Milkweed Seed Collection Protocol

***Notes***: Because we are interested in local adaptation, it is preferable to collect seed from **naturally occurring** *Asclepias syriaca* populations (remnant prairie or naturally colonized sites like old fields) 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. Ideally, seedlings would 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 (see methods for characterizing site conditions). 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 plants at the same site if you would like to participate in the reciprocal transplant experiment. Nevertheless, collecting and submitting a single seed pod from a single plant at your site is a valuable contribution, as long as all the associated data are available.

**Site Selection and Characterization**. You will need to decide what the boundaries of your collection site are, but please restrict the boundaries to the area where *A. syriaca* are found. Step 11 is optional; data will be collected remotely based on coordinates submitted in step 1 of the Seed Collection protocols (Complete only if you want to use this as a learning activity). Many of these methods are based on the EREN PFPP protocols.

1. Choose a site for collection, based on criteria above. Give your site a name (e.g. STOLAF) and record it in all caps on the **Site Data Form** online. Under seed collected, record Y for yes. Leave the transplant column blank.
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>

1. Record the origin of the milkweed plants at the site (N=Native, COL=Colonized Naturally, PL=Planted, UNK=Unknown.
2. 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.
3. 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.
4. Record the type of site (RESP=Restored Prairie, REMP=Remnant Prairie, OF=Old Field, RD=Roadside, FORE=Forest Edge, Other:\_\_\_)
5. 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>

1. 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.
2. Record whether other milkweed species are present at the site. Y=yes, N=no, UNK=unknown.
3. If other milkweed species are present at the site, please list them.
4. 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=non-native grasses, IH=introduced herbaceous plants, IW=introduced woody plants, Other\_\_\_\_\_\_
5. If possible, identify the dominant species at the site based on cover. If unknown, record UNK.
6. 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.
7. OPTIONAL: Record Soil Order and Climate Data for your site. You may complete these methods based on EREN PFPP [**protocols**](http://erenweb.org/new-page/carbon-storage-project/permanent-plot-protocol/pfpp-protocol-files/)**.**

**Seed Collection.** These data will be recorded on the **Seed Collection Data Form**.

1. Identify **at least** **5 plants** 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 plant* using GPS coordinates in decimal degrees. 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/singleplot.html#d4>

If possible, please also mark the plants.

1. Only collect seed pods when they are mature. This occurs 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 will probably not be viable. Collect **at least one seed pod** per milkweed plant, and no more than 1/2 of the pods from an individual plant.
2. When seed pods are ready, collect pods **individually** (one pod in a bag) into labeled paper lunch bags. Label each bag with
	1. The plant number (1-5)
	2. The pod number
	3. The site name
	4. The coordinates of the plant
	5. The date of collection
3. Record all of the collection data for each pod collected.

**Seed Processing**

1. It may be possible to simply strip the seeds from the pappus (fluff/hairs) if the pod is newly opened. If this is not the case, add a coin or two to the paper bag 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.
2. 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.
3. 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.
4. Mail the seeds to:

Emily Mohl

Biology Department

St. Olaf College

1520 St. Olaf Ave

Northfield, MN 55057

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

We will mass and count the seeds upon arrival. We will cold stratify some for experimental study of tolerance. The rest will be labeled and stored in envelopes until they are needed for Part 2—the local adaptation experiment.

**Planting Site Selection.** For Part 2, the local adaptation experiment, you will need to select a site to plant your seedlings. The site should be flat, mowed and at least 4x4 meters to accommodate your plot. You may also create a second plot as a class extension to design your own experiment, as long as the first plot meets these criteria.

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