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Performance of Early Instar Monarch Butterflies (*Danaus plexippus* L.) on Nine Milkweed Species Native to Iowa

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Abstract

Over the past two decades, the population of monarch butterflies east of the Rocky Mountains has experienced a significant decline. Habitat restoration that includes milkweed plants is crucial to boost population numbers in the breeding range. Monarch butterfly larvae use milkweeds as their only host plant, but larval performance on different milkweed species is not well documented. We examined early instar survival and growth on nine milkweed species native to Iowa. These included *Asclepias exaltata* (poke milkweed), *A. hirtella* (tall green milkweed), *A. incarnata* (swamp milkweed), *A. speciosa* (showy milkweed), *A. sullivantii* (prairie milkweed), *A. syriaca* (common milkweed), *A. tuberosa* (butterfly milkweed), *A. verticillata* (whorled milkweed), and *Cynanchum laeve* (honey vine milkweed). In laboratory and greenhouse experiments, larval survival on all nine milkweed species did not differ. Larvae that fed on *C. laeve* plants were an instar behind larvae that fed on any other species, while larvae that fed on *A. verticillata* weighed more than larvae that fed on any other species. Our results show that early instar larvae can survive on all nine milkweed species.

Keywords

monarch butterfly, milkweed, conservation, larval feeding

Disciplines

Ecology and Evolutionary Biology | Entomology | Natural Resources and Conservation | Plant Sciences | Population Biology

Comments

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PERFORMANCE OF EARLY INSTAR MONARCH BUTTERFLIES (*DANAUS PLEXIPPUS* L.)
ON NINE MILKWEED SPECIES NATIVE TO IOWA

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ABSTRACT. Over the past two decades, the population of monarch butterflies east of the Rocky Mountains has experienced a significant decline. Habitat restoration that includes milkweed plants is crucial to boost population numbers in the breeding range. Monarch butterfly larvae use milkweeds as their only host plant, but larval performance on different milkweed species is not well documented. We examined early instar survival and growth on nine milkweed species native to Iowa. These included *Asclepias exaltata* (poke milkweed), *A. hirtella* (tall green milkweed), *A. incarnata* (swamp milkweed), *A. speciosa* (showy milkweed), *A. sullivantii* (prairie milkweed), *A. syriaca* (common milkweed), *A. tuberosa* (butterfly milkweed), *A. verticillata* (whorled milkweed), and *Cynanchum laeve* (honey vine milkweed). In laboratory and greenhouse experiments, larval survival on all nine milkweed species did not differ. Larvae that fed on *C. laeve* plants were an instar behind larvae that fed on any other species, while larvae that fed on *A. verticillata* weighed more than larvae that fed on any other species. Our results show that early instar larvae can survive on all nine milkweed species.

Additional key words: monarch butterfly; milkweed; conservation; larval feeding

Over the last two decades, the populations of monarch butterflies (*Danaus plexippus* L., Nymphalidae) east and west of the Rocky Mountains have experienced a significant decline in overwintering numbers (Brower et al. 2012, Espeset et al. 2016, Inamine et al. 2016). Although recent work has suggested that these declines may not be representative of monarch population size during other stages of monarch phenology or ontogeny (Davis 2012, Davis & Dyer 2015), this decline has been attributed to the loss of milkweed in agricultural fields resulting from the rise of genetically modified crops, increased agricultural herbicide spraying (Oberhauser et al. 2001, Pleasants & Oberhauser 2013), and potentially limited nectar resources (Inamine et al. 2016) as well as the loss of overwintering habitat (The Center for Biological Diversity 2014). Recent models have implicated the loss of milkweeds within the breeding range as the largest threat to the monarch population (Zalucki &

Lammers 2010, Flockhart et al. 2015, Zalucki et al. 2016). Monarchs require milkweed species as larval host plants, but apparently feed indiscriminately on nectar from a variety of plants as adults (Brower et al. 2006). Restoration of monarch habitat within the breeding range is of utmost concern to boost population numbers (Oberhauser et al. 2016); roughly 29 milkweed plants will be needed to produce one adult monarch that will be part of the migratory generation (Nail et al. 2015). For that reason, there have been extensive efforts across federal, state, and non-profit groups to establish monarch habitat to boost monarch numbers. These restoration projects have focused on adding milkweeds to the landscape. Most monarchs found at the overwintering sites have originated in the Midwest (Wassenaar & Hobson 1998, Flockhart et al. 2017) and fed on common milkweed, *Asclepias syriaca* (Asclepiadaceae), as larvae (Seiber et al. 1986, Malcolm et al. 1989). However, there are a number of milkweed

species in the Midwest that were probably used by monarchs before agriculture dominated the landscape and increased the abundance of common milkweed. These other milkweed species could potentially provide important resources, but more information is needed about monarch larval performance on these milkweed species to ensure the most efficient and effective use of resources.

Since the advent of agriculture, milkweeds that grew in-between crop rows in the Midwest (*A. syriaca*) were among the most heavily used monarch host plants in the North American breeding range (Oberhauser 2001, Pleasants & Oberhauser 2013). Virtually all restoration recommendations to date are based on *A. syriaca*, whereas the historic Midwestern grassland and wetland habitats contained several (2–4) milkweed species (Hayden 1919, Pleasants 2015). There are surprisingly few studies that address larval survival on milkweed species with overlapping ranges. Of the studies comparing larval feeding on milkweed species in North America that do exist, Erickson (1973) measured larval performance and nutrition on four milkweed species, while Schroeder (1976) evaluated an energy budget for larvae that fed on *A. syriaca*. Ladner and Altizer (2005) examined growth differences between monarchs collected from eastern and western North America on widely distributed milkweed species; Yeargan and Allard (2005) examined growth differences of larvae that fed on *A. syriaca* and *Cynanchum laeve*; Zalucki et al. (2012) studied the survival and growth of first instars on milkweeds in southern California; Robertson et al. (2015) focused on larval preference among four desert milkweeds native to California; and Agrawal et al. (2015) compared larval performance on a broad range of milkweed species, some of which were native to North America, to determine the impacts of evolutionary history and latex on milkweed defenses and monarch growth. Because most milkweeds native

to the Midwest, especially those with narrow ranges, have not been tested, we examined larval survival on nine milkweed species native to Iowa, which is a high priority area for Midwestern conservation efforts (The Center for Biological Diversity 2014). The species we examined are: *A. syriaca*, *A. incarnata*, *A. tuberosa*, *A. verticillata*, *A. speciosa*, *A. exaltata*, *A. sullivantii*, *A. hirtella*, and *C. laeve*. These species have overlapping ranges (Woodson 1954), varying concentrations of both cardenolides (Woodson 1954, Roeske et al. 1976, Malcolm 1991, Agrawal et al. 2009, Rasmann & Agrawal 2011, Table 1) and quercetin glycosides (Haribal & Renwick 1996, Agrawal et al. 2009), and different habitat requirements (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, Table 2). We examined larval performance on excised leaves and whole plants of the nine species listed above. An investigation of larval performance on excised leaves separates differences in intrinsic leaf qualities, such as cardenolide content, from the latex found in intact plants, while the data from intact plants addresses latex and overall plant architecture as additional factors in larval performance. Understanding larval performance on each of these milkweed species will be useful in choosing milkweed species for monarch habitat restoration efforts across the Midwestern U.S.

METHODS

Monarch larva used in experiments. A monarch butterfly colony was started by collecting 253 monarch eggs and young larvae on *A. syriaca* and *A. incarnata* plants from May 21 to June 9, 2014 from Boone and Story Counties in Iowa. Larvae were reared on *A. syriaca* through the summer growing season in 2014 and *A. curassavica*, a tropical milkweed, from greenhouse-grown plants through the fall and winter of 2014. Adults were allowed to mate and eggs were collected for propagation of the colony on a weekly

TABLE 1. Cardenolide and quercetin glycoside concentration of nine native milkweeds. Chemical concentrations from Woodson (1954), Roeske et al. (1976), Agrawal et al. (2009), and Rasmann & Agrawal (2011)

Milkweed Species	Cardenolides (mg/gram dried leaves) Woodson (1954)	Cardenolides (mg/gram dried leaves) Roeske et al. (1976)	Cardenolides (% Dry Mass) Agrawal et al. (2009)	Shoot Cardenolides (µg/mg) Rasmann and Agrawal (2011)
<i>A. exaltata</i>	0–0.70	0–0.70	.125	0.735
<i>A. hirtella</i>	n/a	n/a	.208	3.289
<i>A. incarnata</i>	0–0.28	0–0.28	.117	0.511
<i>A. speciosa</i>	0.149	0.15	.227	1.112
<i>A. sullivantii</i>	n/a	n/a	.123	2.149
<i>A. syriaca</i>	0.06–2.64	0.06–2.64	.113	1.573
<i>A. tuberosa</i>	0–0.06	n/a	.064	0.070
<i>A. verticillata</i>	0	n/a	.114	0.031
<i>C. laeve</i>	n/a	n/a	n/a	n/a

basis. Twelve generations of colony breeding preceded the beginning of this experiment (Summer 2014–Spring 2015). All of the resulting larvae from colony matings were reared on *A. curassavica* prior to the beginning of this experiment in late spring 2015. Although the colony was exposed to *A. syriaca* in generations prior to this experiment, we do not think that the colony adapted to a particular host plant because monarchs collected from opposite coasts of the U.S. showed no host preference for milkweeds based on geographic location after colony breeding (Ladner & Altizer 2005).

Excised leaf feeding assay. Milkweeds of all nine species were grown from seed without the use of chemical pesticides in a greenhouse at Iowa State University (21.1–35 °C, 16h photophase, and 56% relative humidity (rh)). During each trial, blocks of petri dishes were set up where each block contained 9 petri dishes, with one replicate of each milkweed species and one larva per petri dish. There were six sets of six blocks throughout this assay. For each group of six blocks, plants of each species were randomly selected, stems were cut, leaves were taken above the cotyledon leaf, and the leaves were immediately placed in water. Leaf material was kept cool and transported to the laboratory in wet paper towels, surface sterilized in 10% bleach (sodium hypochlorite) solution for 10 min., and then rinsed 3 times for 10 minutes (30 minutes total) each with cool running water in order to remove potential pathogens, such as OE. Petri dishes (60 mm × 15 mm) were prepared with water-based agar (2.0% w/v agar to water) to keep plant material moist.

Plant species were randomly assigned within a block (each trial= 6 blocks; 6 trials were included for n=36 total blocks). Plant material was placed into each block of petri dishes and one first instar was added to each petri dish. Larvae were kept on trays in an incubator set at 28°C and 40% RH with a 16:8 hr. photophase. Larvae were monitored daily for survival and surface-sterilized leaf material was provided ad libitum; all leaf material was replaced daily. After five days, larvae were removed from the petri dishes. By conducting this assay over a short five-day period, we were able to avoid large reductions in sample size associated with early instar mortality on some host plants (Hóðar et al. 2002). We harvested all larvae after five days throughout the study in order to compare the mass gain and developmental stage for each larva over a fixed amount of time (Agrawal et al. 2015). Larval mass was recorded to the nearest hundredth of a milligram (AND Model GR-202). Head capsule width was measured using a Nikon SMZ 1000 microscope (0.75 × objective, 10× eyepiece with eyepiece grid set with a stage micrometer) and was recorded to the nearest tenth of a millimeter. Instar was

determined from head capsule measurements (Oberhauser and Kuda 1997). All larvae were frozen (-28°C) immediately after weighing.

Whole plant feeding assay. Milkweeds of all nine species were grown from seed without the use of chemical pesticides in a greenhouse (21.1–35 °C, 16h photophase, and 56%rh) at Iowa State University. Seeds were sown into 128-cell plug trays (Landmark Plastics, Akron OH) and then at approx. 6 weeks from germination were transplanted into 3.5 inch square deep perennial pots (Kord, Ontario Canada). Plants ranged from 10–30cm in height depending on milkweed species; milkweeds were 8 weeks old when used in each trial. Each plant was watered and placed into a water-filled waxed-paper cup. One neonate was added to each plant. A mesh pop-up hamper cage (57× 37× 55 cm) was placed over the plant and neonate; a no-see-um netting bag was pulled up over the mesh cage and tied on the top with a wire tie. A block in this case included one whole plant of each of the 9 species growing in the pop-up cage. The total number of blocks was 6 per trial, 36 blocks total.

All blocks were kept on the same bench in the greenhouse (21.1–35 °C, 16h photophase, and 56%rh) positioned in a randomized complete block design (6 groups of 6 blocks as in the excised feeding assay). Greenhouse temperature was recorded hourly via Thermocron sensors (iButton, New South Wales Australia). Larval weight (mg), survivorship, and head capsule width (mm) were recorded after 5 days.

Lipid assay. Lipid content was quantified for larvae used in the excised leaf feeding and larvae used in the whole plant feeding assay. Lipid content was quantified using whole bodies of individual larvae that were 5 days old, a mixture of 2nd and 3rd instars, via colorimetric assays with a sulphophosphovanillin reagent, a method that has been demonstrated to provide consistent results for honey bees (Toth et al. 2005, Toth & Robinson 2005). We homogenized whole caterpillars (n=6 per milkweed species for both the excised leaf feeding assay and the whole plant feeding assay, for a total of 108 larvae analyzed) in 2:1 chloroform:methanol solvent in 12 mL glass vials using glass stirring rods to crush each individual. Samples were then left undisturbed for 17 hours to allow the lipids to be extracted into chloroform methanol. After 17 hours, samples were strained through glass wool to remove particulates and leave only lipids dissolved in chloroform methanol. Extracted lipids were then stored in 1mL of 2:1 chloroform:methanol at -20C. One hundred µL of lipid extract was used in each assay. Each sample was dried completely under a stream of air, then 200 µL of 100% sulfuric acid were added, and

all samples were heated for 10 minutes in a bath of boiling water. Two ml of a sulfophosphovanillin reagent were added to each sample (Toth et al. 2005). Samples were then briefly vortexed and placed in the dark for 15 minutes to allow the reaction to proceed. Three technical replicates of 200 ul of the resulting solution from each sample were measured for absorbance in a Gen5 2.06 multiwell spectrophotometer at 525 nm. The average of the three replicates was used to estimate lipid quantity by treatment. Estimated quantities of lipids were calculated from standard curves, run alongside the samples, based on known concentrations of cholesterol in petroleum ether (Toth & Robinson 2005, Toth et al. 2009).

Statistical analysis. Data were analyzed using R version 3.1.2 (R Core Team 2014). Data were combined across trials (36 blocks total) within each experiment, as blocks were not significantly different from one another. Differences in survival were determined using a log rank test on the Kaplan-Meier survival estimates for larvae fed each milkweed species. A one-way ANOVA was used to assess differences in larval mass and head capsule width between groups relative to the milkweed species they were fed in both excised feeding and whole plant experiments. A Tukey HSD test was used to assess pairwise differences in larval responses among milkweed species. A linear regression was used to assess the relationship between larval mass and cardenolide content, reported in Agrawal et al. 2009, in the excised feeding assay. Mass and head capsule width were not transformed prior to analysis. A one-way ANOVA was used to assess differences in total percent of lipids between groups relative to the milkweed species they were fed in both excised feeding and whole plant experiments. A Tukey HSD test was used to assess pairwise differences in larval lipid percentages.

RESULTS

Excised leaf feeding assay. Larval survivorship varied from 94–100% across milkweed species, averaging 96% across treatments. Survivorship did not differ among milkweed species ($\chi^2=9.8$, d.f. =8, $p < 0.05$). Larval mass was significantly different among milkweed species ($F=11.65$, d.f. =8, $p < 0.001$). Larvae that fed on *C. laeve* weighed significantly less than those that fed on *A. incarnata* ($p < 0.01$), *A. tuberosa* ($p < 0.01$), and *A. verticillata* ($p < 0.01$; Figure 1). Larvae that fed on *A. hirtella* weighed significantly less than those that fed on *C. laeve* ($p < 0.05$), *A. incarnata* ($p < 0.001$), *A. speciosa* ($p < 0.01$), *A. sullivantii* ($p < 0.001$), *A. syriaca* ($p < 0.001$), *A. tuberosa* ($p < 0.001$), and *A. verticillata* ($p < 0.001$; Figure 1). Larvae that fed on *A. exaltata* weighed significantly less than those that

fed on *A. incarnata* ($p < 0.001$), *A. tuberosa* ($p < 0.001$), and *A. verticillata* ($p < 0.001$; Figure 1).

Larval head capsule width was significantly different among milkweed species ($F= 2.56$, d.f. =8, $p < 0.01$) when all instars were pooled; head capsule width was positively correlated with larval weight. This relationship was significant ($r=0.71$; $p < 0.001$). Larvae that fed on *A. incarnata* developed to 4th instars and had the largest head capsule width. Larvae that fed on *A. hirtella* developed to 3rd instars and had a head capsule width that was significantly smaller than those fed on *A. incarnata* ($p < 0.05$) or *A. verticillata* ($p < 0.05$; Figure 2). All other comparisons were not significantly different.

Whole plant feeding assay. Larval survivorship varied from 81–100% across milkweed species, averaging 90% across treatments. Survivorship did not differ among milkweed species ($\chi^2=11.4$, d.f. =8, $p > 0.05$). Larval mass was significantly different among species ($F=6.956$, d.f. =8, $p < 0.001$; Figure 3). Larvae fed *A. verticillata* weighed more than larvae fed any other species (Figure 4) and were significantly different from *C. laeve* ($p < 0.001$), *A. incarnata* ($p < 0.01$), *A. speciosa* ($p < 0.05$), *A. sullivantii* ($p < 0.01$), or *A. tuberosa* ($p < 0.001$). Larvae that fed on *C. laeve* weighed the least. This difference was significant in comparison to *A. hirtella* ($p < 0.001$), *A. exaltata* ($p < 0.05$), *A. speciosa* ($p < 0.05$), *A. sullivantii* ($p < 0.05$), *A. syriaca* ($p < 0.05$), and *A. verticillata* ($p < 0.001$). No other species showed differences in pairwise comparisons.

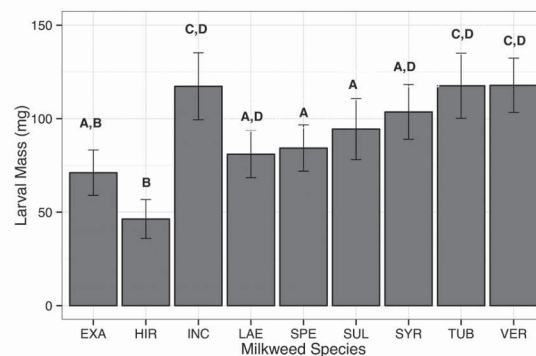


FIG. 1. Differences in mass (mg) among larvae fed excised leaves of nine native milkweed species. This graph represents 6 trials (36 blocks, 315 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=34 larvae), HIR= *A. hirtella* (n=34 larvae), INC= *A. incarnata* (n=35 larvae), LAE= *C. laeve* (n=36 larvae), SPE= *A. speciosa* (n=34 larvae), SUL= *A. sullivantii* (n=35 larvae), SYR= *A. syriaca* (n=35 larvae), TUB= *A. tuberosa* (n=36 larvae), and VER= *A. verticillata* (n=36). Bars that share a letter are not significantly different from each other at $p < 0.05$.

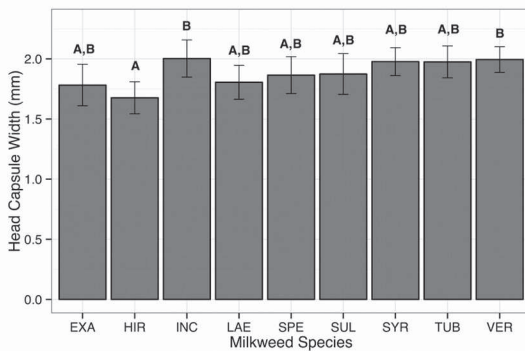


FIG. 2. Differences in head capsule width (mm) among larvae fed excised leaves of nine native milkweed species. This graph represents 6 trials (36 blocks, 315 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=34 larvae), HIR= *A. hirtella* (n=34 larvae), INC= *A. incarnata* (n=35 larvae), LAE= *C. laeve* (n=36 larvae), SPE= *A. speciosa* (n=34 larvae), SUL= *A. sullivantii* (n=35 larvae), SYR= *A. syriaca* (n=35 larvae), TUB= *A. tuberosa* (n=36 larvae), and VER= *A. verticillata* (n=36 larvae). Bars that share a letter are not significantly different from each other at $p < 0.05$.

Larval head capsule width was significantly different among milkweed species ($F = 17.25$, d.f. = 8, $p < 0.001$); head capsule width was positively correlated with larval weight. This relationship was significant ($r = 0.54$; $p < 0.001$). All larvae reached the third instar during the study, with the exception of those fed *C. laeve*. Larvae that fed on *C. laeve* did not reach the third instar. Larvae fed *C. laeve* had a significantly smaller head capsule width in comparison with each of the other 8 milkweed species ($p < 0.001$ for all species). No other species showed differences in pairwise comparisons.

Lipid assay. During excised leaf feeding trials, lipid concentration (lipids as a percentage of total larval mass) was not significantly different among caterpillars that fed on nine different milkweed species ($F = 0.475$, d.f. = 8, $p > 0.05$). However, the percent lipid was different among larvae that fed on different species of milkweed plants in the whole-plant assay ($F = 3.707$, d.f. = 8, $p < 0.01$). Larvae that fed on *A. incarnata* had a higher percentage of lipids than larvae that fed on *A. exaltata* ($p < 0.01$), *A. hirtella* ($p < 0.05$), *A. sullivantii* ($p < 0.05$), *A. syriaca* ($p < 0.05$), *A. tuberosa* ($p < 0.05$), or *A. verticillata* ($p < 0.001$). All other comparisons were not significantly different.

DISCUSSION

Our findings suggest that young monarch larvae can survive on all nine milkweed species. Eight of the nine species could be used for monarch habitat restoration in the Midwest, provided that each species is planted

within its native range and in its appropriate habitat (Table 2). *C. laeve* is not the best choice for such plantings because larvae did not grow as quickly when they fed on this species.

Larvae that fed on excised leaves reached the fourth instar in five days, while larvae that fed on whole plants only reached the third instar in five days in the greenhouse. On average, larval mass after 5 days for larvae that fed on whole plants was 33.4% that of larvae fed on excised leaves. Differences in instar and larval mass are likely due in part to differing temperatures between excised leaf and whole plant experiments. Larvae fed leaf material in petri dishes in the laboratory experienced a stable temperature of 28°C in the controlled environmental chamber while those that fed on whole plants experienced fluctuating temperatures from 23°C to 28°C in the greenhouse. Given that larval growth rates are dependent on temperature (Zalucki & Kitching 1982), the lower temperature in the greenhouse probably resulted in less rapid growth during the whole-plant feeding assay. Larvae that fed on excised leaves also were not exposed to plant latex flow and pressure, which can slow larval growth by up to 50%; larvae in petri dishes also moved less due to a confined space and did not need to negotiate the architecture of the plants (Zalucki & Malcolm 1999, Zalucki et al. 2001a). Larval mortality was minimal throughout the study (96.6% survival excised leaf feeding; 90.4% survival plant feeding), well below ~50% reported elsewhere regardless of whether larvae

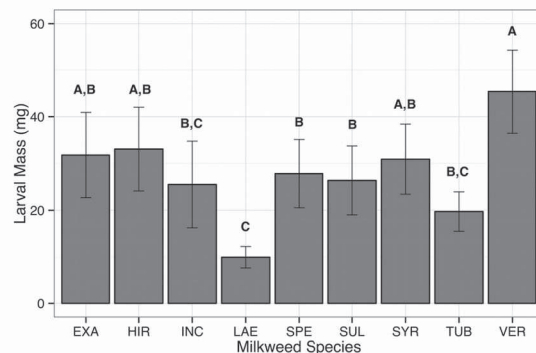


FIG. 3. Differences in mass among larvae fed whole plants of nine native milkweed species. This graph represents 6 trials (36 blocks, 294 larvae total). Each bar represents one milkweed species; error bars depict 95% confidence intervals. EXA= *A. exaltata* (n=31 larvae), HIR= *A. hirtella* (n=32 larvae), INC= *A. incarnata* (n=31 larvae), LAE= *C. laeve* (n=31 larvae), SPE= *A. speciosa* (n=31 larvae), SUL= *A. sullivantii* (n=31 larvae), SYR= *A. syriaca* (n=36 larvae), TUB= *A. tuberosa* (n=34 larvae), and VER= *A. verticillata* (n=36 larvae). Bars that share a letter are not significantly different from each other at $p < 0.05$.

TABLE 2. Summary of milkweed habitat preferences. Information compiled from Woodson (1954), Kaul et al. (1991), Eilers & Roosa (1994), and USDA-NRCS (2017).

Milkweed Species	Common Name	Habitat Preference	Blooming Period	Soil Moisture	Soil Type	Iowa Distribution
<i>Asclepias exaltata</i>	Poke Milkweed	Woodland edges, upland woods	May–August	Moist	N/A	Northeastern Iowa
<i>Asclepias hirtella</i>	Tall Green Milkweed	Prairie remnants, fields	May–September	Mesic to Dry	Sandy and clayey soils	South Central Iowa
<i>Asclepias incarnata</i>	Swamp Milkweed; Rose stream banks, wet woods, swamps, marshes, Milkweed	Wet meadows, floodplains, riverbanks, pond shores along canal banks, and riparian sites	May–August	Moist to Mesic	Clayey soils, neutral to slightly acidic pH	Entire State
<i>Asclepias speciosa</i>	Showy Milkweed	Pastures, meadows, forest clearings, untilled fields, roadsides, and ditch banks	May–September	Moist to Dry	Sandy, well-drained soils, neutral to slightly acidic pH	Western Iowa
<i>Asclepias lincantii</i>	Prairie Milkweed	Mesic prairie, alluvial meadows, floodplains, and level roadsides	June–August	Moist to Mesic	Low, moist soils	Central and Western Iowa
<i>Asclepias syriaca</i>	Common Milkweed	Banks or floodplains of lakes, ponds, waterways, prairies, forest margins, roadsides and waste places	June–August	Moist to Dry	Sandy, clayey, or rocky calcareous soils	Entire State
<i>Asclepias tuberosa</i>	Butterfly Milkweed	Prairies, open woodlands, roadsides, and disturbed areas	April–September	Mesic to Dry	Sandy, loamy, or rocky limestone soil	Entire State
<i>Asclepias verticillata</i>	Whorled Milkweed	Prairies, open woodlands, roadsides, and disturbed areas	April–August	Mesic to Dry	Sandy soils	Entire State
<i>Cynanchum laeve</i>	Honeyvine Milkweed	Alluvial woods, cities, waste areas, disturbed areas	July–October	Moist to Mesic	Sandy soils	Southwestern Iowa

fed on excised leaves or whole plants (Oberhauser & Solensky 2004).

Unlike Ladner and Altizer (2005), we found no difference in larval mass or instar size between larvae fed *A. incarnata* and *A. syriaca* (Figures 1 and 3). However, it is possible that differences in larval growth among milkweed plants may be more pronounced during the final instars. We did see evidence, as they did, that *A. speciosa* may produce lighter larvae, but only when larvae fed on excised leaves (Figure 1). This could suggest that young larvae have difficulty processing milkweed leaves with higher cardenolide content, as *A. speciosa* tends to have higher foliar cardenolides compared to some of the other milkweed species (Table 1; Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, Rasmann & Agrawal 2011) or that these leaves are structurally difficult to eat. We also saw evidence that *A. hirtella* leaves produced lighter larvae than other species (Figure 1), but this could be the result of wilting of the excised leaves during larval feeding or larval difficulty processing leaf material with a high cardenolide content (Table 1; Agrawal et al. 2009, Rasmann & Agrawal 2011). Larvae that fed on *A. hirtella* plants were not significantly lighter than larvae that fed on other species (Figure 3).

Unlike Yeargan and Allard (2005), larvae reared on *C. laeve* plants were significantly smaller and did not grow as quickly as larvae fed other species; larvae fed *C. laeve* did not reach the third instar during the whole plant assay in our study. Our results suggest that larvae can survive on *C. laeve*, but those larvae may not mature as quickly as larvae feeding on other milkweeds. Larvae that fed on *A. verticillata*, a milkweed species that tends to have low cardenolide levels (Figures 1 and 3, Table 1), produced the heaviest larvae. Although we did not measure cardenolide content in our milkweed plants, *A. speciosa* and *A. hirtella* have higher average foliar cardenolides when compared to other milkweed species (Table 1, Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, Rasmann & Agrawal 2011). Cardenolide content is only one factor that could contribute to the variation in larval mass that we observed. Other factors such as differing latex content and flow, differing amounts of larval movement on various milkweed species, and differing plant architecture among milkweed species also likely contributed to the observed differences in larval mass (Zalucki et al. 2001a,b).

Like Cookman et al. (1984), we observed differences in lipid concentration among larvae reared on different host plants. However, in our study larvae that fed on excised leaves did not show a difference in lipid concentration. Our results suggest that *A. incarnata*

may be a more lipid-rich food source for monarch larvae, and that other milkweed species may not be as good a food source for lipid content. Alternatively, monarchs may be able to process toxins from *A. incarnata* more effectively, leading to higher lipid storage (Roeske et al. 1976).

In summary, all nine milkweed species can be used as host plants by the monarch butterfly. Larvae that fed on excised leaves at a controlled temperature weighed more and matured faster than those raised on whole plants in a greenhouse with more variable temperature. Larvae that fed on *A. incarnata* and *A. verticillata* weighed the most, while those that fed on *C. laeve* weighed the least. This is an important finding because milkweeds are needed to boost monarch numbers during the breeding season in the Midwestern U.S (Pleasants & Oberhauser 2013, Flockhart et al. 2015).

Although larvae that fed on *A. incarnata* and *A. verticillata* weighed the most, monarch habitat should include milkweed species with habitat needs that best match the potential restoration site (Table 2). *A. syriaca*, *A. incarnata*, and *A. verticillata* are found across the entirety of Iowa, but *A. syriaca* and *A. verticillata* are found in drier locations than *A. incarnata* (Woodson 1954, Eilers & Roosa 1994). *A. incarnata* is found in wet areas, especially near wetlands and along waterways (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, USDA-NRCS 2017). *A. exaltata* is found in northeastern Iowa in upland woods and along forest edges (Eilers & Roosa 1994). *A. tuberosa* is commonly found in prairie remnants across Iowa, while *A. hirtella* is restricted to mesic remnants in southern Iowa (Eilers & Roosa 1994, USDA-NRCS 2017). *A. speciosa* is found in the western half of Iowa in woodland openings, prairies, and roadside ditches (Woodson 1954, Kaul et al. 1991, Eilers & Roosa 1994, USDA-NRCS 2017). *A. sullivantii* is rare across Iowa, but can be found in mesic prairies and roadsides in mesic soil (Woodson 1954, Eilers & Roosa 1994, USDA-NRCS 2017). *C. laeve* occurs frequently in southwestern Iowa in moist, sandy soils (Woodson 1954, Eilers & Roosa 1994, USDA-NRCS 2017).

In order to provide a complete assessment of the value of different milkweed species, we need to examine adult female egg load and potential fecundity for individuals that have fed on different milkweed species from first instar through adult eclosion. These feeding trails should use mature milkweed plants. We also need to understand the oviposition response and preference of female monarchs for different milkweed species to gauge their potential value in habitat restoration.

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