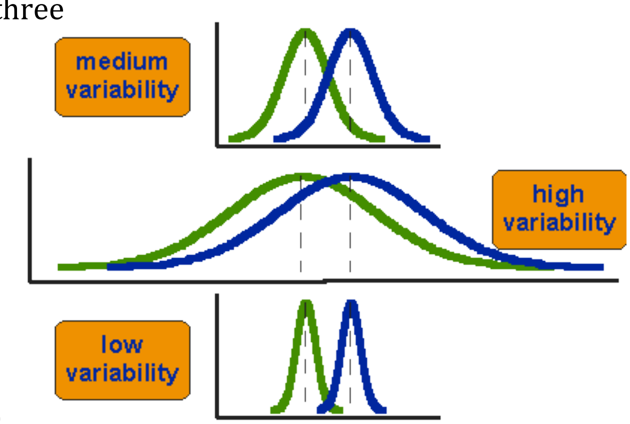

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