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Milkweed Measurement Protocol

Notes: This protocol can be used to measure the wild plants from which you collect your seedpods, as well as your annual measurements on the plants growing in your experimental plot every spring and fall. Data can be collected using essentially the same protocol, with modifications for the wild milkweed measurements shown in square brackets [ ] below. For measuring wild plants, we will count each stem individually. Even though multiple stems may connect underground in the same plant, we can't tell which are related from above ground. Please submit these data on the "Wild Milkweed Seed Collection Data Form." If you wish to measure more wild stems than you collect seed pods from, please continue to give each stem a unique number.

All measurements should be made on milkweed plants annually in the fall and in the spring. In the fall, attempt to measure plants approximately 3-6 weeks prior to the date you collected the seeds originally, with the intention of collecting data as seedpods near maturity, but prior to the release of any seeds. The ideal date may vary annually depending on environmental conditions. In the spring, complete measurements ~2-5 weeks after the date of first frost.

Note that weeds may colonize the pots where you planted your milkweed. Weeds can be problematic because they confuse students who are not familiar with distinguishing milkweed from weeds, especially in the first years of growth when milkweeds are still establishing. We recommend covering the plot with weed suppressor material to facilitate the growth of your milkweeds early on. Also, it is very useful for the lip of the pot to be visible above the soil during sampling. Finally, in the fall, plants are beginning to senesce, so they may be fragile and leaves may fall of easily. Encourage care and ask students to record as notes any damage they inflict on their plant to facilitate resolving accuracy issues once all the data are entered.

Ideally, three students should perform each measurement independently, and the data from each of the measurements should be entered and checked during the data entry process. For all continuous quantitative data EXCEPT total fruit mass, estimate one digit beyond what the measurement tool (ruler, tape measure, etc.) provides. Students should record data in the provided Data Sheet. If possible, these data should be compared against previous measurements to ensure the data are reasonable. The site coordinator should review the data prior to final submission. Missing values, or those deemed unreliable by the site coordinator, should be entered as NA. Please record any notes in the space provided.

For visual step-by-step guides, please see the "Milkweed Measurement Series" videos.

The measurements below are designed to help us understand milkweed fitness, or how well an individual is able to reproduce, and herbivory, or how much an individual is eaten by other organisms. Because milkweeds reproduce for many years, we need to have good measures of growth as well as measures of seeds produced. It would be better to count the seeds, but previous research has shown that the number of seed pods can often provide a good estimate of seed production (La Rosa Dissertation 2015). Herbivores can reduce a plant's fitness by eating parts of the plant.

For each plant, record your site code (using ALLCAPS), plot number, date (month/day), year, plant source ID number, location number, and the initials of the recorder and the measurers. Then measure and record the following.

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I. Fitness Measures

Common milkweed can branch underground to make multiple stems, but these stems are all part of the same plant. For most fitness measurements and all herbivory measurements, we will ask you to record values for the ***whole plant*** *by looking at all of the stems on the plant. For some fitness measurements, we will ask you to record the value for the* ***largest stem*** *only.*

Ia. Whole Plant Measures

A. Number of Stems.

1. Count the number of stems separate emerging from the soil in the pot and record this number. Be sure you are counting only common milkweed stems, and not stems of a weed. Also, a single milkweed stem may have multiple branches. If it branches above ground, these are all counted as part of the same stem.
   [If you are measuring wild plants, count the number of stems within a 5 meter radius of your plant. Because milkweed can spread clonally underground and it is impossible to tell which stems belong to the same plant in the wild, all of the remaining measurements will be made on a single stem.]

B. Total Number of Nodes.

1. A node is the place on a plant where the leaves attach to a stem. Even after the leaves fall off, the nodes remain as bumps on the stem. In milkweed plants, leaves are opposite, so nodes are typically found across from each other, in pairs, going up the stem.

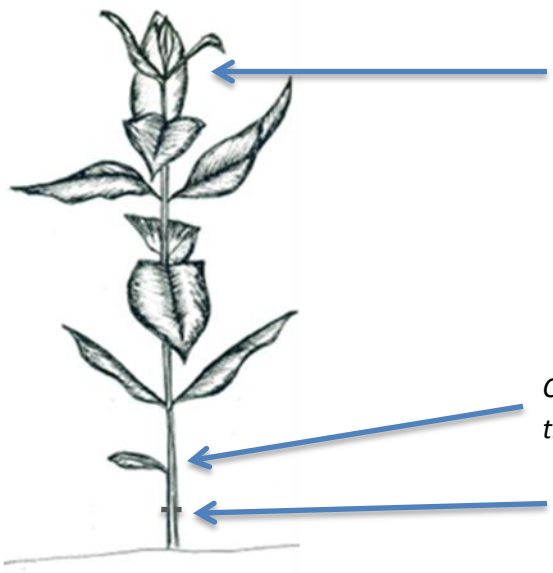

Illustration by Jacqueline Nuzzo

2. Be sure you count each node individually, not each pair of nodes.

3. Starting at the base of the plant where it meets the soil, count each individual node, regardless of whether or not there is a leaf attached.

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4. If the plant has branches, count the nodes systematically on each of the branches and the main stem.

5. If two pairs of nodes are less than 1 mm apart, count the lower pair of nodes but NOT the higher pair of nodes.

6. Record the total number of nodes on the whole plant.

C. Number of Leaves.

1. Starting at the base of the plant where it meets the soil, count each leaf. Even if a leaf is shriveled/brown but remains attached to the plant when you touch it lightly, then count it.

2. Be sure to count each individual leaf, not each pair of leaves.

3. If the plant has branches, count the leaves systematically on each of the branches and the main stem.

4. If two pairs of nodes are less than 1 mm apart, count the leaf/leaves on the lower pair of nodes but NOT the ones on the higher pair of nodes.

D. Total Fruit Number.

1. Starting at the base of the plant where it meets the soil, count each fruit, or seed pod, regardless of how fully formed it appears.

2. Record the total number of fruits on the plant.

E. Total Fresh Fruit Mass (g).

1. NOTE: only the last class or period to perform measurements should measure and record total fresh fruit mass.

2. Remove the fruit, or seed pods, from your milkweed plant by cutting them at the base of the fruit and collect them into a labeled bag or container. Remove all seed pods regardless of how fully-formed they appear.

3. Back in the classroom, mass all of the fruits from one plant together; repeat for all milkweed plants. Record the masses in the data sheet.

4. Dispose of the seedpods as instructed by your teacher. The purpose of harvesting all of the seedpods is to prevent non-local seeds from spreading through the site.

F. Leaf Tissue Chemical Analysis. (In development; probably involves collecting a leaf sample to send in.)

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Ib. Largest Stem Measurements.
For all subsequent fitness measures, if there is more than one stem, record the measurement for the largest stem.

G. Stem Height (cm).

1. Using a meter stick, measure from the base of the plant where it meets the soil to the highest node. Record your measurement in centimeters.

2. It is important not to measure to the tip of the highest leaf; instead, only measure to the highest node. If two nodes are less than 1 mm apart, measure to the lower node- the higher node does NOT count.

H. Stem Diameter (mm).

1. Measure the diameter of the largest stem 1 cm above the soil line using one of the methods below.

a. *Caliper Method:* Using a pair of calipers, gently measure the diameter of the stem at the base of the plant 1 cm above the soil. For more information on using calipers, please see the following video: https://www.youtube.com/watch?v=FNdkYIVJ3Vc.

b. *Alternative Tape Measure Method:* Using a flexible tape measure, measure the circumference of the stem at the base of the plant 1 cm above the soil. Divide your circumference by π (3.14) to obtain the diameter.

c. *Alternative String Method:* Using a piece of string, wrap the string around the milkweed stem at the base of the plant 1 cm above the soil, then measure the piece of string against a ruler to determine the circumference. Divide your circumference by π (3.14) to obtain the diameter.

2. Record the diameter in mm, and then record the method used to measure the stem diameter.

I. Length (cm), Width (cm), and Area of Largest Leaf (cm^2).

1. Locate the largest leaf on the stem. Measure the length as the distance, in centimeters, from the tip to the place where the blade of the leaf ends and meets the petiole, or stem. Measure the width at the widest point in centimeters.

2. (***OPTIONAL***) To calculate the area of the largest leaf, trace the outline of the largest leaf on a piece of graph paper. If there is damage to the leaf, extrapolate to where you believe the leaf should have grown to. Do not draw the stem, or petiole, of the leaf, but end your drawing of the leaf where the blade of the leaf meets the stem. Use care because leaves are easily detached from the plant.

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3. Record whether or not you needed to extrapolate the leaf area due to damage. Choose NA if you did not measure leaf area.

The following steps may be completed when you return to class.

4. Print out the "1 cm Standard Graph Paper." Cut out an area that is 10 cm x 10 cm. Mass the 10 cm x 10 cm area.

5. Cut out the outlines of the largest leaf, and mass it.

6. Using dimensional analysis, calculate the area of the largest leaf.

$$\frac{\text{Mass of 100 square cm}}{\text{Mass of traced leaf outline}} = \frac{\text{100 sq. cm}}{\text{Area of leaf}}$$

7. Record the area of the largest leaf.

II. Herbivory Estimates
*Herbivory estimates should be made by evaluating the **whole plant**, not just the largest stem.*

A. Number of Leaves with Chewing Damage.

1. Examine leaves for chewing damage. Chewing damage is identifiable as an area where leaf tissue was obviously removed, usually leaving a ragged edge. Leaf damage might be on the edge of the leaf or an area inside of the leaf.

2. Record the number of leaves with chewing damage.

B. Number of Leaves with Weevil Damage.

1. Examine leaves for weevil damage. Weevils damage leaves by chewing the midrib on the underside of the leaf, leaving holes and dried latex behind.

2. Record the number of leaves with weevil damage.

C. Number of Leaves with Leaf Miner Damage.

1. Examine leaves for leaf miner damage. Leaf miner damage is identifiable as a small "bubble" on the leaf inside which the leaf miners burrow and leave their droppings behind. Often they are discolored compared to intact leaf tissue.

2. Record the number of leaves with leaf miner damage.

D. Number of Leaves with Mollusk Damage.

1. Snails and slugs are mollusks that leave slime trails behind them. They typically cause skeletonized damage on the leaves, meaning they cut out lots of little holes on the inside of the

leaf. Don't confuse this with damage by the colonies of tussock moth caterpillars. You'll know it's mollusk damage if you see the slime trails.

2. Record the number of leaves with mollusk damage.

E. Presence/Absence of Insect-Imposed Stem Damage.

1. Examine the stem for insect-imposed stem damage. Scars on the stems of milkweed plants typically indicate that weevils have been around, with sometimes severe impacts on plant growth. Weevils are known to damage the stems in one of two ways, by leaving a long **trench**, or by leaving a series of rings around the stem, called a **girdle**.

2. Select whether there was a trench, girdle, other damage, or no damage

F. Presence/Absence of Leaf Curling.

1. Some milkweed plants have highly curled leaves. This can be the result of viruses, aphids, or spider mites. Record YES if it is present and NO if it is absent.

G. Presence/Absence of Leaf Spots.

1. Dark spots or patches can occur on milkweed leaves for many reasons, ranging from disease to ozone damage to sticky patches of mold. Record YES if it is present and NO if it is absent.

H. Presence/Absence of Invertebrates.

1. Determine if there are any invertebrates on the plant- they may be on the stem, top of the leaves, or underside of the leaves.

2. Determine which invertebrates are present using the attached guide and photos. For each of the following groups, record YES if it is present and NO if it is absent:

a. Monarch caterpillars
b. Milkweed bugs (note that larvae look different)
c. Tussock moth larvae
d. Red milkweed beetles
e. Weevils
f. Aphids
g. Ants
h. Ladybeetles
i. Bees
j. Spiders
k. Snails/slugs
l. Other

3. If you have aphids on your plant, record the color. Select all that apply.

a. Bright Yellow
b. Green or Brown
c. Clear or Orange
d. NA

I. Notes. Record notes about things that happened or you observed but did not record elsewhere. For example, you can record that you accidentally removed a leaf or broke part of the plant. Also record any questions you have developed to share with your class.

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Thank you!