

Milkweed Seed Collection Protocol

Notes: Because we are interested in local adaptation, it is important to collect seed from **naturally occurring** *Asclepias syriaca* populations (remnant prairie or naturally colonized sites like old fields or roadsides) rather than planted populations. For the local adaptation experiment, it will be necessary to transplant milkweed seedlings into a common area where you will be able to measure them annually with students. It's great if seedlings can be transplanted into the original collection site (in which case you fill out just one Site Data Form), but any local site with similar conditions will provide useful information. It is more important to collect naturally occurring seeds than to collect seeds from the site into which you will plant. If you collect from a different site than you plant, you will simply fill out two Site Data Forms, one for collection and one for transplantation.

We ask that you collect seed pods from 5 different stems that are ≥ 5 meters apart. Nevertheless, if that is not possible, we will do our best to work with the seed pods you can collect, as long as all the associated data are available.

Site Selection and Characterization. Please record this information on the **Site Data Form**. You will need to decide what the boundaries of your collection site are.

1. Choose a site for collection, based on criteria above. Give your site a 3-6 letter name (e.g. STO or STOLAF) and record it in all caps on the **Site Data Form** online. Under seed collected, record Y for yes. You may transplant seeds at the same site or a different site. If you know you will be transplanting at this site, you may mark yes under "Transplant Site". Otherwise, you may mark know, or UNK, if it is unknown where you will transplant your seeds.
2. Identify common milkweed, *Asclepias syriaca*, at the site. As there are multiple milkweed species that may co-occur, be sure to collect seeds only from *Asclepias syriaca*. Similar-looking species include showy milkweed, *A. speciosa*, and poke milkweed, *A. exaltata*. In addition to the curriculum materials, the following resources may be useful to help identify *A. syriaca*, or common milkweed:
<http://www.monarchwatch.org/milkweed/guide/syriac.htm>
<http://extension.psu.edu/pests/weeds/weed-id/common-milkweed>
http://www.wildflower.org/plants/result.php?id_plant=ASSY
3. Record the origin of the milkweed plants at the site (N=Native, COL=Colonized Naturally, PL=Planted, UNK=Unknown.)
4. If seeds were planted, please note where the seeds were Collected or Purchased. If not planted or not applicable, record NA. If unknown, record UNK.

5. If planted, record the year the milkweed established at the site. If seeds were sown in the fall, record the following year as the year of planting. If not planted, record NA. If unknown, record UNK. If planting occurred over multiple years, record a list or a range.
6. Record the type of site (RESP=Restored Prairie, REMP=Remnant Prairie, OF=Old Field, RD=Roadside, FORE=Forest Edge, Other:___)
7. Record the size of the site in hectares. To convert acres to hectares, divide the size in acres by 2.47. You may use these directions to find the size of your site.
http://erenweb.org/wp-content/uploads/2012/08/EREN-PFPP_Appendix-III_WebSoilSurveyAreaCalc_30July2012.pdf
8. Estimate the number of flowering stems of common milkweed, *A. syriaca*, at the site. Record L for less than 30, M for 30-1,000, and H for >1000. If it is not possible to estimate, record NA.
9. Record whether other milkweed species are present at the site. Y=yes, N=no, UNK=unknown.
10. If other milkweed species are present at the site, please list them.
11. Identify the dominant type of vegetation at the site, based on cover. If the site is an edge/boundary, you may record two of the following separated with a slash(/). NG=native grasses, NH=native herbaceous plants, NW=native woody plants, IG=introduced grasses, IH=introduced herbaceous plants, IW=introduced woody plants, Other_____
12. If possible, identify the dominant species at the site based on cover. If unknown, record UNK.
13. Disturbance History. Record the most recent year each of the following types of disturbance has occurred at the site: burning, mowing, plowing, spraying with herbicide. If the disturbance has never occurred, record 0. If the history is unknown, record UNK. Record other notes or other types of disturbance in the disturbance notes column.
14. Take a picture of the site of collection site where a milkweed plant is included but the surrounding vegetation is as well. Upload it to the photos folder. Label the file with the site name;_your initials;_date.
15. Complete the soil sampling steps below.

Soil Sampling

1. Select about 3 different locations within your site from which to collect soil.
2. Collect > 1 cup of soil from these locations
3. Mix the soil in a bucket or container, and bring it back to your lab or classroom.
4. Use a small amount of soil (about 10 g) to calculate soil moisture as mass of water in the sample/mass of dry soil.
 - a. Use an aluminum crucible or other heat resistant container to hold the soil. Crumbling the soil will help it to dry more efficiently.

- b. Mass the crucible and record the mass to the nearest hundredth of a gram:
 - c. Add soil to the crucible and record the mass (add about 10 grams of soil):_____
 - d. Calculate the wet mass of the soil as the difference (c-b). Record it here: _____ and in the google Site Data Form.
 - e. Place the sample and in an oven at 105 degrees C for ~48 hours. Allow the samples to cool, weigh to the nearest hundredth of a gram.
 - f. Subtract the mass of the crucible (step b) from the new mass, and record the dry mass of the soil in the google Site Data Form.
 - g. Calculate soil moisture as mass of water (d-g)/mass of dry soil (g), and record in google Site Data Form.
4. Dry the remaining soil, collect it, and send it to Emily Mohl at the address on the top of the page.
 - a. Label your soil sample with the site name and date of collection.

Note: If you collect and plant at separate sites, please send a soil sample for each site.

Seed Collection. Please print and take the Seed Collection Data Sheet and at least 5 Wild Milkweed Data Sheets with you to the field. Also be sure to bring your milkweed measurement guide. You will mail in the Seed Collection Data Sheet when you send in your seeds, and you will submit the data online on the **Wild Milkweed Seed Collection Data Form**, completing one form for each stem from which you collect seed pods.

1. Identify **at least 5 stems** that are producing seed pods and are separated by at least 5 meters. *A. syriaca* is clonal, so the distance is intended to ensure that collections are from genetically distinct individuals. Record the location of *each stem* using GPS coordinates in decimal degrees. It is often possible to find GPS coordinates on your phone. Please follow the directions on the Great Lakes Worm Watch site for how to find and record coordinates using GPS (Step 5) or iTouch Maps (Step 6): http://greatlakeswormwatch.org/team/single_plot.html#d4.
2. Only collect seed pods when they are mature (see Figure). Pods are ready when the seam splits when you press on it. If the pods are not ready yet, you may put a rubber band on the pods to help prevent seeds from dispersing before you can collect the pod. Avoid collecting



- pods with milkweed bugs or milkweed bug damage, as these seeds may not be viable. Collect **at least one seed pod** per milkweed stem, and no more than 1/2 of the pods from an individual plant.
3. When seed pods are ready, collect pods **individually** (one pod in a bag) into labeled paper lunch bags. Label each bag with
 - a. The stem number (1-5)
 - b. The pod number
 - c. The site name
 - d. The coordinates of the plant
 - e. The date of collection
 4. Record all of the collection data for each pod collected. Record any notes for each seed pod.
 5. For each of the stems from which you collect seeds, please complete a set of Wild Milkweed Measurements using the Milkweed Measurement Protocols and the Wild Milkweed Data Sheet.
 6. As with other milkweed measurements, if students are taking the data, we ask that at least 3 students/groups of students make independent measurements on the same plant.
 7. Enter the data into the Wild Milkweed Seed Collection Form. You will complete the form at least once for each stem from which you collect seed pods. If additional measurements are taken on a stem, they may be submitted using the same form, simply recording "0" for the number of pods collected.
 8. Send the processed seeds (below) along with your Seed Collection Data Sheet to Emily Mohl at the address at the top of the page..

Seed Processing. We prefer to receive seeds that are separated from the "fluff".

9. It may be possible to simply strip the seeds from the pappus (fluff/hairs) if the pod is newly opened.
10. If this is not the case, add a coin or two to with the seeds to a paper lunch sack and shake vigorously. Cut a small hole in the corner to pour out the seeds. The procedure may need to be repeated several times until most seeds have been collected.
11. Allow seeds to dry completely if they are not already dry. Put the seeds into a Ziploc bag labeled with the same information as identified in step 3 above. Use a separate bag for each pod collected.
12. Record any notes about the collection or processing of each seed pod on the data entry forms online: you should complete one form for each seed pod collected.
13. Mail the seeds and the printed and completed Seed Collection Data Sheet to:
Emily Mohl
Biology Department
St. Olaf College

Emily Mohl
mohl@stolaf.edu; 773-729-0617 (c)

St. Olaf College-Biology Dept.
1520 St. Olaf Ave. Northfield, MN 55057

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14. Send any questions to mohl@stolaf.edu.

We will mass and count the seeds upon arrival. They will be stored until they are needed for the local adaptation transplant experiment. In March, we will send each participating site 5 packets of seeds mixed with sand. These can be stored in the refrigerator until you are ready to cold stratify them with water. At about 2.5 months prior to planting, you need to add water to dampen the sand in each bag before returning them to the refrigerator for 4 weeks.

Planting Site Selection. For Part 2 of the local adaptation experiment, you will need to select a site to plant your seedlings. The site should be flat, mowed and at least 4x5 meters to accommodate your plot. You may wish to create up to 3 additional plots, depending on resources available to you and your seed germination rates.

Contact Emily Mohl at mohl@stolaf.edu or 773-729-0617 with any questions. Thank you!