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**Muza, Katte E.** ***Invertebrate Diversity Found on Common Milkweed (Asclepias Syriaca) During Wisconsin Growing Season***

## Abstract

Common milkweed (*Asclepias syriaca*) is studied for its defense mechanisms and species interactions. It is a host to a variety of species throughout the growing season, with distinct species attracted to various parts of the plant. I observed both established and first year plants from May through September 2017, to determine the diversity of the invertebrate community found on the milkweed throughout the growing season. The information was broken into months, then analyzed to determine species richness and any overall trends. I identified 22 species from ten taxonomic groups. The highest species richness occurred in July and August. The most common groups of insects were aphids and weevils, which were observed in 11 out of 12 time periods. I observed that the invertebrates stayed mainly on the established milkweed until the first-year plants had grown larger. A positive correlation of r = 0.78 between height and species richness supports this. I also found a positive correlation between average species richness and percentage of leaves with chewing damage (0.27). This information could help with further research on how insect populations interact with and affect milkweed and when protection, such as insecticide, should be applied.

## Acknowledgments

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## Chapter I: Introduction

The milkweed family (*Asclepiadaceae*) is a distinctive plant family known for its interactions with certain insect species. Common milkweed (*Asclepias syriaca*) is prevalent in North America, with a range from Texas north into Canada. Milkweed is often found wherever it can find adequate sunlight and soil, appearing in pastures, roadside ditches, lawns, and so on. Along with this wide range, the species has been known to show evidence of location adaptations to help them survive in their specific microhabitat and microclimate (Woods, Hastings, Turley, Heard, & Agrawal, 2012). Some of the traits that exhibit local adaptation in some populations include a higher tolerance of water stress, suitable temperatures ranges, and herbivore defense mechanisms (Couture, Serbin, & Townsend, 2015).

The purpose of this project was to inspect the species richness of insects which use common milkweed. Multiple kinds of insects feeding on the milkweed in several ways could cause competition between them and further cause a natural decline in one species as the presence of another species increases. This was seen in one study where aphid populations declined with the addition of monarch caterpillars (Ali & Agrawal, 2014). Also, as the plant is eaten, it will cause the production of inducible defense mechanisms, such as latex or cardenolides, which can negatively affect some but not all species of herbivores. For instance, latex will deter swamp milkweed beetles (*Labidomera clivicollis*) more than monarch caterpillars *(Danaus plexippus*) (Van Zandt & Agrawal, 2004).

Lastly, some species, such as pollinators or other specialists, might not be observed on or near the plant at times in the season when their preferred part is not present. An example of this would be the big milkweed bug (*Oncopeltus fasciatus*), which feeds primarily on the milkweed seeds, not appearing near the plant until at least the flowers bloom (Ralph, 1976). Likewise, it would also make sense for predatory species, such as spiders and ladybugs, to not appear until after a food source has appeared. This in turn can act as a limiter for other species which otherwise could use the milkweed (Koch, Hutchison, Venette, & Heimpel, 2003). Similarly, different parts of the plant or plant community will also attract different invertebrate species. Pollinators as adults will spend more time in the flower umbels, while the caterpillars will be found on the actual leaves. Likewise, many beetles affect either the roots or the seed pods.

In addition to differences in insect communities over the course of the growing season, there may be differences between individual plants of differing ages. As a perennial, milkweed plants can live several years, with usually two years needed before the plant will begin to reproduce and create seeds. Before then, the plant invests more in building its root systems. The differences in how resources are use could affect how invertebrates use the plant, and one experiment found that smaller milkweed plants were focused on more by herbivores (Scheidel & Bruelheide, 2004).

The objectives for the study were to look at both the overall trends in the insects using the milkweed and to see if there is a difference in the use between established and first year milkweed. For the total insect use of the milkweed, most studies focus on only one or a few of the better-known insects instead of the myriad of the total using the plant. Therefore, it will be of interest to see how the entire community of invertebrate species use the plant. When comparing first year to established plants, because it is a perennial it could be of interest to see how insects affect milkweed during parts of their lifecycle; such as root production in first-year and reproduction with the established. I hypothesized that first-year milkweed would be used more often than older plants, because their leaves would not be as tough to eat as the older plants or filled with as much latex.

**Statement of the Problem**

Most studies on milkweed focus more on a few select species or on its defense mechanisms, not on the complete use of the plant. Along with this, most studies look at solely established milkweed and not fully how species can affect the milkweed during different times of the plant's life.

**Purpose of the Study**

To determine what the invertebrate species richness is on common milkweed during the growing season.

To determine if there are any trends for how milkweed is used by invertebrates over the course of the season.

To compare how invertebrates' use first year plants to established plants.

**Assumptions of the Study**

Assumptions of the study is that invertebrates will have equal opportunity to use both the established and first year plants. The study also assumed that the growing season that year was average and that the soil and location used was typical for where milkweed grows across Wisconsin.

# Chapter II: Methodology

In this section, I will explain certain attributes about where the study took place and how the study was conducted. This includes such aspects as the steps from growing the first year plants, what variables were collected and how information was collected.

## Location, Soil, Temperature

The milkweed studied in this project was grown at the local Menomonie community gardens. The soil in the lot was roughly 94% 512C Drammen loamy with 6% other components, on a 6 -12% slope (Web Soil Survey, n.d.). The land is tilled every fall in September, and if wanted the lots can be fertilized by the land users. Protecting the entire community garden was a fence to keep out larger herbivores and gopher traps to deter burrowing herbivores. Next to the garden plot on the west was a vacant plot filled with a mixture of native and left over planted plant species. Some of these species included giant sunflower, *Bromus* species, crab grass, and others. To the north of the plot was a used garden which was mulched and maintained. On the east and south sides of the garden was it common lawn turf.

In the area during the study time, the average temperatures ranged between a low of 10.89ºC to a high of 24.11ºC. The average rainfall during this time was 100.58 inches per month (Climate Menomonie - Wisconsin and Weather averages Menomonie, n.d.). During periods where it did not rain for several days, the milkweed was watered manually to ensure that the planted ones did not die due to water stress.

## Procedure

**First-year milkweed germination and planting process.** In April 2017, I floated the milkweed seeds in water for roughly one week, then those which sprouted a root were planted in soil in the greenhouse at the UW-Stout campus. The seeds were kept in the greenhouse for several weeks, until they were between 50 and 75 mm tall, then moved to the community garden where those in good health were planted in a random order in the plot in May. I planted the milkweed seedlings one meter apart in fabric bags to help deter rhizomes from the plant or surrounding plants from expanding too far and being confused with other milkweed plants. However, as seen later in the project wild specimens penetrated one bag.

Of the seeds in the beginning, only a small number germinated. Of those which were counted as germinated, only fourteen were alive at the time to plant in the community garden, and only six survived through the summer. Along with this, of the six milkweed plants grown, five had defects in the main stem, with the stem being twisted and giving more of a bush look than the typical strait stem normally seen in common milkweed.

**Data Collection and Analysis**

From May 20th through September 15th of 2017, I checked the milkweed several times a week and collected data on the types of herbivore and herbivore activity observed. To help determine overall health of the plants, other aspects of the plants were also recorded, including the number of stems, number of leaves, number of nodes, number of seed pods, width of stem, height of tallest stem, area of largest leaf, number of leaves with chewing damage, presence of stem damage, presence of leaf curling, and presence of fungal spots. The herbivore information was collected at least once a week with the number of and height of stems and number of both total leaves and chewed leaves. I collected the other information at least twice a month.

For analysis, I broke the dates down into months. I then used this information to determine what the species richness was during those times and to see if there were any trends. I assessed relationships by finding correlations between insect use and the general traits of the plants. I also used linear regression/correlation tests to determine how signs of herbivory affected the overall health of the plants. I quantified signs of herbivory as percentage of leaves with chewing damage. Variables looked at for overall health include the height of the main stem, and percentage of eaten leaves.

For this study, the first-year plants were considered first year if they were planted while the established was the plants which came up from the year before in the plot. Due to the small sample size, they were compared qualitatively instead of using statistical analyses.

# Chapter III: Results

In this section I describe the results from the observations throughout the growing season and how correlation tests provide evidence of the relationships between the plants and the invertebrates.

## General Species Richness

During the growing season, a total of 22 distinct species were observed from ten different taxonomic groups. From these groups, the highest amount of species richness occurred from mid-July through mid-August. The species richness was lower from late-May to early-June and again in mid-September. Some species such as weevils, ants, and aphids were observed as part of the species richness for most of the growing season, with weevil damage appearing throughout the season. In the cases of ants and aphids, they were observed and added to the species richness, but their population dropped as the season progressed (Figure 1).

## General Trends Observed

The flowering period of the milkweed colony seems to have played a role in the species richness observed. Pollinator species such as wasps, honeybees, and butterflies, along with caterpillars, were only observed from mid-July through late August. During this time, the milkweed plants were either flowering or had recently ended flowering. Likewise, other species, such as the large milkweed bug (*Lygaeidae*) and the red milkweed beetle (*Tetraopes tetrophthalmus*), did not appear until around this time. Ladybug appearance seemed to coincide with when pollinators laid their eggs, with ladybugs not appearing until shortly after monarch eggs were observed.

While aphids were observed during most of the season, I observed yellow aphids, or Oleander aphids (*Aphis nerii*), more than the other species, appearing in 73% of the time frames.

I did not observe any clear aphids after mid-July, and I only saw green aphids in late June. Later in the summer the overall aphid population diminished, especially during late July through mid-August, when there were only a few patches of aphids compared to earlier in the season.

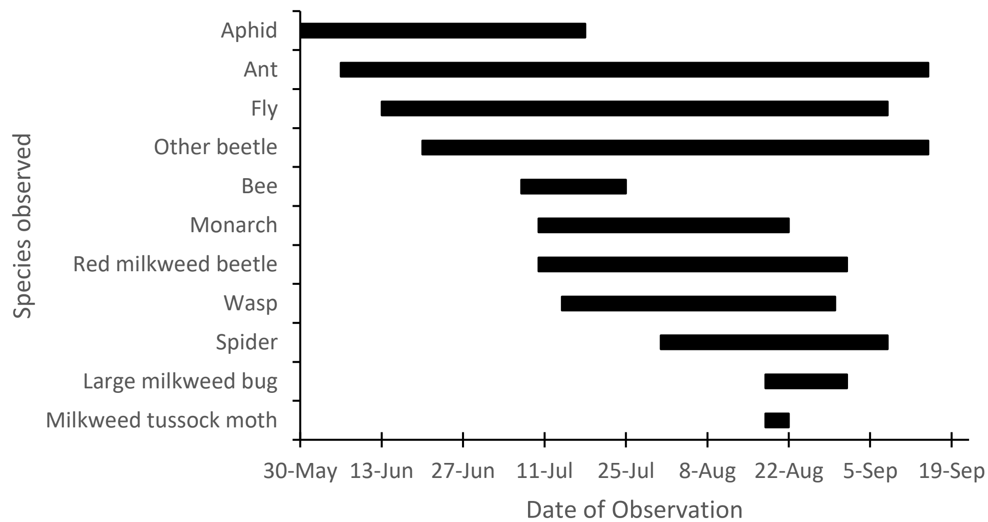


*Figure 1.* Invertebrate presence observed on milkweed during the 2017 growing season.

**Species Richness Observations**

Throughout the growing season, the established milkweed plant held most of the observed species. Most species left the newly planted milkweed alone until they were larger, appearing from mid-July through September.

To see what variables could have affected species richness, I first looked to see when the species richness was highest. From graphing the average species richness by month, it appears that the average species richness steadily increased until its peak in August, then fell in September (Figure 2). I also looked to see if there was a positive correlation between the percentage of leaves with chewing damage and the species richness observed on those plants (Figure 3). I found a weak positive correlation with a linear correlation coefficient of 0.278 and a 95% confidence interval of (0.18, 0.86), although the relationship may be non-linear. Stem height was also positively correlated with invertebrate species richness (Figure 4), with a linear correlation coefficient of 0.78 and 95% confidence interval of (0.35, 0.94). There was an outlier observed, where a taller plant suddenly became shorter due to damage from a storm. If this outlier were removed, the linear correlation would be higher.

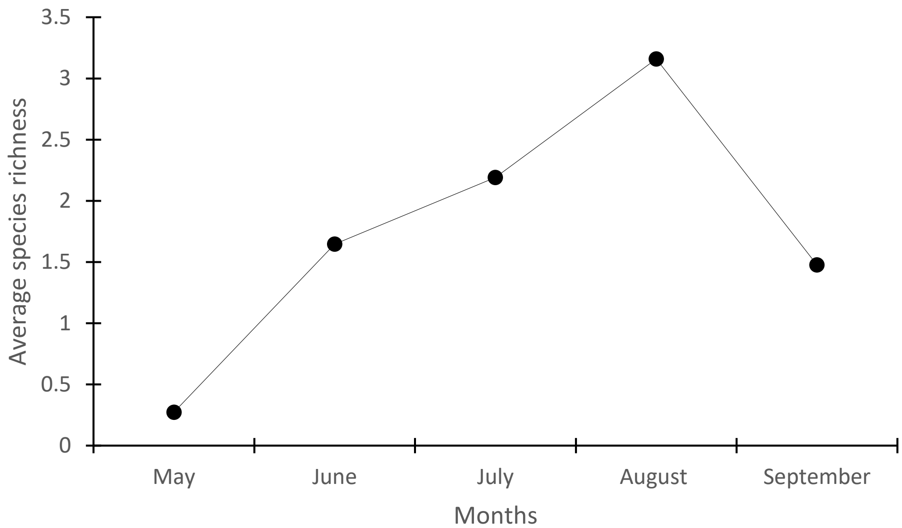


*Figure 2.* Average invertebrate species richness per milkweed plant, by month.

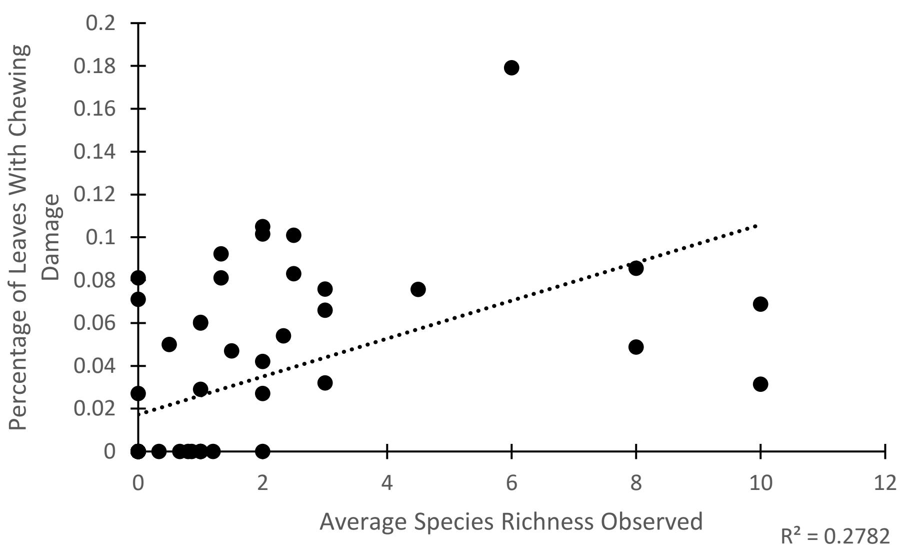


*Figure 3.* Average species richness compared to percentage of leaves chewed per plant.

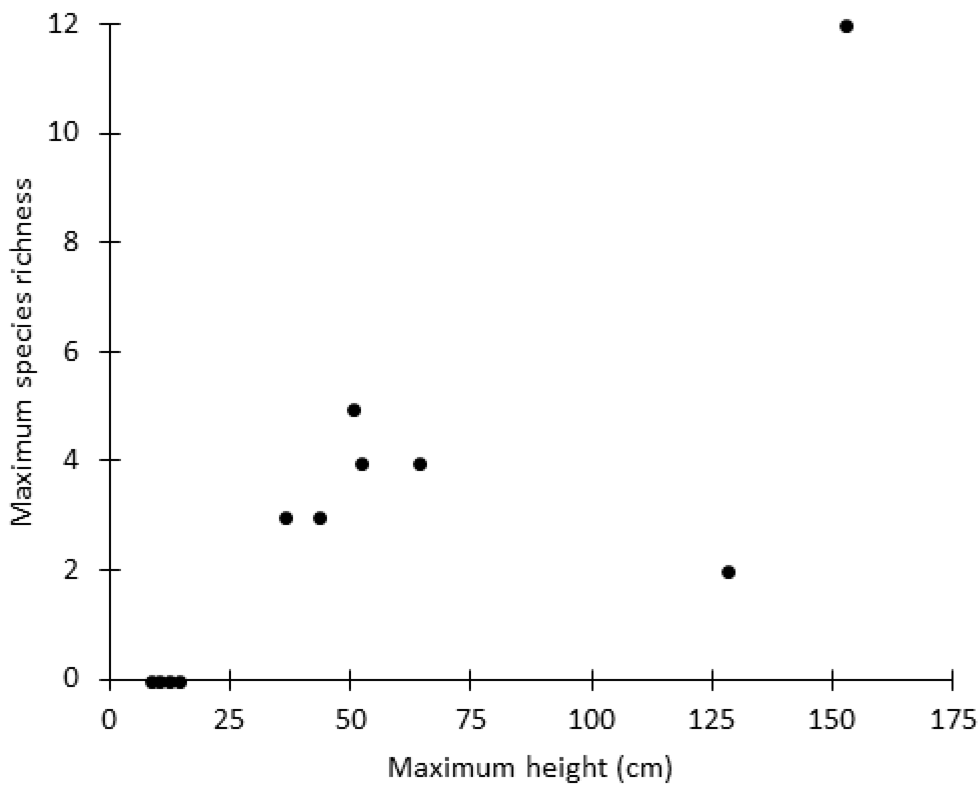


*Figure 4.* Relationship between the maximum height obtained during the growing season and the maximum invertebrate species richness observed in a single observation.

# Chapter IV: Discussion, Conclusion and Recommendation

In this section, I address what the results means in terms of the study and how it can be beneficial to future study and conservation. I will also discuss what can be done in future studies to make the results stronger.

## Discussion

In this study I determined that from July and August is when there was the highest invertebrate species richness. Some species were seen throughout the season, while others only appeared during certain times. Species richness may have been lower in the beginning and end of the season since at those times there was a cooler temperature and not as large a selection of types of food from the milkweed available. I also observed that percentage of leaves with damage was weakly correlated with species richness, but more data are needed to clarify that relationship.

From the beginning of the project, there was low germination success and survival of seedlings. Other studies indicate that some possible reasons for the low germination rate include that they germinate better when scarred, which we did not do, and that fluctuating the temperature between 15-25 degrees Celsius can help with germination (Evetts & Burnside, 1972; Farmer, Price, & Bell, 1986). Many of the seedlings had defects in their stems as they grew. These defects may have been from when they were grown in the greenhouse, since there was little wind to strengthen and prepare the stems for their permanent location.

During the growing season, the species richness was highest in the middle of the season. The main increase was from the amount of pollinator species during the flowering part of the season. From what I observed, there appeared to be certain trends for how particular species appear and use the milkweed. In most of the cases, it was likely related to when their specific food is available. This makes sense, since it would be unusual for a species to go to a plant where there is no food for them, especially when it has potentially dangerous defense mechanisms.

Species richness was positively correlated with the height of the main stem. This was different from the idea that the younger (smaller) plants would be targeted due to their softer leaves. One probable reason for the observed correlation could be that since they were too young to flower they were overlooked by some species, ultimately lowering the species richness observed on them. Like the correlation to plant height, the high correlation to number of leaves to species richness could be due to the fact that the plants with more leaves simply had more area for the species to use. Another contributing factor was that the highest leaf count was from a plant which had multiple stems via rhizomes.

One limitation of this study was that I was unable to estimate the abundance of various invertebrate species, limiting the kinds of inferences possible. If I were to repeat this experiment in the future, I would try to quantify the abundance of different insects that were observed at a time. Weighing their biomass or counting individuals would have allowed me to assess if there truly was a change in abundance over time or if they stayed constant. Another weakness of this project was my learning and improvement in insect identification over the course of the season. Due to this, there is a chance that some species or signs earlier in the study appeared which I did not recognize as a sign of insect use, creating a bias in the data collection compared to later in the summer. To help deter this, I initially had some pictures of common threats, such as caterpillars and signs of weevil and slug damage, but I found I needed to do additional research to identify other signs and species.

Currently, the information gathered can be useful for future milkweed projects because it tells us when to expect distinct species of herbivores and how their population are in west-central Wisconsin. In the future, it would be interesting to try to replicate this experiment in various locations throughout the area, or across Wisconsin. This would be interesting, because then we could see if the results observed were simply in this location or if they are similar to other places, since milkweed tends to adapt to its local microclimate. If they were consistent across the state, then it may prove more significant for what was learned in this study. It would also be interesting to see if there is any difference from the observed results with other species of milkweed.

If future research is conducted, I would also recommend doing additional checks during the night and of the roots periodically, along with during the day. This would give a better idea of what species use the plant across more than just diurnal time periods. This is especially true since it could give a better idea of insect use by moth species and certain beetles which spend more time underground.

**Conclusions**

Based on my observations, during late July and August when the plants are flowering, milkweed is used most frequently by the largest amount of distinct species. Due to this, if there are concerns about the health or establishment of milkweed, this time is when the plants should be protected. Damage from herbivores did appear to increase with the species richness, but this did not appear to greatly affect the plants because the richness was positively correlated with stem height. With this in mind, it seems more important to ensure the overall health of the plant and not simply one aspect of the plant.

**Recommendations**

When planting new milkweed in an area it is a good idea to start with slightly older plants which will reach a flowering stage sooner. This will ensure proper vegetation for species which need the nectar or seed pods, especially if those are species being targeted. If certain invertebrates are of interest, then it is also important to keep in mind when they will be present and insure that the food source is available and in good health during those times.

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