

Milkweed Germination and Planting Protocol

Notes: Each school will receive 300 seeds total: 100 from their “home” site, or ecotype, and 50 from each for four other ecotypes. The seeds will be a mix of seeds from different pods collected at the site. Seeds will come mixed with a small amount of sand, and they will be labeled with the Code Name and Plant Source ID number of the site from which they were collected (Site Number). You will also receive a link to a **Seed Source Datasheet** with the code names and numbers, as well as the coordinates, of all sites participating. From here on out, use the Plant Source ID number rather than the site Code Name to reduce bias. At the end of the experiment, you can switch back to Code Names to identify which sites your plants came from for your analysis.

You should cold stratify and germinate all seeds, but you will only plant the fifth “back up” ecotype in the field if not enough seedlings are available from one of the other ecotypes. You will need 5 plants from each of 4 ecotypes to plant in each plot (only one plot is required). Be sure to keep seeds from each ecotype labeled at every step!

Important: Please record the timing of each of the steps that follows on the Plant Timeline Data Sheet, and once you have planted your seedlings in the field, enter all the dates into the **PLANT TIMELINE FORM** online.

Cold Stratification. Cold stratification of seeds is important to their germination and should be done before the germination protocol. If you need to store your seeds before you begin cold stratification, it is best to store them in the refrigerator, *but this alone is not cold stratification*.

1. About 2-2.5 months before the average date of last freeze in your area, cold-stratify your seeds. To do this, add enough water to each bag of sand so that the sand becomes damp but not sopping wet. Gently mix the seeds with the sand inside the bag. The goal is to have all of the seeds in contact with moist sand during the period of cold stratification. Label the bag with the date of cold stratification, and store in a refrigerator for 3-4 weeks in a location where the seeds won't be disturbed too much.

Randomization. You MUST follow the randomization protocol to set up your pots or plug trays before you begin planting!!!

Randomizing Seedling Configuration. When seeds are growing in either a greenhouse or classroom, it is important to randomize their placement so that no confounding factors, like light or temperature differences, influence the growth patterns we see between ecotypes. There is a sample randomization sheet available as an excel file available as a model. **PLEASE follow these guidelines carefully and ask if you have questions!**

1. Determine where your seedlings will grow; it may be in a greenhouse or in the back of your classroom. Be sure there is enough space for all of the flats to sit on a flat surface with enough light. We recommend planting in deep plug trays. If you use plug trays with 32 cells/tray, you will need 10 trays. If you don't have enough space for that, you may opt for a 72-cell plug tray.
2. Decide how you will group your seeds. You will want to plant your seeds in batches of the same ecotype that you can label. If you are planting in a 32-cell plug tray, each row of 4 cells may constitute one group, and you can label each row. If you are planting in a 72-cell plug tray, you may label groups of 6 cells.
3. To randomize the order you will plant seeds into the plug trays, first open an empty Excel file.
4. Create a data sheet with columns for Plant Source ID number, Seeds, and Batch.number.
5. Calculate the number of batches you will need for each ecotype. Divide the number of seeds from an ecotype (either 50 or 100) by the number of cells in each group (eg. 4 or 6). This tells you the number of batches you will need for each ecotype.

Excel can perform these calculations for you easily.

- a. Simply enter the number of seeds you have for each ecotype.
- b. Then create an equation to divide amount in the cell with the number of seeds (in this case cell B2) by the number of cells per group. In the example below, there are 4 cells per row. When you hit return, the equation should disappear and the result should appear in the cell.
- c. Click on the cell. Find the small box in the bottom right of the cell, click on that, and drag down to the bottom row to fill the equation down. This tells you how many batches you will need for each ecotype.

	A	B	C
1	PlantSour	Seeds	Batches
2	8	100	=B2/4
3	4	50	
4	7	50	
5	1	50	
6	9	50	
7			

	A	B	C	D
1	PlantSour	Seeds	Batches	
2		8	100	25
3		4	50	12.5
4		7	50	12.5
5		1	50	12.5
6		9	50	12.5
7				
8				

6. To complete your randomization, create three new columns: one for BatchNumber, one for PlantSourceID (ecotype), and one for RandomNumber.

7. Fill in the data sheet like the example shown below, except yours will continue for up to 80 rows.
 - a. First, enter the sequence of 1-n for the number of batches you need for each ecotype (round up to a whole number).
 - b. Next, fill in the ecotype number next to the sequence of batches for that ecotype.

E	F	G
BatchNumber	Plant.SourceID	RandomNumber
1	1	
2	1	
3	1	
4	1	
5	1	
6	1	
7	1	
8	1	
9	1	
10	1	
11	1	
12	1	
13	1	
1	4	
2	4	
3	4	
4	4	
5	4	
6	4	


8. Once you've filled in the Batch and Plant SourceID columns, go to the random number column.
9. Click on the box next to the first batch of the first site. Type in "=RAND()."
10. Hit "enter."
11. Click and drag to fill this first box all the way down to the bottom of the data table, as shown below.

E	F	G	E	F	G
BatchNumber	Plant.SourceID	RandomNumber	BatchNumber	Plant.SourceID	RandomNumber
1	1	0.145962501	1	1	0.589961186
2	1		2	1	0.252413071
3	1		3	1	0.689660429
4	1		4	1	0.984556443
5	1		5	1	0.285380299
6	1		6	1	0.588684397
7	1		7	1	0.783663603
8	1		8	1	0.112484756
9	1		9	1	0.409179212
10	1		10	1	0.378734361
11	1		11	1	0.693184677
12	1		12	1	0.218833717
13	1		13	1	0.611846019
1	4		1	4	0.960860517
2	4		2	4	0.510346448
			3	4	

12. Highlight **ALL the data in ALL** three columns in this data table. This is an important step!



Sort

13. Go to “Data,” and click on the  button at the top. Choose a custom sort.
14. Under “Column: Sort By,” select “Random Number.” Click “OK.” If you don’t see this option, make sure the box that says “My list has headers” is checked.
15. Your data table should now be sorted by the random numbers, with the smallest one at the top. The PlantSourceID numbers are now sequenced in a random order, as shown in the figure below.
16. Note that if you click or type in the data sheet, the Random Number column will reshuffle. This is no cause for alarm- the other two columns will not reshuffle. This explains why the random numbers do not appear to be sorted from low to hi in the figure below, even though that was the criteria used to sort this data sheet.

	E	F	G
BatchNumber	Plant.SourceID	RandomNumber	
10	7	0.729567278	
25	8	0.17364823	
1	7	0.081039518	
19	8	0.770805101	
8	9	0.846337201	
6	8	0.187894646	
12	4	0.426143532	
4	7	0.5851034	
11	9	0.073779427	
5	7	0.997646777	
2	9	0.119447359	
9	1	0.995250898	
7	8	0.741964709	

17. Use this list to label the groups in your plug trays in sequential order. This will result in different combinations of plants growing next to each other, so that some unmeasured factor, such as a cold spot in the room, is not confounded with ecotype.
18. For example, using the table above, I would label the first row of 4 plugs with Ecotype 7. The second row would be Ecotype 8, 3rd row Ecotype 7, 4th row Ecotype 8, 5th row Ecotype 9, and so on.

Planting seeds into plug trays.

1. When cold stratification is complete and you are ready to plant.
2. Fill the plug trays with potting soil. Use Hoffman seed starter potting and planting mix, available at [Amazon](https://www.amazon.com) and other stores.
3. Use a finger to create a small depression in each pot.
4. Follow the labels you created during the randomization process to plant seeds. For example, in the first row, I would plant 4 seeds from Ecotype 7, one seed in each plug cell. In the second row, I would plant 4 seeds from Ecotype 8, one per cell.
5. CHECK carefully to make sure you plant seeds in the appropriate cells. We typically plant all the seeds from one ecotype first, and then follow with another.
6. Cover the seeds lightly with dirt after planting.
7. Insert a toothpick or make a mark on the label to indicate when you have finished planting each row of a given ecotype..

8. Repeat procedure, ensuring that you plant seeds in the appropriate rows for their ecotype, until all seeds are planted.
9. Water regularly to keep the soil damp (but not too wet) and keep seedlings in well-lit, warm conditions until ready for transplant. If the plants are in a tray, you can water them from the bottom to avoid disturbing young seedling growth; however, you do not want them standing in water for long. It's important not to let the soil dry up too much at this stage, but you don't want saturated soil either. Domes can be helpful.
10. Once you know how many seeds from each ecotype have germinated, please record these data in your row on the **Seed Source Datasheet**. Record the number of seeds from **your home** site that germinated (out of 100) in the NH.germinated column, and the number of seeds that germinated (out of 50) from source 1, 2, 3, and Extra populations in the N1.germinated, N2.germinated, N3.germinated, and NExtra.germinated columns, respectively.

Transplanting to the field. Transplanting to the field should occur when seedlings are approximately 3" tall and when danger of frost has passed.

****Please remember to record the timing of these steps on the PLANT TIMELINE FORM****

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