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Monarch butterfly larvae and airborne chemicals in relation to identifying milkweed

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ABSTRACT

Monarch butterflies rely heavily on the milkweed plant. As young caterpillars they only eat the leaves of the milkweed plant. Later in life, while they don't need to eat, they do again call on the milkweed plant to deposit their eggs. An added benefit to this mutualistic relationship is that thanks to exclusively eating milkweed, the butterfly obtains a mild toxin, making them safer in the wild, deterring predators with their bright colors letting them know they are a threat and are not worth the trouble. In some situations, larvae can consume all the leaves around them, so they must travel to the next nearest milkweeds plant. Caterpillars have common senses like smell, taste, touch, sound, and sight. However, their vision is limited to light/dark detection with little or no image formation. Using their other senses, they need to identify the next plant and because of their slow speed, it would be beneficial to get it right the first time. I tested their use of airborne chemicals to locate milkweed by using y-maze trials, with options of either a milkweed plant and a non-milkweed plant. After the trials were ran there was no significant evidence for the caterpillars relying on such chemicals. During the trials the larvae seemed to use random chance and luck more than their senses to travel to their next meal. This means their sense of smell may be weaker than previously thought, and that they might use their other common senses to lead them to the next milkweed plant, though insect vision poor in their pupal state taste could play a larger role than previously expected. This would mean that they use taste to determine if they in fact found the correct plant.

Keywords: *Milkweed, Monarch Butterflies, Larvae*

INTRODUCTION

The monarch butterfly (*Danaus plexippus*) and milkweed plant have one of the most famous mutualistic relationships in the animal kingdom. The entire life cycle if the monarch butterfly revolves around the milkweed plant. They are born there, eat their, and will die soon after they lay their eggs their once again. The larvae gain a mild toxin from eating exclusively the milkweed plant, this is why they are so bright and colorful, telling potential predators that they are a threat and shouldn't be preyed upon. This helpful trait while aiding them to survive limits their diet and forces them to have very limited food resources.

Without the other each are in danger of going extinct extremely quickly. With rising temperatures and fewer plants, the monarch butterflies are struggling to survive, as witnessed in populations east of the Rocky Mountains (Pocius 2018). Increased urbanization is causing their milkweed populations to decline, which has been directly linked to problems with slowing monarch population recovery (Pleasants and Oberhauser, 2013; Flockhart et al., 2015; Pleasants et al., 2017, Knight et al., 2019). Efforts to help preserve milkweed in their main migration pathway has been a focus to help increase population numbers (Pocius 2018).

Monarchs (*Danaus plexippus)* are well known for taking part in a near 3000-mile migration annually where they travel every fall to breed in the southern United States. Through their migration they encounter a multitude of different species of milkweed, some native and some non-native to their birthplace. Milkweed host plants commonly grow on crop field margins, potentially exposing them to a range of agrochemicals such as neonicotinoid insecticides used in corn and soybean production (Olaya-Arenas & Kaplan, 2019; Pecenka & Lundgren, 2015) (Olaya-Arenas et al., 2020).

Caterpillars have the same five common senses humans have. Touch, taste, smell, sight, and sound. Though insect sight, especially in their early stages is poor, only allowing for recognition of light or dark, but not sharp edges or shapes (Péter 2020). This suggests they use their other senses to locate food. Perhaps taste is what tells the young caterpillar they have found the correct plant. Alternatively, smell could play a role in directing the larvae from a distance, allowing them to find food from far away.

The aim of this study is to assess the ability of monarch butterflies (*Danaus plexippus*) to locate a nearby milkweed plant for potential feeding. Are they directed by a chemical in the air that points them in a specific direction, or do they utilize a different method? If we better understand their feeding ecology, we could use this in conservation efforts for the declining butterfly. Currently there is little study into this question. We know very little on what attracts monarchs (*Danaus plexippus*) to the milkweed plant, leaving us to wonder how caterpillars locate the correct plant once leaving an already consumed host plant. Is their sense of smell their strongest sense and how effectively can they utilize it the help them locate another food source?

MATERIALS AND METHODS

To better understand the relationship the larvae, have with the milkweed plant and how they can so reliably locate a new plant to feed on, I have investigated the possibility that they use airborne cues.

Milkweed leaves were collected from the wild and stored in a refrigerator until use in trials in order to keep them fresh. Monarch butterflies were ordered as larvae from (www.monarchwatch.org). Once delivered, they were maintained in the lab by placing a fresh wet paper towel every other day and adding milkweed leaves *ad libitum*. Once the caterpillars got about to the 3rd or 4th instar, they were used in the choice trials described below.

To test the hypothesis, I removed their food source for about a half a day prior to the start of trials, making sure they aren't going to starve but will be ready to eat when the experiment starts. Next, I took one larva at a time, placed it in the "Y" tube. . One tube arm led to a small sample of milkweed leaf, ready for the larvae to find it. The other tube arm led to a plant leaf unfamiliar to them called sliver squill (*Iedebouria socialis*) which in theory the larvae should have no interest in visiting much less eating. The squill plant has no close relation to the milkweed plant so there should be a distinction made by the caterpillars if they in fact detect airborne chemicals to find food. I flipped a coin to determine which plant would go on which side of the tube this way the larvae wouldn't just choose one side every time a gave the trials more randomness. I crumpled both the milkweed and non-milkweed leaves, making sure I broke parts of the leaf allowing for the aroma that was to be excreted by the leaf to be more easily brought up into the air. At each end of the y-tube arm was a small computer fan that created a gentle breeze directed down the tube bringing the airborne chemicals of each plant choice towards the larvae. Larvae were watched to determine which arm of the y-tube they chose to crawl down. I repeated this process 3 times for every larva, resetting the larvae to the beginning of the tube each time and re-flipping the coin to decide which side the milkweed would be place on. The tubes obstructed the view of the caterpillars to the plant, this was done on purpose to eliminate the use of any visual signal on their decision.

I tested a total of 22 caterpillars three times each. In each trial, caterpillars were nearly equally likely to head to either plant at the end of the y-tube arm (Figure 1). These choices were not significantly different from random choice. When confronting the data, there is a slight lean towards the milkweed path being chosen more often but not in any significant way. On average, just due to random chance, we would expect the that each path or plant would be chosen. Similar to flipping a coin there is a 50/50 chance you will get either option. The results showed that 55% of the time the caterpillar chose the correct path and successfully made it to the milkweed. However, the other 45% they chose wrong and would have unnecessarily expanded resources in the wild if they were looking for a new source of food. To determine this, I used a t-test to quantify the data. This resulted in a 0.22655, substantially low when the number should have been closer towards the 0.5 mark, telling me there is little reason to believe there was anything airborne, at least at the time, that was attracting them to a side and directing them to their food. In fact, the larvae didn't seem too interested in the milkweed, most larvae that traveled down the tunnel eventually ended up on the celling of the tube without even visiting the milkweed. The same can be said about the other end of the tube they in fact never visited the other leafy plant they instead again crawled to the celling and stopped. This could show that they are trying to find a spot to chrysalis or weren't interested in the milkweed instead looked for a path out.

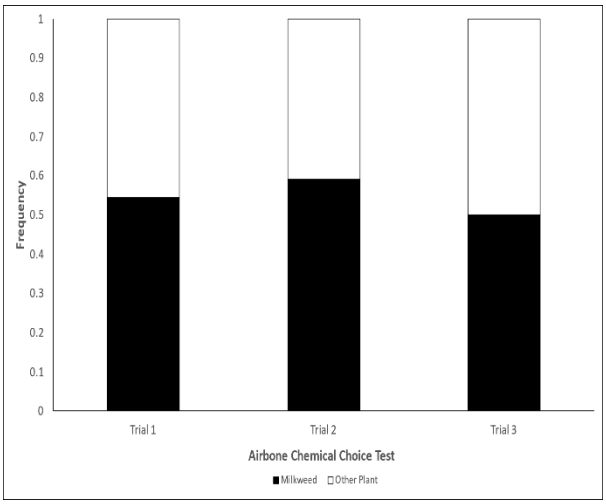

Figure 1. The figure above shows the three trials carried out and the height of the black bar is the relative prevalence of the larvae choosing the milkweed. All the bars are around the same size showing little evidence that the caterpillars are receiving signals to direct them in the correct way.

DISCUSSION

In this study, I found no evidence that Monarch caterpillars use airborne chemicals to locate potential milkweed plants for food.

It is my idea that several factors played into why the process of data collection was so frustratingly slow and inconclusive. After the fact I wondered if I had starved the larvae for a day before running the trials would the volition of the larvae be stronger and more obvious as they travel to finally eat after food deprivation. On top of that the day of leaving them alone would have given them a much needed, no

maintenance day where I didn't go in their enclosure and pock them around allowing them to destress and return to a healthy state.

Considering the size of a full-grown milkweed plant is relatively large, one plant could sustain a couple clutches of eggs into adult hood. However, the plants are becoming hard to come by due to habitat loss, so I would expect the insects to have a more definitive mode of identifying the plant they rely on for survival. The results paint a different picture than what I envisioned in the beginning.

The larvae were removed from their enclosures when they were in the 4th instar stage, which is the final stage before they begin getting in position for their chrysalis stage. At this time the caterpillars should have been ready to eat, working to fill their energy reserves for their transformation. The observed lack of a preference, may be as simple as the larvae taste test leaves until it finds the familiar taste of milkweed. They may also still receive signals in the air to help point them in the correct direction, but they may be dampened like their vision. Giving themselves time to make it to the next plant so that they can eat may be a constant threat that the larvae need to juggle daily in the fight to survive.

To better help larvae that may struggle finding their next host plant, it may be important to plant a cluster of milkweed plants in a given area. A butterfly garden is an ideal place for monarchs (butterflies in general) to deposit their eggs and they are typically filled with milkweed. This meaning for larvae that find themselves running out of room on their original plant they can move to a nearby one with a much higher probability of success in finding the correct plant. Similarly, roadside grassland and wildflower reserves are vital to sustaining the monarch population. They provide a natural habitat that has less of a chance for human interaction. Simply leaving these areas "alone" or managed, if necessary, has the potential to not only help monarch butterflies but nearly every other living thing in the area. In order to help the environment, we need to look at smaller habitats and start there.

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