Common Milkweed Adaptive Clines Throughout Northeastern US

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Rochester Institute of Technology: Environmental Science Capstone

Abstract

The goal of this study contribute to research surrounding plant adaptation in urban and marginal areas, we assessed five different ecotypes of Common Milkweed (*Asclepias syriaca*) planted experimentally on RIT property to evaluate how adaptability contributes to success of the plant.This species is commonly utilized by the monarch butterfly, so understanding its adaptability will be informative for pollinator-friendly seed mixes, such as the Seneca Park Zoo Society's. After collection, we sent our data to a national dataset Educational Research as Ecological Network (EREN), to be used in a comparative study of local and nonlocal common milkweed strains based on physical attributes such as number of leaves, stem diameter stem height and other. The end goal is to inform SPZS of these findings for their seed mix and how they compared nationwide.

Introduction

In light of climate change and rapidly changing ecosystems due to human development, many studies have attempted to understand clinal adaptation and how adaptation occurs over large geographic scales. Plants must learn to adapt to new environments to avoid extinction. This leads to adaptive clines existing where environmental conditions differ, where the organisms characteristics will differ to increase fitness in the new environment.

To understand adaptive clines, transplantation experiments are used to demonstrate that trait expression due to varying environmental conditions can be tied to fitness components in a non-local versus local environment. Northern milkweed populations have been shown to emerge earlier, grow more slowly and have become more resistant to herbivory (Woods et al. 2012). The Ecological Research as Education Network (EREN) attempts to build off of this and other similar studies to understand how common milkweed (Asclepias syriaca) adapts to new environment conditions and herbivory pressures. The EREN project is led by Dr. Emily Mohl of St. Olaf's College to determine if common milkweed is locally adapted and to determine if there are regional changes in characteristics and adaptation. This project has 30 total participants in which 24 participated in part 1 where wild common milkweed characteristics were examined and seeds were collected in various habitat types. Then 18 participants moved onto part 2 of the study which germinated the collected seeds and created transplantation experimental plots to study local and non-local common milkweed fitness. Our goal was to participate in part 2 of this study and examine all participant data from both part 1 and 2 of this expansive study. We examined if performance measurements differed between various habitat types in wild common milkweed and if there was a difference in performance measurements between local and non-local common milkweed in participants experimental plots.

We hypothesize that the habitat type in which the wild milkweed was examined will have no impact on the fitness of the common milkweed, and therefore the traits between habitat types will be similar. We also hypothesize that the locally sourced milkweed in our RIT transplantation experiment plot, and in all participants experimental plots, will have higher fitness than the non-locally sources seeds.

Methods

The following protocol was adapted from the Ecological Research as Education Network (EREN) project methods (Appendix 1 and 2). The 2017-2018 capstone group completed the nationwide naturally occuring milkweed collection, germination and transplantation method as outlined below, and the 2018-2019 capstone group completed all other methods and analysis. The project had 30 participants in total, 24 of which participated in part 1 an 18 of which participated in part 2 (Figure 1).

Figure 1: EREN participant locations, 24 of which completed part 1 and 18 of which completed part 2 by planting their own Common Milkweed Plot.

Nationwide Naturally Occuring common milkweed seed collection and performance

Seed pods from naturally occuring common milkweed were collected at 2 sites in Rochester, NY; the northbound off ramp outside of Mount Morris on I-390 and the Seneca Park Zoo. At each site, the site habitat type was recorded (Restored prairie, remnant prairie, old field, roadside, forest edge, or other) and the size of the site was recorded. The number of flowering stems of common milkweed stems within the site were estimated. Five individual common milkweed plants that were at least 5 meters apart were sampled per site and physical characteristics were recorded. Physical characteristics included the number of stems, leaves, nodes, fruit, and flowering umbels, the stem height and diameter, largest leaf size, and herbivory observations. After observations, the seed pods from each plant were collected, the seeds were stripped from the pod, separated from the fluff and dried after returning to the lab. Additionally, soil samples were collected and dried at 105℃ in the lab. After drying, the seeds and soils were sent to Dr. Emily Mohl at St. Olaf's College for further analysis.

In addition to our capstone group at RIT, there were 23 other participants who completed the same seed collection and performance measurement protocol. We analyzed the results from 21 of the total participants to determine if there was a significant difference in the performance measurements between Naturally Occuring common milkweed growing in various habitat types. All participant data was averaged for each habitat type with 15 individuals or more. This resulted in the analysis of old fields (n=81), restored prairies (n=15) and roadsides (n=15). Gardens, railroad right-of-ways and wetlands were omitted due to the small sample size (n<15). The same milkweed characteristics were measured as explained above. Data that was marked “not collected” was not included and marked as “NA”. Data that was marked “NA” was changed to “0” if applicable (for example, if there were 5 leaves, and the number of leaves chewed was marked NA, it was assumed the number of leaves chewed was none or “0”).

Germination and transplantation

Of the 24 participants in the seed collection protocol, 18 continued onto the germination procedure, including the 2017-2018 capstone group. We received common milkweed seeds from 5 different locations (referred to as “ecotypes”), one of which was our local ecotype (ecotype 12) and 4 were non-local ecotypes (ecotype 10 from Bridgewater, MA, ecotype 11 from Kenosha County, WI, ecotype 14 from Fenner, NY, and ecotype 18 from St. Johnsbury, VT). We germinated at least 50 of each ecotype in the greenhouse from February to May. The percent germination (defined as breaking the soil surface) by May 1st was recorded and analyzed to determine potential differences among ecotypes. Five individuals of four ecotypes (local ecotype 12, and non-local ecotypes 10, 11 and 14; non-local ecotype 18 was omitted.) were then transplanted to a 20 m^2 plot on RIT’s campus near the observatory. Growth and survival will be monitored for 3 years in conjunction with the broader EREN study.

RIT Local common milkweed performance measurements

In the fall of 2018, as per the 3 year broader EREN study, we completed the milkweed Measurement Protocol (as completed with the naturally occuring common milkweed outlined above). We completed the protocol for all 20 transplanted common milkweed plants at the RIT observatory. Physical characteristics included the number of stems, leaves, nodes, fruit, and flowering umbels, the stem height and diameter, largest leaf size, and herbivory observations. The goal was to investigate if there was a significant difference in the performance measurements of common milkweed seeds collected locally (our Rochester ecotype) versus seeds collected non-locally (3 non-local ecotypes). The ecotypes were examined by latitude and distance to seed collection site.

Nationwide non-local common milkweed performance measurements

All 18 participants that took part in the germination part of the broader EREN project (Part 1) also completed the common milkweed Measurement protocol as outlined above. To understand if other participants were seeing differences in local versus non-local traits, we examined 16 out of 18 participants Fall Measurement data. When conducting the analysis several assumptions were taken into account. The assumptions include adding 0 values to the data that had missing values for some of the sites such as Augustana and Oakhill. For example data that had no value or NA values were replaced by “0”. We made sure that all the participants had each habitat type as 15 individuals type or more. We then averaged all the values for each specific variables for the different sites. Those values include the average number of leaves, average stem height, average stem diameter as well as longest leaf length and largest leaf width.We did not use the number of chewed leaves nor the number of damaged leaves because most of the sites did not have those values entered. A linear regression was then ran on comparing the average of the different variables as a function of the distance between where the seeds were sourced and where they were planted. We then reported the r-square values for each of the models to determine the significance of the different response variables.

Results

Nationwide Wild common milkweed performance in various habitat types

Stem height was found to not significantly vary between the habitat types (p=0.25). There was also no significant difference between stem diameter (p=0.45). Significant variation in both the restored prairie and roadsides likely led to no significant difference between the three habitat types. It appeared that the roadside had slightly more leaves on average, although it was found that there was no significant difference in the average number of leaves between the three habitat types (p= 0.32). Roadside common milkweed also had slightly more fruit on average than restored prairies and old fields, although this was found to be insignificant (p= 0.07). Lastly, when examining herbivory, there was substantial variation in the data and therefore no significant difference in large herbivore damage or weevil damage between habitat types. (Figure 2) (p =0.92 and 0.59, respectively).

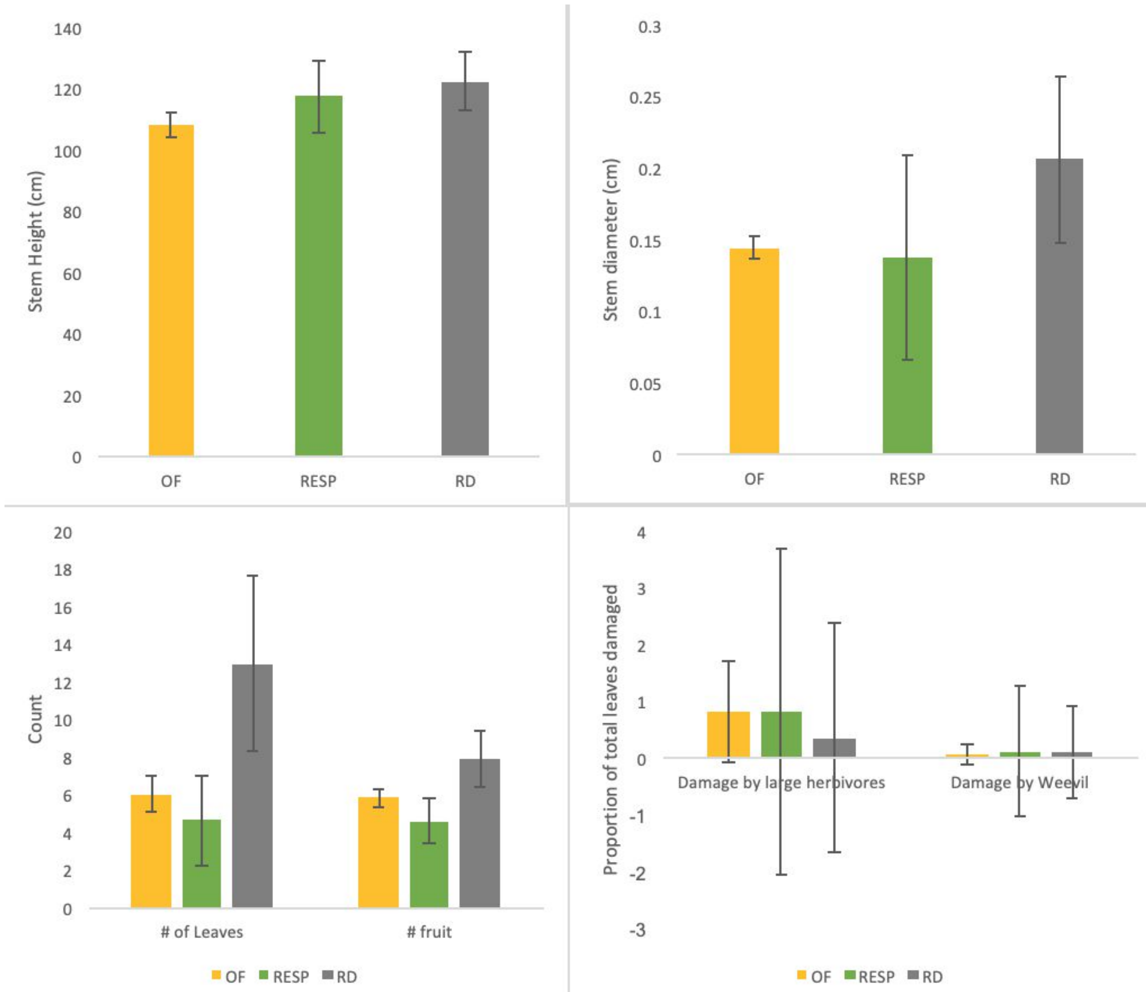

Figure 2: Wild common milkweed performance measurements in old various habitat types including fields (OF), restored prairies (RESP), and roadsides (RD), top left: average stem height (+/- SE), top right: average stem diameter (+/- SE), bottom left: average number of leaves and number of fruit of (+/- SE), bottom right: proportion of the average number of leaves per habitat type that were damaged by large herbivores and by weevil (+/- SE), p > 0.05, for all comparisons.

Germination rates

When examining the germination rates of the 5 ecotypes, ecotype 11, which was from Kenosha County, WI, had lower germination than the other 4 ecotype (Figure 3). The other 4 ecotypes had similar germination rates, of approximately 70-85%. Ecotype 11 was sourced from the farthest away location, 1075 km from where it was germinated at RIT. Ecotype 12 was the locally sourced common milkweed from 51 km away in Mt. Morris, NY. Ecotype 10, 14 and 18 were sourced 663, 175 and 668 km away, respectively.

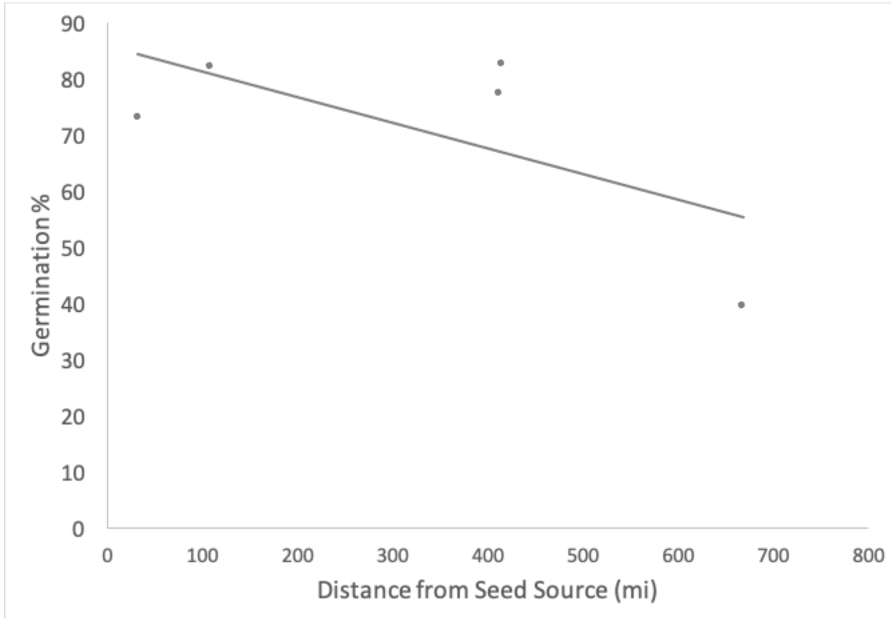

Figure 3: Average common milkweed germination percentages for all 5 ecotypes examined, (ecotype 10 from Bridgewater, MA, ecotype 11 from Kenosha County, WI, ecotype 14 from Fenner, NY, and ecotype 18 from St. Johnsbury, VT, (n_{10}=49, n_{11}=48, n_{12}=92, n_{14}=45, n_{18}=52).

RIT 2018 Fall Local milkweed performance measurements

Our data suggest that there is no significant difference between any of the 4 transplanted ecotypes when examining number of leaves, stem height, stem diameter, leaf dimensions, and herbivory data. There is a trend towards ecotype 11 (Kenosha County, WI) having slightly poorer performance when examining many of these characteristics, but this is not statistically significant ($p > 0.05$ for all comparisons).

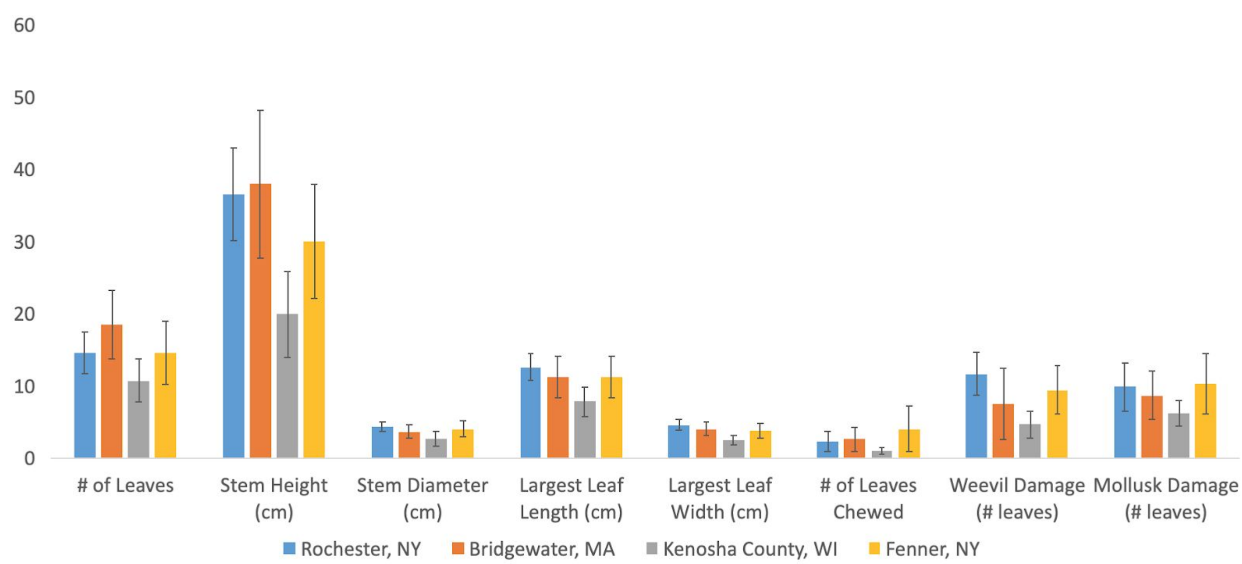

Figure 4: Common milkweed performance measurements grown at the RIT observatory as part of the 3 year EREN study where the Rochester, NY sample was locally sourced and the other three locations were non-locally sourced (p>0.05).

National 2018 Fall Milkweed Adaptation

When examining all participant data to determine if locally sourced common milkweed has increased performance compared to non-locally sourced, it was found that there was no significant difference in performance measures. There was no significant trend when examining the number of leaves by the distance from where the seeds were collected and where they were planted (Figure 5). The number of leaves was fairly consistent at all distances examined. The same was true for stem height, where there was no significant difference in stem height by distance and the data was quite variable (Figure 6). Similarly, the stem diameter was relatively consistent across all distances with some outliers (Figure 7). Lastly, the largest stem length and width also did no significantly vary across distance from seed source and planting location (Figure 8 and 9). Overall, it was found that there was no significant difference in any of the characteristics examined suggesting that there was no difference in performance between locally and non-locally sourced common milkweed.

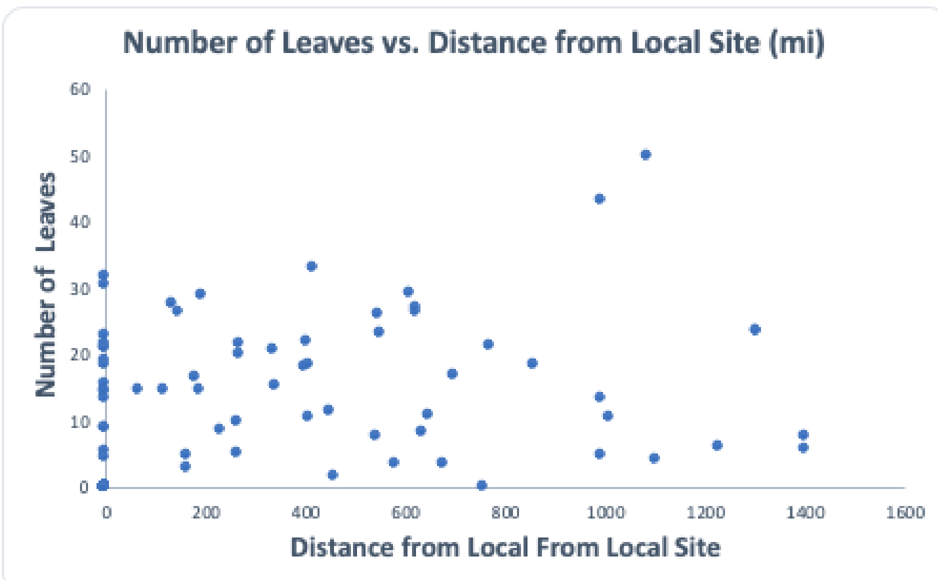

Figure 5: The average number of leaves per common milkweed ecotype as a function of the distance from where the seeds were sourced and where they were planted (mi), r^2=0.36.

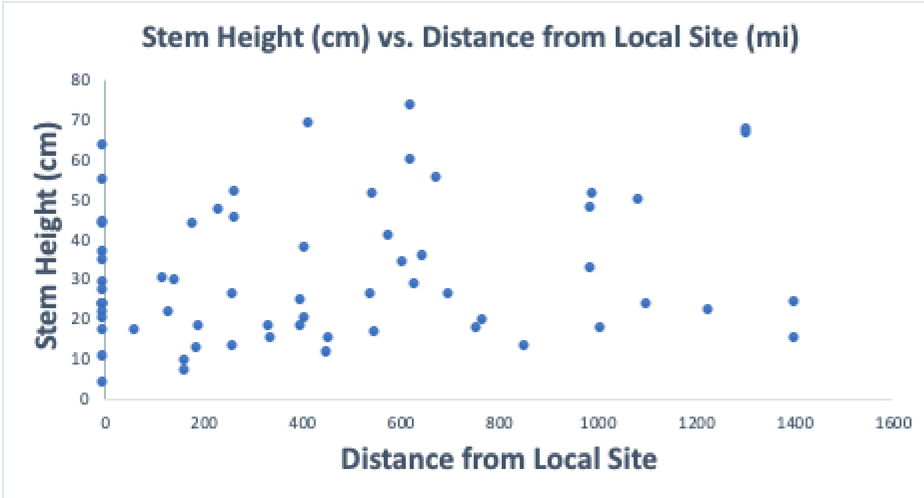

Figure 6: *The average stem height per common milkweed ecotype as a function of the distance from where the seeds were sourced and where they were planted (mi),* r^2*=0.39.*

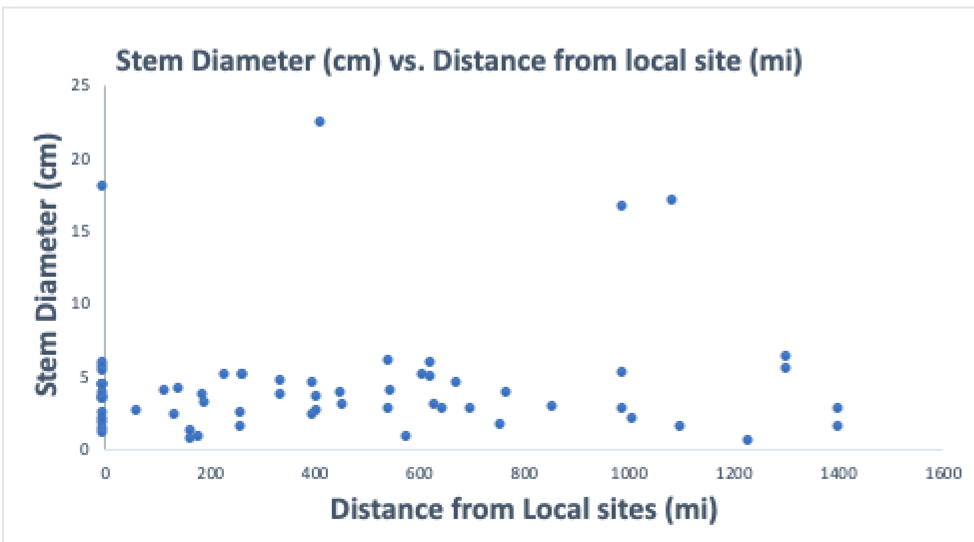

Figure 7: *The average stem diameter per common milkweed ecotype asa function of the distance from where the seed were sourced and where they* r^2*=0.0063*

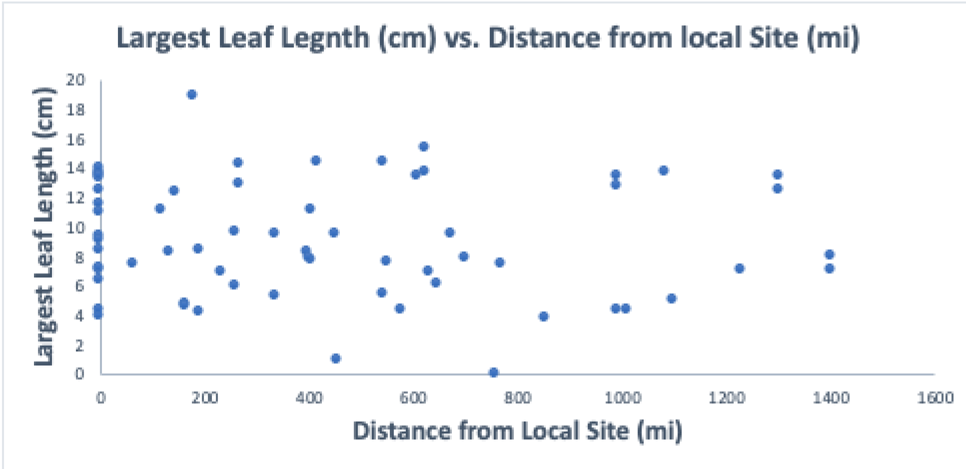

Figure 8: *The average length of the largest leaf per common milkweed ecotype as a function of the distance from where the seeds were sourced and where they were planted (mi),* r^2*=0.007.*

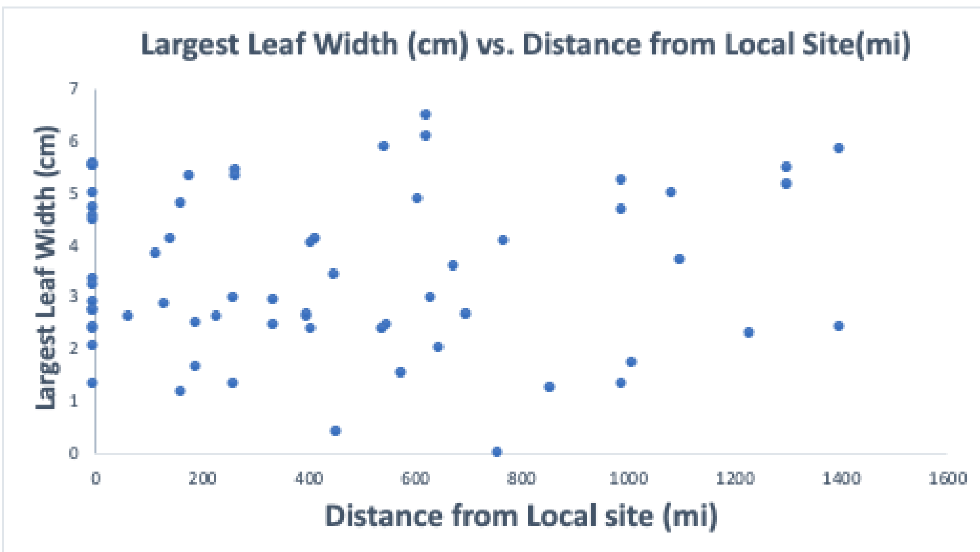

Figure 9: *The average largest leaf width per common milkweed ecotype as a function of the distance from where the seeds were source and where they were planted (mi),* r^2*=0.0015.*

Discussion

It was determined that habitat type had no significant impact on common milkweed performance, where similar performance measurements were observed in all three examined habitat types. This suggests that common milkweed is not heavily impacted by environmental conditions and is able to survive in disturbed areas such as roadways to the same extent as a more rural habitat, such as old fields.

Our data also suggests that there is no significant difference in the fitness of local versus nonlocal common milkweed. This means that when growing common milkweed it is not important to source seeds in a similar location to where you plan to plant them. Our results suggest that seeds can be planted in Rochester that are sourced from 700 mi away and still have similar traits to seeds sourced in Rochester. A similarity in performance measures were seen for all characteristics examined for all other participants examined. Where no local plant in any experimental plot had greater performance measurements than the non-locally sourced plants.

Our results contradict Woods et al. (2016), who observed that 13 traits that were examined between different naturally occurring common milkweed populations significantly differed across the geographic scale examined. The sites extended from Knoxville, TN to Quebec Canada ranging 1200 mi. Geographic clines existed in the seed mass and seed number, number of stems, biomass, latex production, and herbivory. Clines may have been observed due to the extent of the area examined and the variation in the plots and the variation in latitude that existed between sites. Although our sites varied by distance, latitudinal differences were small compared to those observed in the study completed by Wood et al. (2016).

In future studies about this data, latitudinal differences should be examined, rather than distance to determine if more significant results are seen. Depending on the latitudinal gradient of the EREN project as a whole, results may not be observed.

The data contained significant variability between all individual plants analyzed which may have lead to insignificant results. Variability in experimental plots, both at RIT and by the other 17 participants, may have been observed due to the young age of the plants. The common milkweed was germinated by all participants over the winter of 2017-2018, making the plants less than a year old at the 2018 fall measurement time. More significant results may be viewed in future measurement events.

As the data was compiled from many participants, some assumptions had to be made about the respondents data. These assumptions were clearly stated so that as future data and corrections become available the methods can be adjusted and future calculations can be completed. These likely did not dramatically impact the results, but could have led to slight errors.

Our data suggests that the SPZS seed mix does not need to be collected locally to where it will be planted as we saw no significant difference between the local and nonlocal milkweed in our RIT plot. This can suggest our client SZPS can create on seed mix to be used all throughout the Western New York Region. When comparing the RIT study to the nationwide dataset, we can conclude that roadsides have the ability to provide viable habitats for pollinator friendly gardens. This can only suggest that the SPZS seed mix would perform as well on roadsides as in other habitats types.

References:

Woods, E.C. et al. 2016. “Adaptive Geographical Clines in the Growth and Defense of a Native Plant Published by : Wiley Stable URL : Http://Www.Jstor.Org/Stable/41739362 Accessed : 06-04-2016 04 : 45 UTC Your Use of the JSTOR Archive Indicates Your Acceptance of the Terms & Condit” 82 (2): 149–68.

APPENDIX INDEX

1. EREN milkweed Measurement Protocol

2. EREN milkweed Measurement Data Sheet

APPENDIX 1: EREN milkweed MEASUREMENT PROTOCOL

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Rev. 9/15/16

milkweed Measurement Protocol

For each plant, record your site code (using ALLCAPS), plot number, date (month/day), year, time, plant source ID number, location number, and the initials of the recorder and the measurers. Also record the weather conditions, selecting all that apply from the following list: Sunny, Cloudy, Rainy, Windy, Other.
Then measure and record the following.

I. Performance Measures

Common milkweed can branch underground to make multiple stems, but these stems are all part of the same plant. For most performance 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 performance 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 there is no plant present, record a 0, and record NA for other values below. [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.

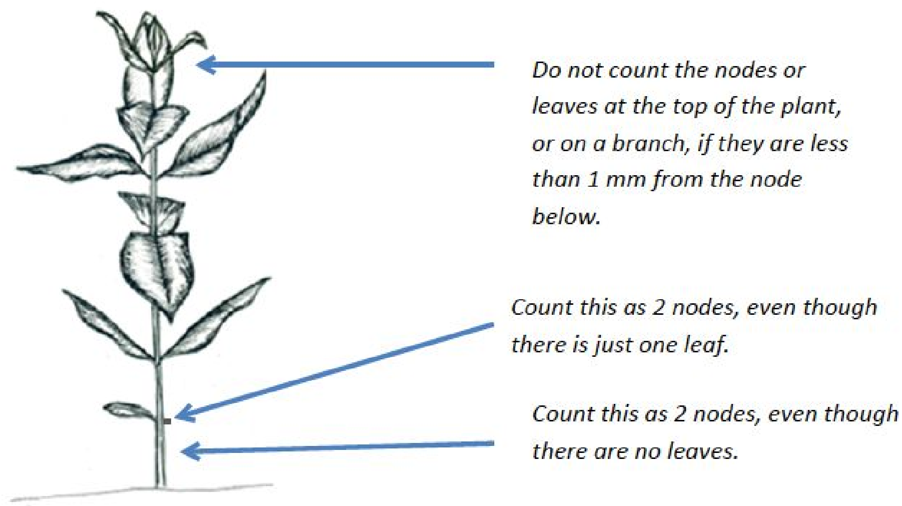

Illustration by Jacqueline Nuzzo

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.

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 Number of Flowering Umbels.

1. milkweeds produce flowering umbels with multiple flowers in the same cluster. Count the number of clusters, or umbels, on all the stems. NOTE: In the spring and fall, there are likely to be no flowering umbels.

2. Record the total number of flowering umbels on the plant. If there are none, record 0.

F. Total Fresh Fruit Mass (g).

1. NOTE: only the last class or period to perform measurements should measure and record total fresh fruit mass. [This step should NOT be performed for wild milkweed plant measurements.]

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 fullyformed 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.

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.

3. 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 (cm2).

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.

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, specifically, damage by weevils. 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

3. If there is stem damage that was not due to weevils, mark it as non-insect imposed stem damage. This could be damage due to mammalian browsing, molluscs, accidents, or any other damage not identifiable as insect damage. Note if the apex (top) of the stem has been removed, or if the damage is just on the surface of the stem.

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. Japanese Beetles
m. Other (record in notes section)

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.

Thank you!

APPENDIX 2: EREN milkweed MEASUREMENT DATA SHEET

Milkweed Lab Data Sheet

Site:		**Plot Number:**		**Date:**		**Plant Source ID:**		**Location #:**	
				Time:					
Recorder:		**Measurers:**				**Weather:**			
Ia: Whole Plant Measures- take all stems of the plant into account.						*Measurer initials for this section:*			
# of stems:				# of leaves:			# flowering umbels:		
total # of nodes:				# of fruits:			Fresh fruit mass (g):		
Ib: Largest Stem Measurements- take only the largest stem into account.						*Measurer initials for this section:*			
Stem height (cm):			Largest leaf length (cm):			Largest leaf width (cm):			
Stem diameter (mm):			Largest leaf area ***(Optional:*** trace on graph paper, mass, calculate; cm2):						
Diameter method: calipers [ ] tape measure [ ] string [ ]					Area estimated/extrapolated?			Y [ ] N [ ]	
II: Herbivory Estimates-take all stems into account						*Measurer initials for this section:*			
# of leaves with chewing damage:		*Insect-imposed stem damage?*	Trench Girdle Other None	*Monarch caterpillars*	Y [ ] N [ ]	*Weevils*	Y [ ] N [ ]	*Bees*	Y [ ] N [ ]
# leaves with weevil damage:		*Non insect-imposed stem damage?*	Y [ ] N [ ] Apex [ ]	*Milkweed bugs*	Y [ ] N [ ]	*Aphids*	Y [ ] N [ ]	*Spiders*	Y [ ] N [ ]
# leaves w/ leaf miner damage:		*Leaf curling?*	Y [ ] N [ ]	*Tussock moth larvae*	Y [ ] N [ ]	*Ants*	Y [ ] N [ ]	*Snails/ slugs*	Y [ ] N [ ]
# of leaves with mollusk damage:		*Leaf spots?*	Y [ ] N [ ]	*Red milkweed beetles*	Y [ ] N [ ]	*Lady-beetles*	Y [ ] N [ ]	*Japan-ese Beetles*	Y [ ] N [ ]
If aphids present: color? Select all that apply. Bright Yellow [ ] Green/Brown [ ] Clear/Orange [ ] NA [ ] Other [ ]									
Notes:									
Rev 9/6/18									