JOURNAL OF
EMERGING INVESTIGATORS

Article

Milkweed sustainability in the Sonoran Desert: *A. erosa* is more water-efficient compared to two other species

Norbu Shastri[1], Y. Gloria Yueh[2]

[1] Basis Phoenix High School, Phoenix, Arizona

[2] College of Graduate Studies, Midwestern University, Glendale, Arizona

SUMMARY

Monarch butterfly population counts have drastically decreased since the 1980s, and this decline is tied heavily to destruction of Monarch hostplants, milkweed. The Sonoran Desert supports over a dozen species of milkweed, all possible hosts for Monarch caterpillars. The purpose of this experiment was to identify the most water-efficient species of milkweed for propagation in the Sonoran Desert to support Monarch butterfly populations. This experiment tested likely candidates for propagation in the climate of Arizona and Northern Mexico. Three milkweed species (Arizona milkweed, Desert milkweed, and Tropical milkweed) grown in two different volumetric water concentrations were tested. Desert milkweed had the highest germination rate, survival rate, plant mass, and water retention capability. Thus, Desert milkweed was most effective at absorbing and utilizing water resources compared to the other species. Interestingly, Desert milkweed's average height was taller in the lower volumetric water level, but for overall plant mass, the higher the water level, the larger the mass. Desert milkweed is the best candidate for widespread propagation in the Sonoran Desert. Given that this research identified a native species of milkweed that can grow with minimal resources, future conservation efforts could use Desert milkweed as a prime species for mass-propagation conservation projects with minimal strain on local resources. The conclusions we drew will aid Monarch conservationists in preserving Monarch habitats during drought conditions.

INTRODUCTION

Since the discovery of the Monarch butterfly overwintering grounds in Mexico in the 1980s, population studies of the North American Monarch population have identified an alarming decrease in monarch survival rates due to several factors, namely heavy losses of their host plant milkweed across the continent (1). Monarch migratory populations have decreased by up to 90 percent from historic levels in the last three decades, in both Eastern and Western Monarch populations (2).

The Monarch butterfly, *Danaus plexippus*, is an insect in the order *Lepidoptera* found across North America. Adult butterflies have black bodies with dotted white patterns, and their wings are a dark orange hue, with thick black veins and borders on each wing. Caterpillars are striped yellow, black, and white with two pairs of black tentacles, feed exclusively on milkweed plants in the *Asclepias* genus, and grow up to about two inches at maximum length. Monarch caterpillars spend up to two weeks in the larval stage before forming a chrysalis, which are hard and appear green with gold spots and a black line near the apex. Adult butterflies emerge from their chrysalis within about twelve days of formation, after which their lifespan varies; individuals among the three generations of Monarchs that follow the migratory generation tend to live only two to six weeks, but the migratory generation itself can live up to nine months (3). A similar related species known as the Queen butterfly, *Danaus gilippus*, is also found in the Sonoran Desert and solely feeds on milkweed; however, they do not migrate (4).

Monarchs conduct an annual migration of thousands of miles across contiguous North America, flying from as far as Canada and Maine to Northern Mexico in a single generational journey. The Monarch butterfly hibernates over a four-month winter in Mexico, amassed in groups of hundreds of thousands of butterflies along the trunks of forest trees (5). Come spring, they begin their journey back, which culminates with the original migratory population laying eggs on milkweed plants in the Southern United States, where the next three generations of butterflies will begin the trek home to the uppermost extremities of temperate North America (5). With its iconic orange and black wings, the Monarch is an important cultural symbol for Americans, and protecting its habitat is imperative. Furthermore, Monarchs are an important part of local ecosystems: they pollinate many species of flowering plants and serve as food sources for birds and other insects. Losing these once numerous insects from the environment would undoubtedly have multi-level repercussions throughout the ecosystem.

Milkweed, a common, leafy plant that has dozens of varieties, is often viewed as a pest by agriculturists and land regulators due to its affinity to spread quickly and its poisonous sap (6), but it is the only plant that Monarch caterpillars can feed off and deposit eggs upon (6). In the United States, milkweed populations are in constant threat of destruction on all fronts, especially in the open fields and grasslands of the Midwest that Monarchs rely on as anchor points in their migration (7). Finding a solution to propagate milkweed plants in the Sonoran Desert with the least number of expended resources is the goal of this experiment.

The three milkweed species chosen for the study were *Asclepias erosa* (*A. erosa*, Desert milkweed), *Asclepias angustifolia* (*A. angustifolia*, Arizona milkweed) and *Asclepias curassavica* (*A. curassavica*, Tropical milkweed) (8, 9, 10). All three species were selected for their prevalence in Arizona: Arizona milkweed and Desert milkweed are both native to

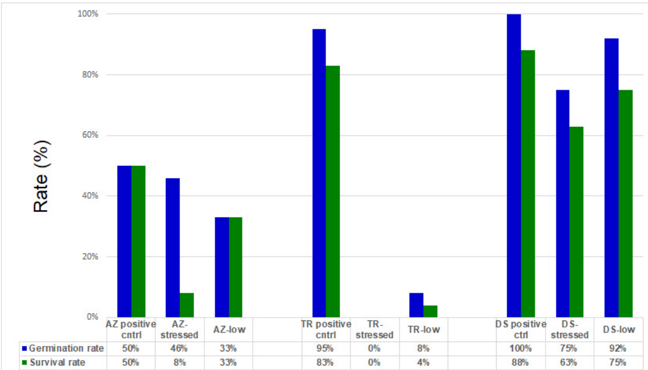


Figure 1: The Germination and Survival Rate of the Three Tested Milkweed Species under Two Soil Moisture Levels. Positive controls were conducted by saturating the soils daily for the entire span of the experiment. The experimental and positive control groups each had an n-value of 24 seeds per water level per species. The negative control n-value was 2 seeds per species. Three milkweed species were examined: Arizona (AZ), Tropical (TR) and Desert (DS) milkweeds.

Arizona (11), and Tropical milkweed is established in Arizona and is originally from nearby Mexico. Tropical milkweed is commonly found as an ornamental in urban gardens, and therefore is the easiest to obtain and grow. All three species can be readily found across Arizona and grow well with adequate water. It is hypothesized that Desert milkweed will have the highest germination rate and the highest growth rate with the least amount of water compared to Arizona milkweed and Tropical milkweed due to its high commonality and success in the open Sonoran Desert (8).

Our data supported the hypothesis that Desert milkweed indeed showed the highest germination rate and survival rate compared to Arizona milkweed and Tropical milkweed. The growth rate, measured by weight and height under the two tested soil moisture levels (12,13), was correlated with the observation of germination rate. In addition, Desert milkweed appeared to have a higher water retention ability (14) compared to the other two species. This was an important discovery towards further widescale milkweed propagation in the Sonoran Desert, as it showed that Desert milkweed can be grown more resource-efficiently than other milkweed species for Monarch conservation.

RESULTS

Desert milkweed grown in both "stressed" and "low" (volumetric water contents: <10% for stressed and 10-14% for low) conditions had the best germination rates (75% and 92%, respectively) and survival rates (63% and 75%, respectively (**Figure 1, Table 1**). The Tropical milkweed groups had the lowest germination rates (0% and 8%) and survival rates (0% and 4 %). The germination rates were calculated as the percentage of seedlings that sprouted divided by the initial number of seeds planted, and the survival rates were calculated as the percentage of plants still alive by the 35th Day of the experiment divided by the initial number of seeds planted. In between, the "stressed" Arizona milkweed group had a higher germination rate than the "low" Arizona milkweed group (46% and 33%); however, the majority of the seedlings in the "stressed" group did not survive after 35 days (dropped from 46% germinated to 8% surviving). The negative controls for all species yielded zero germination. A Two-way ANOVA analysis was performed to assess which milkweed species and which soil moisture produced the greatest germination rate and the survival rate. The soil moisture seemed to have a higher impact on the germination rate (p-value of 0.03601) and the survival rate (p-value of 0.02826), compared to the species (p-values of 0.69 and 0.20, respectively). In all three species, the net survival rate was lower under "stressed" conditions.

Experimental Group	# of seeds planted	# of seeds germinated	# of seedlings survived after 35 days
AZ-stressed	24	11	2
AZ-low	24	8	8
TR-stressed	24	0	0
TR-low	24	2	1
DS-stressed	24	18	15
DS-low	24	22	18

Table 1: The Number of Seeds Germinated and the Number of Surviving Seedlings after 35 days for AZ (Arizona milkweed), TR (Tropical milkweed) and DS (Desert milkweed). Two soil moisture levels are denoted as "low" (10-14% volumetric water level) and "stressed" (<10% volumetric water level).

Among the three tested milkweed species, Desert milkweed had the highest average whole plant weight (**Figure 2**). When the soil moisture was at 10-14% volumetric water level ("low"), Desert milkweed had the highest average weight, about four times as its Arizona milkweed counterparts and almost double its Tropical milkweed counterparts.

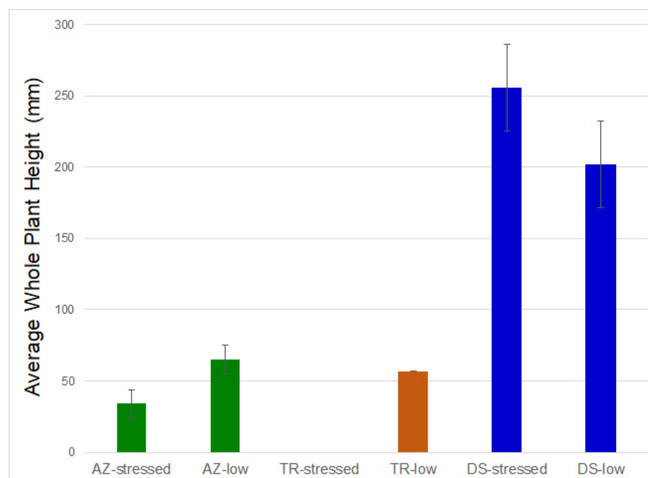


Figure 2: Average Whole Plant Fresh Height (mm) on the 35th Day under Two Soil Moisture Levels: Stressed (<10% VMC) and Low (10-14% VMC). Standard deviation bars are depicted. Three milkweed species were examined: Arizona (AZ), Tropical (TR) and Desert (DS) milkweeds.

Experimental Group	Mean Final Height (mm)	Standard Deviation (mm)	Range (mm)	Minimum (mm)	Maximum (mm)	Count (N)
AZ-stressed	34.00	9.90	14	27	41	2
AZ-low	65.88	9.94	31	44	75	8
DS-stressed	255.67	7.84	97	205	302	15
DS-low	201.67	31.99	129	137	266	18

Table 2: Descriptive Statistics analysis of plant height (mm) of Arizona (AZ) and Desert (DS) milkweeds under low or stressed soil moisture levels conducted on Day 36.

This demonstrated Desert milkweed's superior ability to incorporate water resources into plant mass, suggesting it is best equipped for the Sonoran Desert's low water availability. Interestingly, the Desert milkweed under the "stressed" condition had the highest average height (255.6700 mm) of all the plants, including the same species with the higher soil moisture (**Figure 2, Table 2**). Yet, these plants (DS-stressed) were lighter with an average weight of 276.9600 mg when compared to the DS-low average weight of 395.7300 mg (**Figure 3, Table 3**). This observation led to further analysis of the weight distribution between the shoot and the root of the Desert milkweed (**Figure 4**). The DS-low plants had a higher average shoot to root ratio (4.61 to 1) than the average DS-stressed ratio (2.73 to 1), suggesting that the DS-low condition promoted more shoot mass growth than the DS-stressed ones, despite being shorter. In addition, the average fresh root weight in the "stressed" group was higher than the "low" group (74.3070 mg vs. 70.5920 mg), suggesting the need to absorb more soil moisture under extremely low soil moisture conditions.

Based on our data, an interesting observation was that the plant mass of Desert milkweed seemed to have a 1-2% higher water incorporation than the other species, which may be associated with its better ability to survive in an arid environment; more experiments are needed to confirm this implication (**Figure 5**). It seems that Desert milkweed is best equipped to incorporate environmental water into plant mass.

DISCUSSION

The Monarch butterfly uses milkweed as its sole host plant, and due to decreasing availability of milkweed supply across the nation, finding a species of milkweed that can quickly grow with minimal water resources and is native to its area is imperative. The results of our experiment show that Desert milkweed ranked highest among all criteria of height, water-content, and mean weight. Desert milkweed was therefore the most efficient at translating limited resources into growth. From these results it is fair to extrapolate that

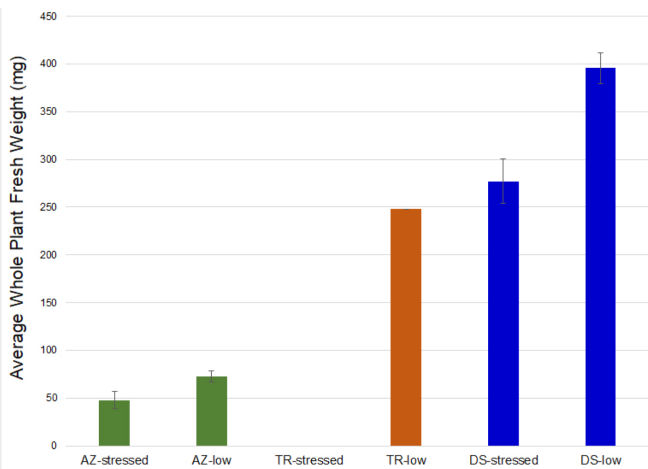


Figure 3: Average Whole Plant Fresh Weight (mg) at the 35th Day under Two Soil Moisture Levels: Stressed (<10% VMC) and Low (10-14% VMC). Three milkweed species were examined: Arizona (AZ), Tropical (TR), and Desert (DS) milkweeds**.**

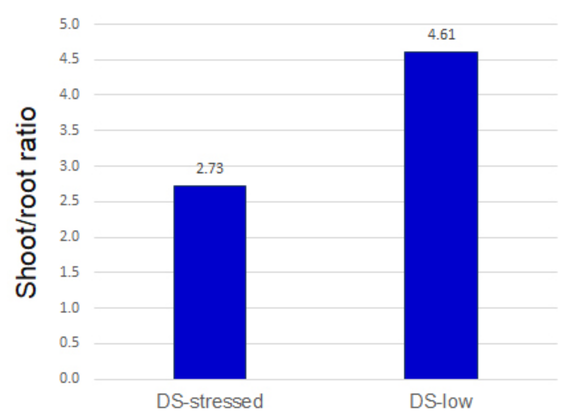
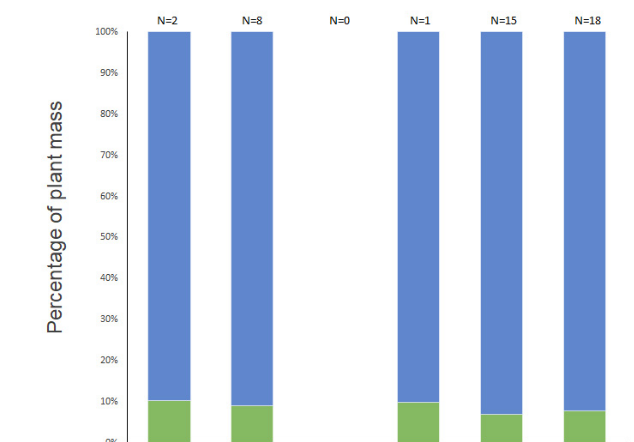


Figure 4: Shoot to Root Ratio Weight Analysis of Desert milkweed under the Two Soil Moisture Levels. Ratio calculation is derived from Table 3.

Experimental Group	Mean Weight of Wet shoot (mg)	Mean Weight of Dry shoot (mg)	Mean Weight of Wet root (mg)	Mean Weight of Dry root (mg)	Mean Weight of Whole plant-wet (mg)	Mean Weight of Whole plant-dry (mg)
AZ-stressed	35.6750	3.6250	11.9250	1.3000	47.6000	4.9250
AZ-low	59.7625	4.8875	12.750	1.6625	72.5125	6.5500
TR-stressed	0	0	0	0	0	0
TR-low	197.9500	20.3000	49.9500	4.2500	247.9000	24.5500
DS-stressed	202.6500	15.4267	74.3067	3.7067	276.9567	19.1333
DS-low	325.1389	26.8111	70.5917	3.8667	395.7306	30.6778
Experimental Group	Standard Deviation of Mean Weight of Wet shoot (mg)	Standard Deviation of Mean Weight of Dry shoot (mg)	Standard Deviation of Mean Weight of Wet root (mg)	Standard Deviation of Mean Weight of Dry root (mg)	Standard Deviation of Mean Weight of Whole plant-wet (mg)	Standard Deviation of Mean Weight of Whole plant-dry (mg)
AZ-stressed	10.6750	0.1250	1.7250	0.2000	8.9500	0.3250
AZ-low	4.5655	0.6787	1.2823	0.2304	5.5394	0.8757
TR-stressed	0	0	0	0	0	0
TR-low	0	0	0	0	0	0
DS-stressed	16.4760	1.4267	11.0824	0.3819	23.3268	1.5500
DS-low	15.2332	1.1002	5.2725	0.3631	15.9951	1.1096

Table 3: Descriptive statistics analysis of plant mass (mg) of shoot and root before and after the dehydration process in all three species conducted on Day 36. Three milkweed species were examined: Arizona (AZ), Tropical (TR) and Desert (DS) milkweeds.



	AZ-stressed	AZ-low	TR-stressed	TR-low	DS-stressed	DS-low
Water Content (%)	90%	91%	0%	90%	93%	92%
Dry Plant Mass (%)	10%	9%	0%	10%	7%	8%

Figure 5: Comparison of Water Content in Each Milkweed Species under the Two Soil Moisture Levels. Dry plant mass percentage calculation was derived from the average dehydrated whole plant weight divided to the average fresh/wet whole plant weight. The percentage of water content is calculated using 100% minus the calculated dry weight percentage.

Desert milkweed is the ideal candidate of the three tested species for future propagation in the Sonoran Desert on a wide scale. This experiment has demonstrated the sheer advantage in water and resource conservation that Desert milkweed holds above its peers in the genus. Given that Desert milkweed is commonly found in open desert without high rainfall, it is likely that the results of this experiment are reflective of this species' adaptations to low water conditions.

There are many future directions towards which this project can be taken. Repeating this study with a larger sample size will strengthen its conclusions. Additionally, future experiments could potentially determine the reason that Arizona milkweed germinated at a higher rate under more stressed water conditions, and why Desert milkweed grew taller with less water. Differing levels of water deprivation could be tested to determine the ideal water resource to optimize growth. Possible future experiments to strengthen this conclusion and better utilize these findings may include studies on the space occupied by the roots of Desert milkweed and the effects of filtered or unfiltered water on these plants, to further identify ways to conserve resources towards the goal of monarch conservation. Furthermore, additional studies about the underlying attraction of Monarch egg-laying females to different species of milkweed could augment conservation efforts by further specifying ideal candidates for wide scale planting. If data from a milkweed-affinity study could be combined with a milkweed resource efficiency study, an even clearer milkweed candidate for conservation purposes could be identified. As our experimentation identified Desert milkweed as the most efficient candidate, many of these future experiments would likely involve this species.

MATERIALS AND METHODS

Desert milkweed seeds were obtained from the Desert Botanical Garden in Phoenix, Arizona, and Arizona milkweed seeds and Tropical milkweed seeds were from seed pods collected on the plants raised in the Phoenix, Arizona home garden. Seeds were soaked in purified water at room temperature (between 75-78°F) for eight hours before the experimental setup. The negative control included 2 seeds from each species planted in dry soil pellets (manufactured by Jiffy). The positive control was comprised of twenty seeds of each species planted in soil with an average of 35-42% volumetric water content (VMC), ideal for most plant growth (13, 14). For the experimental groups, the soil pellets were fully saturated with warm water at approximately 120 degrees Fahrenheit before planting. For each milkweed species, two seeds were planted in each pellet, and a total of forty-eight seeds of each species in 24 pellets were set up, divided into two trays of 12 pellets per tray. From Day 2 to Day 5, the soil moisture was kept at 35-42% VMC. Starting from Day 6 of planting, two volumetric water contents were tested for daily watering: <10% (stressed) and 10-14% (low). Soil moisture was measured using the Soil Moisture Sensor (ECO WITT WH0291 Sensor). All trays were put in a roughly 14-hour/10-hour light-dark cycle in a temperature regulated home environment (between 75-78 °F). After germination, the shape and the height of cotyledon were recorded, and the humidity level and temperature were monitored daily. Measurement of the height of each seedling required unfurling the seedling until the stem was straightened up. These steps were repeated for the duration of 35 days from the beginning of planting. At the end of the 35th day, the plants were transported, and the rest of the experiments were conducted in a university research laboratory. Each plant was carefully removed from the soil pellet to avoid damaging the roots. On the 35th day, the full lengths of shoot and root were measured and recorded. Each root was washed in distilled water and dried with a Kimwipe

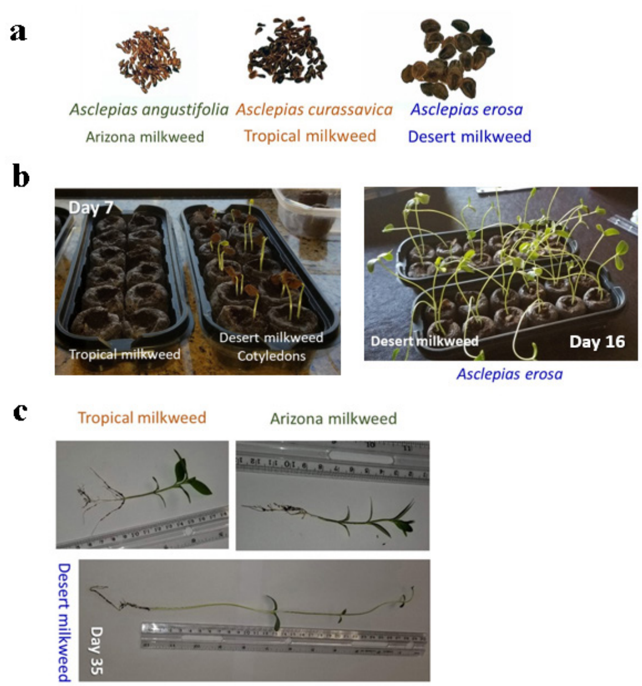


Figure 6: Experimental Setup. (a) Seeds of Arizona milkweed, Tropical milkweed, and Desert milkweed, (b) Growth and development of Tropical milkweed and Desert milkweed on Day 7, and Desert milkweed on Day 16, (c) Growth and development of Arizona milkweed, Tropical milkweed, and Desert milkweed on Day 35, as compared to a ruler measuring millimeters.

before it was severed from the shoot with a scalpel (**Figure 6**). Each shoot and its corresponding root were weighed in its individual Falcon tube using an analytic scale (AND GR-202, d = 0.1 mg). Individual empty Falcon tubes were previously weighed and recorded also. The weight of the fresh shoot or fresh root was calculated based on the difference of these two recordings. After the fresh weight measurements, the Falcon tubes were then placed in an oven at 90° Celsius for 24 hours. On Day 36, measurements of the dry weight of shoots and roots, using the same method described previously, were conducted. Statistical analysis was conducted using the Excel descriptive statistics feature. A Two-way ANOVA Test indicated that there was a significant effect from the soil moisture content on the germination (p-value of 0.03601) and survival rate (p-value of 0.02826) of seeds. This test and a subsequent post-hoc test did not find a significant effect of the specific species studied on either germination or survival rates.

ACKNOWLEDGEMENTS

We thank Ms. Sandor (Basis Phoenix) for providing the feedback on this study. We thank Taara Y. Shastri (Basis Phoenix) for establishing the VWC measurements. We thank Midwestern University for providing the research laboratory to conduct this study.

Received: July 30, 2021
Accepted: December 10, 2021
Published: March 21, 2022

REFERENCES

1. Inamine, Hidetoshi, *et al*. "Linking the Continental Migratory Cycle of the Monarch Butterfly to Understand Its Population Decline." *Oikos*, vol. 125, no. 8, Apr. 2016, pp. 1081–91, doi.org/10.1111/oik.03196.
2. Pelton, Emma M., *et al*. "Western Monarch Population Plummets: Status, Probable Causes, and Recommended Conservation Actions." *Frontiers in Ecology and Evolution*, Frontiers, 3 July 2019, www.frontiersin.org/articles/10.3389/fevo.2019.00258/full.
3. Flockhart, D. T. Tyler, *et al*. "Unravelling the Annual Cycle in a Migratory Animal: Breeding-Season Habitat Loss Drives Population Declines of Monarch Butterflies." *Journal of Animal Ecology*, edited by Tim Coulson, vol. 84, no. 1, June 2014, pp. 155–65, doi.org/10.1111/1365-2656.12253.
4. Saul-Gershenz, Leslie, *et al*. "Ecology of the Western Queen Butterfly Danaus Gilippus Thersippus (Lepidoptera: Nymphalidae) in the Mojave and Sonoran Deserts." *Insects*, vol. 11, no. 5, May 2020, p. 315, doi.org/10.3390/insects11050315.
5. Reppert, Steven M., and Jacobus C. de Roode. "Demystifying Monarch Butterfly Migration." *Current Biology*, vol. 28, no. 17, Sept. 2018, pp. R1009–22, doi.org/10.1016/j.cub.2018.02.067.
6. Birnbaum, S. S. L., and P. Abbot. "Insect Adaptations toward Plant Toxins in Milkweed-Herbivores Systems - a Review." *Entomologia Experimentalis et Applicata*, vol. 166, no. 5, Mar. 2018, pp. 357–66, doi.org/10.1111/eea.12659.
7. Pleasants, John M., and Oberhauser, Karen S. "Milkweed Loss in Agricultural Fields because of Herbicide Use: Effect on the Monarch Butterfly Population." *Insect Conservation and Diversity*, vol. 6, no. 2, Mar. 2012, pp. 135–44, doi.org/10.1111/j.1752-4598.2012.00196.x.
8. J. Rosatti, Thomas, and Carol A. Hoffman. "Asclepias Erosa." *Ucjeps.berkeley.edu*, 2013, ucjeps.berkeley.edu/eflora/eflora_display.php?tid=14371.
9. Pegram, Kimberly V., and Natalie A. Melkonoff. "Assessing Preference and Survival of Danaus Plexippus on Two Western Species of Asclepias." *Journal of Insect Conservation*, vol. 24, no. 2, Dec. 2019, pp. 287–95, doi.org/10.1007/s10841-019-00197-z.
10. Wade, Lizzie. "Plan to Save Monarch Butterflies Backfires." *Www.science.org*, 13 Jan. 2015, www.science.org/content/article/plan-save-monarch-butterflies-backfires.
11. Kearney, Thomas H., & Peebles, Robert H. Arizona flora with supplement. *University of California Press, Los Angeles,* 1960.
12. Gutierrez, Mario, *et al*. "Association of Water Spectral Indices with Plant and Soil Water Relations in Contrasting Wheat Genotypes." *Journal of Experimental Botany*, vol. 61, no. 12, July 2010, pp. 3291–303, doi.org/10.1093/jxb/erq156.
13. Xu, Zhenzhu, *et al*. "Plant Responses to Drought and Rewatering." *Plant Signaling & Behavior*, vol. 5, no. 6, June 2010, pp. 649–54, doi.org/10.4161/psb.5.6.11398.
14. Harris-Valle, Citlalli, *et al*. "Physiological Response of Cucurbita Pepo Var. Pepo Mycorrhized by Sonoran Desert Native Arbuscular Fungi to Drought and Salinity Stresses." *Brazilian Journal of Microbiology*, vol. 49, no. 1, Sept. 2017, pp. 45–53, doi.org/10.1016/j.bjm.2017.04.005.