

The MMMILC Project Protocol (v. 2015-06-22)

1. Training and certification

- a. All participants in the MMMILC Project must complete basic training and the certification exam before collecting data.

2. Milkweed assignments

- a. A set of milkweeds has been randomly assigned to each observer pair for each week of the field season. Each participant is responsible for checking the weekly assignment list each week. The weekly assignment list will be on the shared Google document.
- b. Collect data for this project anytime during the week, making arrangements to coordinate with your partner for field work. All field work should be conducted with at least two people unless absolutely necessary. If you are unavailable for field work, you are responsible for finding another certified participant to take your place for the day.

3. Inventory your field kit

- a. Before going into the field, check the contents of your field kit. Each kit should contain 1 clipboard, 10 blank datasheets, a protocol, pencils, one set of dial calipers, a milkweed field guide, a folding ruler, and a map of the field site.
- b. If you are planning to do field work over the weekend, be sure to check one out of the shared MMMILC Project locker.
- c. Restock the field kit as necessary. Blank forms are available in the filebox; when the blank forms run out, please print out or photocopy new ones. If any field kit equipment is damaged or missing, please email Louie Yang at lhyang@ucdavis.edu.

4. Datasheets

- a. *Use a new datasheet for each day.* Be sure to fill out all fields for all sheets, including the daily observations if you use multiple sheets. You will be entering these data into an online spreadsheet at the end of each day - please write as neatly as possible!

5. Data

- a. **Daily data:** At the start of each field trip, record the following daily observations:
 - i. *names* = names of both observers; note who is recording and who is measuring.
 - ii. *kit.ID* = include the number of the field kit that you used. The field kit number should be on the back of each kit.

- iii. *weather* = a brief description of the current weather, including temperature (in degrees F to the nearest 5°), cloud cover (0-100% to the nearest 10%), wind (in mph to the nearest 5mph) and precipitation (*none*, *light* or *heavy*).
 - iv. *start.time* = approximate start time for the field trip, in 24h format (e.g., "9:30" or "17:15")
 - v. *end.time* = approximate end time for the field trip, in 24h format (e.g., "9:30" or "17:15")
 - vi. *mm.dd.yy* = the date of the observation, written in "mm/dd/yy" format
- b. **General data:** At the beginning of each observation, record (and double-check) the milkweed ID number, and note the general status of the plant. Please check to make sure that the plant is outside of the seedling tube; if not, please remove the wooden stake from the ground and rotate it 180 degrees before putting it back in the hole. The milkweed will then end up adjacent to the sleeve on the outside. Please pull any weeds encroaching on the milkweed; this will help the milkweed, and also make it easier to take your observations.
- i. *milkweed.ID* = the milkweed ID number on the tag, irrigation tubing, or seedling tube
 - ii. *milkweed.status* = If the plant has not emerged aboveground, write "N.E." or if all aboveground parts are dead, write "DEAD". If the plant is otherwise missing (e.g., the planting site is covered with a pile of mulch), indicate "MISSING", and explain in the notes. Otherwise, indicate the percentage of green vs. senescent tissue (0-100% GREEN). When visually estimating data, it is often useful for the pair to generate two independent assessments, then discuss your estimates to arrive at a consensus. Your goal should be accuracy, not precision; in other words, focus on the big picture and don't worry about differences of a few percent.
- c. **Monarch data:** Next, carefully inspect all stems and leaves for evidence of monarch eggs or caterpillars, paying particular attention to the undersides of leaves at the apex of each stem. You may need to carefully move leaves in order to inspect all surfaces of the plant, but try to minimize disturbance. You will be collecting the following data:
- i. *stage.length* = Write "None" if no monarchs are present. For each monarch observed, include the stage and the length in millimeters to the nearest 0.1 mm, separated by a hyphen (e.g., "L1-4.3"). Use the following stage codes *only*: egg (E), first instar (L1), second instar (L2), third instar (L3), fourth instar (L4), fifth instar (L5), pupa (P). You can record live eggs as "E", without a length, but do not count hatched or damaged eggs; if you see multiple eggs, record each one (e.g., "E,E,E"). Measure the length of each larvae using calipers, but try not to disturb the caterpillar; if the caterpillar is curled or inaccessible, estimate the straight length using a caliper for reference. If there are multiple monarchs, separate each stage-length observation by a

comma (e.g., "L1-4.3, L2-9.4"). If you observe adult monarch butterflies (AD) flying near or landing on a plant, record the observation in the Notes section (you don't need to record the size of adult butterflies).

- d. **Milkweed data:** Begin by visually estimating overall leaf damage, the number of flower umbels, and the number of seed pods. You will collect the following data for each plant:
- i. *leaf.dam* = visually estimated percent of leaf area of the entire plant (0-100%) removed by chewing herbivores; this measurement does not include damage due to disease, senescence, people, etc. Look for entire missing leaves, and be sure to factor those into your estimate of percent leaf damage. Discuss your estimate with your partner to reach a consensus estimate.
 - ii. *umbel.count* = estimated number of umbels; only include umbels with at least one open flower (not buds); don't include umbels where all the flowers have dried out.
 - iii. *pod.count* = estimated number of green seed pods larger than 1cm; do not count small, shriveled pods; do not count fully open pods that have already dispersed their seeds.
 - iv. *stem.count* = number of living stems > 5 cm; count side stems >5 cm as separate stems; do not count dead stems. For stems that are partially senescent, consider only the green part of the stem.
 - v. *length.by.stem* = length in cm of all living stems > 5 cm to nearest cm, separated by commas; measure the straight-line distance from stem base to apex. Do not measure stems < 5 cm. If there are more than 10 stems, select 10 representative stems being sure to avoid biased selection (do not select only the largest stems, or the smallest stems, or the healthiest stems, etc; aim to choose the most *representative* set of 10 stems); order does not matter.
 - vi. *stem.dia* = stem diameter at ground level, measured with calipers to the nearest 0.1 mm; keep calipers level when measuring; use the broad part of the calipers if possible to avoid crushing stem; if there are multiple stems, randomly select up to 10 stems; order does not matter.
- e. **Other observations:** This is a section to record all other per-plant observations, including observations about the arthropod community, the soil, or any procedural notes. This data section is fun, and could be very time-consuming, but is not the core focus of this research – try to spend at least 90% of your time on the milkweed and monarch observations.
- i. *notes* = record presence and estimated abundance of other terrestrial invertebrates or anything unusual about the plant. Also note if you or your partner accidentally damaged the plant or caterpillars. For extremely

abundant arthropods, estimate abundance to the nearest 10. If you find an unfamiliar species, try to identify it quickly and provide a short descriptive name. In addition, you can take a picture and ask for assistance identifying your find when you next meet with your mentor. Common species include:

1. small milkweed bug (*Lygaeus kalmii*) = SMMB
2. oleander aphid (*Aphis nerii*) = OLAP
3. large milkweed bug (*Oncopeltus fasciatus*) = LAMB
4. milkweed leaf beetle (*Chrysochus cobaltinus*) = CHRY
5. milkweed longhorned beetle (*Tetraopes* spp.) = TETR
6. jumping spider (Salticidae) = JUSP
7. crab spider (Thomisidae) = CRSP
8. ants (Formicidae) = ANTS
9. predatory fly larvae (Syrphidae) = SYRP
10. lacewing adults, larvae, or eggs (Chrysopidae) = LACE

6. Data entry and quality control

- a. Enter all data into the shared Google spreadsheet. All field data should be entered by the same participants that collected the data, before the end of the day on the same day as data collection.
 - i. Enter data into two spreadsheets for each trip:
 1. trip log: this spreadsheet gives a summary of each trip, mostly using the daily data from your datasheet.
 2. data: this spreadsheet records the actual plant-level data for the project. This should include all of the remaining data fields on your datasheet.
- b. Paper copies of these data should be deposited in the class filebox by the end of the observation week. Each pair should staple together all datasheets for a weekly set of milkweeds. These paper copies provide a data archive of last resort.

7. Data visualization

- a. We will periodically review and analyze the data for the season, and offer occasional summary reports on the progress of the season at each of the surveyed sites.