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Monarch butterflies do not place all of their eggs in one basket: oviposition on nine Midwestern milkweed species

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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 in overwintering numbers. Habitat restoration that includes planting milkweeds is essential to boost monarch numbers within the breeding range. Milkweeds are the only host plants for larval monarch butterflies, but female oviposition preference for different milkweed species, especially those with overlapping ranges, is not well documented. We examined the relative inclination to lay eggs on nine milkweed species native to Iowa (no choice), and oviposition preference (choice) among the four most commonly occurring Iowa species (*Asclepias incarnata*, *Asclepias syriaca*, *Asclepias tuberosa*, and *Asclepias verticillata*). In both experiments, eggs were counted daily for four days. The milkweeds tested were *Asclepias exaltata* (poke milkweed), *Asclepias hirtella* (tall green milkweed), *A. incarnata* (swamp milkweed), *Asclepias speciosa* (showy milkweed), *Asclepias sullivantii* (prairie milkweed), *A. syriaca* (common milkweed), *A. tuberosa* (butterfly milkweed), *A. verticillata* (whorled milkweed), and *Cynanchum laeve* (honeyvine milkweed). When females were given only a single species on which to lay eggs, there were significant differences among milkweed species in the average number of eggs laid; *A. incarnata* had the highest average egg count. When females were given a choice among *A. incarnata*, *A. syriaca*, *A. tuberosa*, and *A. verticillata*, there were also differences among milkweed species in the number of eggs laid; again, *A. incarnata* had the highest average number of eggs laid. Additionally, females laid more total eggs when four plants of different milkweed species were available than when there were four plants of a single milkweed species. Our results show that monarch butterflies will lay eggs on all nine milkweeds, but that there are clear preferences for some milkweed species over others.

Key words: *Asclepias;* conservation; *Danaus plexippus;* egg laying; habitat restoration; Iowa; milkweed preference.

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Introduction

The monarch butterfly (*Danaus plexippus* L.) population east of the Rocky Mountains has experienced a significant decline in overwintering numbers over the past two decades (Brower et al. 2012, Stenoien et al. 2016). This decline has been attributed to multiple factors including the loss of milkweed, the only host plants of monarch larvae (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pleasants 2017, Pleasants et al. 2017, Zaya et al. 2017). Recent models have implicated the loss of habitat, including milkweeds, within the breeding range as the largest threat to the monarch population (Zalucki and Lammers 2010, Flockhart et al. 2015, Zalucki et al. 2016). Because the majority of monarchs that overwinter in Mexico originate from the Midwest (Seiber et al. 1986, Wassenaar and Hobson 1998, Flockhart et al. 2017), restoration of monarch habitat in this region, especially on marginal agricultural lands (Thogmartin et al. 2017), is essential to increase population numbers (Oberhauser et al. 2016). Federal, state, and nonprofit groups have undertaken efforts to reestablish monarch habitat. These projects have focused on adding milkweed plants to the landscape.

In the past, milkweeds that grew in crop fields in the Midwest (*Asclepias syriaca*) were among the most heavily used monarch host plants in the North American breeding range (Malcolm et al. 1993, Oberhauser et al. 2001, Pleasants and Oberhauser 2013). Increased use of glyphosate herbicide in corn and soybean fields in conjunction with glyphosate-tolerant crops has all but eliminated *A. syriaca* from crop fields (Pleasants and Oberhauser 2013). Although historic Midwestern grassland and wetland habitats contained multiple milkweed species (Hayden 1919, Pleasants 2015), virtually all restoration recommendations to date are based on *A. syriaca* (Pleasants and Oberhauser 2013, Landis 2014, Pleasants 2017). Monarchs could potentially use multiple milkweed species for oviposition, but more information is needed about monarch oviposition preference and behavior on these milkweeds to ensure that these plants could contribute to population growth.

We examined monarch oviposition on nine milkweed species native to Iowa because it is a high priority area for Midwestern conservation efforts (The Center for Biological Diversity 2014) and because most milkweeds native to the Midwest, especially those with narrow ranges, have not been included in prior oviposition studies. The species we examined were as follows: *Asclepias exaltata* (poke milkweed), *Asclepias hirtella* (tall green milkweed), *Asclepias incarnata* (swamp milkweed), *Asclepias speciosa* (showy milkweed), *Asclepias sullivantii* (prairie milkweed), *A. syriaca* (common milkweed), *Asclepias tuberosa* (butterfly milkweed), *Asclepias verticillata* (whorled milkweed), and *Cynanchum laeve* (honeyvine milkweed). These species look very different (Fig. 1), have overlapping ranges (Woodson 1954), and have different habitat requirements (Woodson 1954, Kaul et al. 1991, Eilers and Roosa 1994). They also have varying concentrations of cardenolides (Woodson 1954, Roeske et al. 1976, Malcolm 1991, Agrawal et al. 2009, Rasmann and Agrawal 2011) and quercetin glycosides (Haribal and Renwick 1996, Agrawal et al. 2009), both of which could influence oviposition through nectar feeding (Manson et al. 2012, Jones and Agrawal 2016) and contact chemoreception via sensilla on their middle and front legs and antennae or ovipositor dabbing (Zalucki et al. 1990, Arikawa 2001), respectively.

Few prior studies have focused explicitly on monarch oviposition preference across multiple native milkweed species. Those studies that did contribute to this knowledge examined milkweed chemical composition, both cardenolides (Zalucki et al. 1990) and quercetin glycosides (Haribal and Renwick 1996, 1998*a*, *b*), in relation to monarch oviposition and post-alightment behavior. These studies laid the foundation for later preference experiments considering plant chemicals as a factor in oviposition behavior. Other work focused on monarch use and preference across regional milkweed species in North America (Cohen and Brower 1982, Calvert 1999, Bartholomew and Yeargan 2002, Casagrande and Dacey 2007) and established that monarchs use some milkweed species over others within localized areas, specifically in Texas (Calvert 1999), Florida (Cohen and Brower 1982, Zalucki et al. 1990), and Kentucky (Bartholomew and Yeargan 2002). Prior work also identified that swallowworts (*Vincetoxicum nigrum* and *Vincetoxicum rossicum)*, milkweed relatives, did not act as a monarch population sink because few eggs were laid on these species (DiTommaso and Losey 2003) and that monarchs with different natal origins (California and Michigan) did not display oviposition preferences for the milkweed species from their natal region (Ladner and Altizer 2005). To better identify how monarchs choose to distribute their eggs when multiple milkweed species are present on the landscape, we used both choice and no-choice experiments with young plants of different milkweed species.

We examined the inclination to lay eggs on young plants of the nine milkweed species listed above in a no-choice experiment to determine the

Fig. 1. Pictures of each of the nine milkweed species used in the no-choice experiment representing differences in plant architecture. Milkweed species are pictured in the field during the summer of 2017 at eight weeks old. Names in gray were also used in the oviposition preference experiment.

egg laying baseline for each. This was done for several reasons: Previous work did not provide a baseline egg laying rate on different milkweed species, no work has been done on the egg laying rate (eggs laid by a female per day per plant) on any of these nine milkweed species, monarchs are adaptable and use multiple host milkweed species throughout their annual cycle (Agrawal 2017), and many previous studies did not compare the same milkweed species. Following the no-choice experiment, we conducted an oviposition preference test using four broadly distributed native milkweeds: *A. incarnata*, *A. syriaca*, *A. tuberosa*, and *A. verticillata* because these species are most common across Iowa (Woodson 1954, Eilers and Roosa 1994).

Materials and Methods

Female monarchs used in experiments

Females used in the experiments were obtained from a monarch butterfly colony that was started by collecting 312 monarch eggs and young larvae on *Asclepias syriaca* plants from 6 June to 28 July

2015 from Boone, Hamilton, and Story Counties in Iowa. Larvae were reared on *A. syriaca* through the summer growing season and *Asclepias curassavica*, a tropical milkweed, from greenhouse-grown plants through the fall and winter. Each generation of adults was tested for *Ophryocystis elektroscirrha* (O.E.) before entering the colony; individuals that tested positive for this parasite (under 5) were frozen. Adults were allowed to mate and eggs were collected for propagation of the colony on a weekly basis. Twelve generations of colony breeding preceded the beginning of this experiment. Individuals from generations 13–15 were used in these experiments. Inbreeding should not affect monarch preferences, as colony breeding of multiple generations of monarchs did not influence monarch oviposition in prior experiments; there is no evidence that inbreeding influences oviposition preference, even when colonies are formed through continuous matings of monarchs collected from different locations (Ladner and Altizer 2005).

Females were allowed to eclose and dry; all females were tested for O.E. before they were placed into a breeding cage; no females that tested positive for O.E. were used in these experiments. Females were allowed to mate and feed, but had never encountered a milkweed plant prior to the beginning of the experiment. Females used in both experiments were between 7 and 11 d old.

Milkweed plants used in experiments

Milkweeds of all nine species were grown from seed (Prairie Moon Nursery, Winona Minnesota, USA) without the use of chemical pesticides in a greenhouse (21.1–35°C, 16-h photophase, and 56% rh) at Iowa State University. Seeds were sown in 128-cell plug trays (Landmark Plastics, Akron, Ohio, USA) and then at approximately 6 weeks from germination were transplanted into 3.5 inch square deep perennial pots (Kord, Ontario, Canada). Plants ranged from 10 to 30 cm in height depending on milkweed species; milkweeds were 8–12 weeks old when used in each trial; all plants used within one trial were of the same age. Groups of 48 plants were transported to the laboratory (19.5–34.5°C, 16-h photophase, and 50% rh) 24 h before the beginning of each oviposition trial. Plant height and leaf number were recorded for each milkweed plant; leaf dimensions for the two largest leaves were recorded on each plant used in the no-choice experiment. Each set of four plants was used for one four-day trial to keep plants in good condition; only one female used each group of four plants in both oviposition experiments.

Relative inclination to lay eggs

Plants were placed into the four corners of $4' \times 4' \times 4.5'$ breathable plastic cages (Plant-House 4, Flowerhouse, Clio, Michigan, USA); plants were watered daily. Each trial consisted of 12 cages with four plants of the same milkweed species in each cage (e.g., four plants of *A. syriaca* in each cage). There was one full trial for each of the nine milkweed species resulting in 8–12 replicates per milkweed species (there was some loss of monarchs during the trials); light, temperature, and relative humidity fell within the same ranges for all trials. A dish lined with a circular sponge and filled with artificial nectar (Gatorade, Pryor, Oklahoma, USA) was placed in the center of each cage; sponges were included to aid butterfly feeding. One mated female monarch never exposed to a milkweed plant was introduced to each cage and allowed to lay eggs for 4 d. At the end of each day, the total number of eggs on each plant was counted. Contrary to Drury and Dwyer (2005), we did not observe females avoiding plants on which eggs were already present, although no eggs were present at the beginning of this experiment. All eggs were removed daily from the plants to prevent larval feeding/injury to the milkweeds as plant damage can result in chemical defense induction in some milkweed species (Agrawal 2017), which could influence monarch oviposition preference. Only females that survived all four days of each trial were included in the analysis.

Oviposition preference

Plants and female monarchs were reared and treated as described above except that in this case, each of the four plants in a cage was a different species. Only four of the most common Iowa milkweed species were tested in this experiment: *Asclepias incarnata, A. syriaca, Asclepias tuberosa, and Asclepias verticillata* (Woodson 1954, Eilers and Roosa 1994, USDA-NRCS 2017). Plants were placed into each cage in a randomized order to reduce issues of plant adjacency.

Trials lasted four days; all monarch eggs were removed from the milkweed plants daily. This experiment contained 14 replicates with 12 cages included in each replicate for a total of 168 females and 672 milkweed plants, 168 of each milkweed species. Only females that survived all four days of each trial and laid at least 50 eggs were included in the analysis.

Statistical analysis

Data were analyzed using R version 3.1.2 (R Core Team 2014). Data were combined across replicates within each experiment, as replicates were not significantly different from one another. Daily egg counts from each female were combined across each four-day trial, as there was no significant difference in the number of eggs a female laid on day one vs. days two, three, or four when all milkweed species were combined or analyzed individually. Differences in egg counts were determined using a Poisson regression (Kaitala 1996, Mery and Kawecki 2002) with individual butterfly as a random effect and milkweed species as a fixed effect. Pairwise differences were determined by comparing least square means for each milkweed species; P-values were adjusted using Tukey's range test for multiple comparisons. Leaf widths were averaged for each plant, and eggs were totaled for each plant over the course of the four-day trial. Egg totals were square-root-transformed for normality, and a Pearson correlation was used to determine the correlation between total number of eggs per plant and the average leaf width per plant. The square root of the number of eggs per centimeter of plant height was analyzed using a mixed effect ANOVA for the oviposition preference study (Ladner and Altizer 2005). Pairwise differences in eggs per cm of plant height were tested using a t-test with a Bonferroni correction. Proportions of egg counts from both studies were arcsine-square-root-transformed and analyzed using one-way ANOVA (Ladner and Altizer 2005). Pairwise differences in transformed proportions were assessed using a Tukey's test. The total number of eggs laid per female per plant was compared across experiments for milkweed species included in both experiments (*A. incarnata, A. syriaca, A. tuberosa, and A. verticillata*) using a Poisson regression as described above.

Results

Oviposition inclination

Female monarchs laid eggs on all nine milkweed species, but laid more eggs on plants of some milkweed species than others (Fig. 2). When milkweed species was included as a fixed effect and individual butterfly was included as a random effect, milkweed species had a significant effect on the number of eggs laid per female. Females laid 26.8 times more eggs on *Asclepias incarnata* than on *Asclepias tuberosa* ($z = 4.27$, $P < 0.01$; Fig. 2) and 22.6 times more eggs on *A. incarnata* than on *Asclepias verticillata* ($z = 4.4$, $P < 0.01$; Fig. 2). Females laid more eggs on *Asclepias sullivantii* than on *A. verticillata* ($z = 3.35$, $P < 0.05$; Fig. 2) and *A. tuberosa* ($z = 3.19$, $P < 0.05$; Fig. 2). There were no significant differences in the number of eggs laid based on female age in days ($z = -1.44$ to 1.53; $P = 0.998$–1.00). There was no significant relationship between the total number of eggs laid per plant and the average leaf width or length ($r = 0.056$, df = 517, $P > 0.2$). Plant height and leaf number were not significant predictors for the number of eggs laid per species.

Oviposition preference

When given four different milkweed species at the same time, female monarchs laid eggs on all four but the number of eggs laid on each milkweed species was significantly different when individual variation in fecundity was included as a random effect and milkweed species was a fixed effect in a Poisson regression (Fig. 3). Females laid 1.7 times more eggs on *A. incarnata* than on *Asclepias syriaca* ($z = 25.49$, $P < 0.01$), 14.9 times more eggs than on *A. tuberosa* ($z = 61.90$, $P < 0.01$), and 4.5 times more eggs than on *A. verticillata* ($z = 54.24$, $P < 0.01$; Fig. 3A). Females laid nine times more eggs on *A. syriaca* than on *A. tuberosa* ($z = 54.14$, $P < 0.01$) and 2.7 times more eggs than on *A. verticillata* ($z = 40.63$, $P < 0.01$; Fig. 3A). Females laid 0.31 times fewer eggs on *A. tuberosa* than on *A. verticillata* ($z = -27.52$, $P < 0.01$; Fig. 3A). When the data were examined as eggs per centimeter of plant height, *A. tuberosa* and *A. verticillata* were different from *A. incarnata* and *A. syriaca* ($F = 34.7$, df = 3, 9874, $P < 0.01$); *A. incarnata* had the highest number of eggs per cm of plant height (Fig. 3B; $P < 0.01$). Females laid 53.7%, 31.3%, 11.5%, and 3.5% of their eggs on

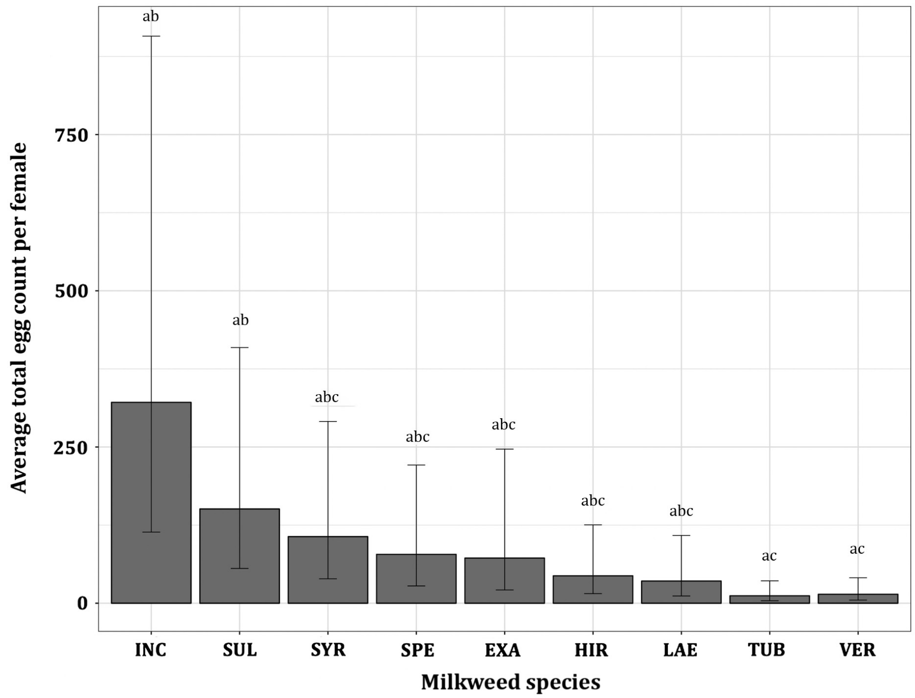


Fig. 2. Average total eggs counted per female over the course of four days when relative inclination to lay eggs was examined. Each bar represents one milkweed species: EXA = *Asclepias exaltata* (N = 8 females), HIR = *Asclepias hirtella* (N = 11 females), INC = *Asclepias incarnata* (N = 11 females), LAE = *Cynanchum laeve* (N = 10 females), SPE = *Asclepias speciosa* (N = 12 females), SUL = *Asclepias sullivantii* (N = 12 females), SYR = *Asclepias syriaca* (N = 12 females), TUB = *Asclepias tuberosa* (N = 11 females), and VER = *Asclepias verticillata* (N = 12 females); error bars represent 95% confidence intervals. Females laid more eggs on *A. incarnata* than on *A. tuberosa* ($P < 0.01$) and *A. verticillata* ($P < 0.01$); females laid more eggs on *A. sullivantii* than on *A. tuberosa* ($P < 0.05$) or *A. verticillata* ($P < 0.05$) in a pairwise comparison of least mean squares. *P*-values were adjusted using the Tukey method for multiple comparisons. Bars that do not share a letter are significantly different from each other.

A. incarnata, A. syriaca, A. tuberosa, and *A. verticillata*. When proportions of eggs laid on each milkweed species were compared, all egg proportions were significantly different from one another (Fig. 3C; $F = 68.92$, df = 3, 336, $P < 0.001$). Egg numbers did not increase with the number of leaves per plant on any milkweed species.

When the number of eggs laid per female on each plant in the inclination to lay trials and preference trials was compared, females laid 2.5 times more eggs when there were multiple species of milkweeds present compared to only one. Egg counts were significantly higher in the preference trials compared to the no-choice trials when the four milkweed species included in both experiments (*A. incarnata, A. syriaca, A. tuberosa, and A. verticillata*) were combined (Fig. 4; $z = 4.34$, $P < 0.001$).

Discussion

Our findings suggest that monarch butterflies will lay eggs on all milkweed species tested in no-choice experiments although they are more inclined to lay on some species than others (Fig. 2). In choice experiments, females still lay eggs on all four species available but prefer some milkweed species over others with preference generally mirroring the pattern exhibited in the inclination to lay experiment (Fig. 3). Interestingly, monarchs females laid more total eggs during the choice experiment when a diversity of milkweeds were present in each cage than would be expected based on the no-choice experiments (Fig. 4). In no-choice tests, we saw the highest egg counts on *Asclepias incarnata* followed by *Asclepias sullivantii*

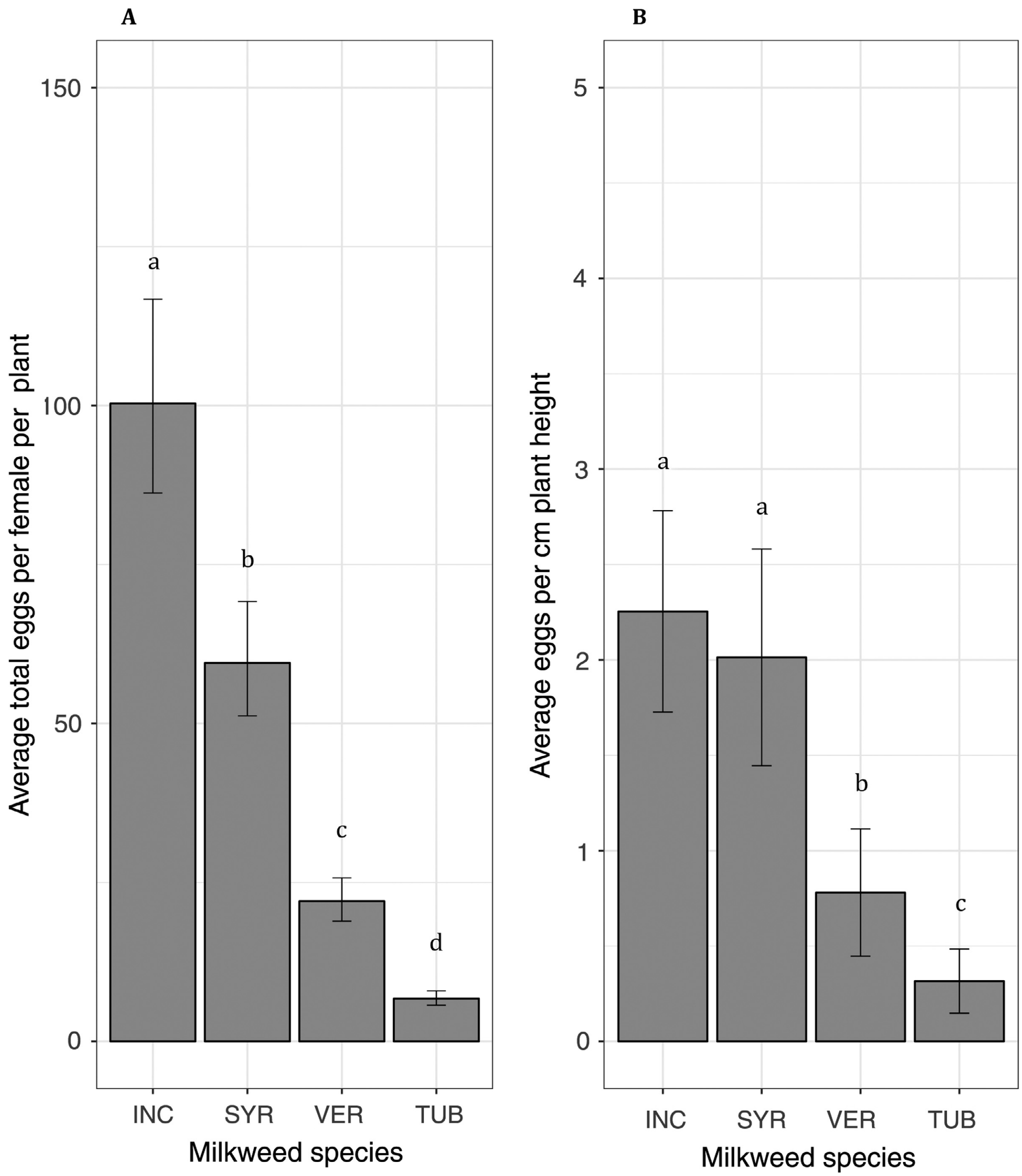
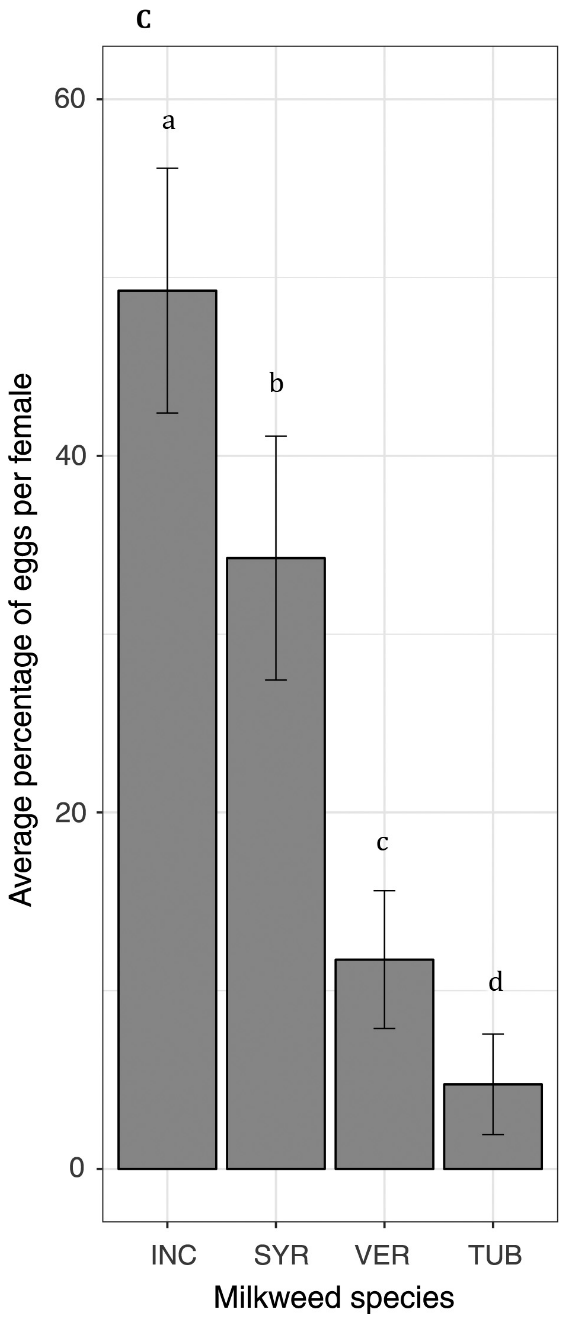


Fig. 3. Average total eggs laid per female on each plant (A), the untransformed average number of eggs per centimeter of plant height (B), and the average percentage of eggs laid on each milkweed species (C) from the oviposition choice study. Each graph represents eggs counted from 85 females and 340 milkweed plants. Each bar represents one milkweed species; error bars represent 95% confidence intervals. In A and C, all four milkweed species are significantly different from each other (pairwise *t*-test (a) Tukey HSD (c), $P < 0.01$). In B, *Asclepias syriaca* and *Asclepias incarnata* are significantly different from *Asclepias tuberosa* and *Asclepias verticillata* ($P < 0.01$).



(Fig. 3. *Continued.*)

and *Asclepias syriaca* (Fig. 2). In preference tests, over half of all eggs laid were on *A. incarnata* (Fig. 3). Females laid fewer eggs on *Asclepias tuberosa* and *Asclepias verticillata* in both preference and no-choice tests (Figs. 2, 3), even though larval survival was high on both of these species in prior experiments (Pocius et al. 2017*a, b*).

It is important to note that monarchs use multiple different milkweed hosts each year throughout their annual cycle (Agrawal 2017). Although these milkweed species appear on the landscape in different proportions, monarchs do not specialize on one milkweed species even when both have co-evolved within a smaller region (e.g., eastern vs. western North America). Monarchs from both the eastern and western populations exhibited the same oviposition preferences when given access to milkweed species from both eastern and western North America (Ladner and Altizer 2005). Our results support the adaptability of monarchs even when milkweed species were closer in proximity than usually seen in the field. Females used all four milkweed species in each preference trial and females laid more eggs

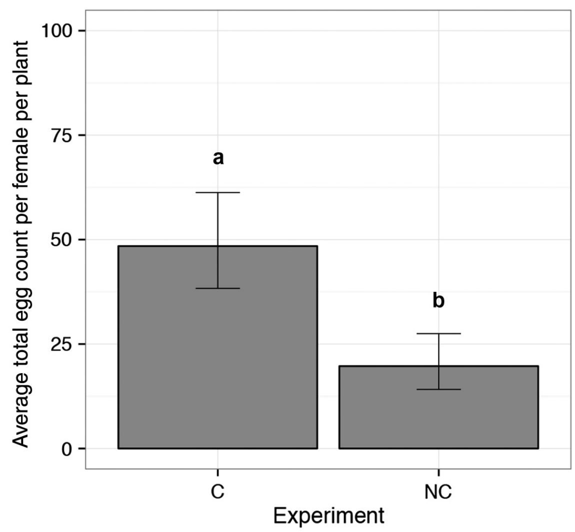


Fig. 4. Average total eggs laid per female on each plant when all eggs from *Asclepias incarnata*, *Asclepias syriaca*, *Asclepias verticillata*, and *Asclepias tuberosa* were combined. This graph represents eggs counted from 130 females and 520 milkweed plants. Each bar represents the average total egg count from one experiment (C = oviposition preference or choice trials, NC = inclination to lay eggs or no-choice trials); error bars represent 95% confidence intervals. The total number of eggs per female per plant was significantly different among the experiments; more eggs were present on average in the oviposition preference tests ($P < 0.01$). Each female had four milkweed plants on which to lay eggs (C = one plant each of four different species, NC = four plants of the same milkweed species).

overall when a mix of milkweeds were present in each cage than when a single species was present (Fig. 4). Because of monarchs' broad use of host species, other explanations for oviposition preference must be explored.

One possible explanation is the difference in secondary plant compounds across milkweed species. Across the monarch breeding range, monarchs encounter a variety of milkweed hosts with different plant architecture and chemical concentrations (Zalucki 1986, Malcolm et al. 1989, Agrawal 2016). Cardenolide and quercetin glycoside concentrations are plant characteristics thought to influence both monarch oviposition and larval performance in prior studies (Zalucki et al. 1990, Malcolm 1991, Haribal and Renwick 1998*a*, Ladner and Altizer 2005, Agrawal et al. 2015). Adult females have been shown to reject high cardenolide hosts even though monarch larvae sequester cardenolides for their own defense as they feed on milkweed plants (Oyeyele and Zalucki 1990, Zalucki et al. 1990, Haribal and Renwick 1998*a*). Females may reject these high cardenolide hosts in response to chemical cues. High cardenolide levels have been linked with low larval survival and slower development rates (Erickson 1973, Zalucki et al. 2001*a, b*, Zalucki et al. 2012). As such, there may be chemical cues that affect oviposition choice. Alternatively, high quercetin glycoside level located on the leaf surface (Agrawal 2017) stimulates oviposition; monarchs respond to these chemicals as part of host plant recognition and females have laid eggs in response to the presence of these chemicals without a plant (Haribal and Renwick 1996).

In our study, the least preferred milkweed species *A. tuberosa* (no choice; Fig. 2) and *A. verticillata* (choice; Fig. 3A) both have low cardenolide levels recorded in the literature (Roeske et al. 1976, Agrawal et al. 2009, 2015, Rasmann and Agrawal 2011), but *A. verticillata* has a higher level of quercetin glycosides than *A. tuberosa* (Agrawal et al. 2009). Females may be able to sense these chemical differences by dabbing their ovipositor on the underside of a leaf prior to oviposition (Zalucki et al. 1990, Arikawa 2001). Additionally, *A. tuberosa* has a layer of trichomes, which may inhibit oviposition or decrease a female's ability to sense leaf chemicals. *A. incarnata*, the most preferred milkweed in both experiments (Figs. 2, 3A, B), also has a relatively low level of cardenolides compared to some of the other species tested (*Asclepias speciosa* and *Asclepias hirtella*), but has a higher level of quercetin glycosides than *A. tuberosa* as reported in the literature (Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, 2015, Rasmann and Agrawal 2011) and lacks leaf hairs. *Asclepias syriaca* also has similar cardenolide levels to *A. incarnata*, but slightly lower levels of quercetin glycosides as reported in the literature (Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, 2015, Rasmann and Agrawal 2011), which could contribute to the differences we observed in egg totals between these two milkweed species. Interestingly, *A. incarnata* and *A. verticillata* have very similar levels of quercetin glycosides (Agrawal et al. 2009). Although these plant chemicals play a role in oviposition preference, additional plant traits undoubtedly contribute to egg laying preference.

Other plant characteristics that may play a role in female oviposition preference include leaf trichomes, leaf morphology (overall dimensions), and overall plant architecture (height, number of leaves, etc.) We counted fewer eggs on the narrow-leafed milkweeds (*A. tuberosa* and *A. verticillata*) in both the no-choice and oviposition preference tests although the total number of eggs laid on each plant is not correlated with leaf width. Observations of ovipositing females showed that *A. verticillata* plants bent under the weight of female monarchs and that the strength of the stems and size of the leaves may present a physical challenge to oviposition (V. Pocius, *personal observation*). In the wild, females may encounter more robust stalks of *A. verticillata*. Thus, presenting females with young plants in the laboratory may have artificially reduced the number of eggs laid on this species.

Females laid a moderate number of eggs on *Cynanchum laeve*, the only vine included in this study. This species was not significantly different from the highly preferred or highly unpreferred milkweed species. It is difficult to explain why females did not utilize this milkweed species more often in the oviposition inclination experiment because the cardenolide and quercetin glycoside concentrations for this plant are currently unknown. The structure of each individual vine also may have been difficult for females as the wider leaves are often tangled in the stem in young plants (V. Pocius, *personal observation*).

Although egg counts were highest on *A. incarnata,* restoration efforts should focus on planting a variety of milkweeds, not just the milkweed species with the highest egg counts reported here because plant quality is important for both monarch larvae and adults. Specialization on one milkweed species is not the optimal strategy for female monarchs; weather conditions, like temperature and precipitation, can have massive impacts on the quality of milkweed plants. For example, *A. incarnata* thrives in wet years, but plants deteriorate in drought conditions (V. Pocius, *personal observation*). Conversely, *A. hirtella* thrives in drier conditions. Females need to have multiple milkweed species to place their eggs on the most viable milkweed hosts during each breeding season.

These milkweed species will perform best in sites that match their habitat requirements. All nine milkweeds tested in our experiments favor different habitats. For example, *A. syriaca, A. incarnata, A. tuberosa,* 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 and Roosa 1994, USDA-NRCS [United States Department of Agriculture, Natural Resources Conservation Service] 2017). See Pocius et al. (2017*b*) for a summary of milkweed distributions. Given the differences in flowering time and plant maturation phenology, a suite of different milkweed species may provide a broader set of resources across the flight season compared to only one milkweed species. Additionally, females laid more eggs when a diversity of milkweed species were present in their environment. Because our conservation goals include increasing the number of eggs laid per female to boost monarch numbers, adding a diverse array of milkweeds to restorations is likely to increase the number of eggs laid in these locations.

Future research should investigate adult female egg load (number of mature eggs contained in the ovaries daily) and potential fecundity for individuals that have fed on different milkweed species in order to assess the value of different milkweeds on the landscape. Future trials should use mature milkweed plants so that monarchs encounter buds, blooms, and differing leaf quality (young and mature leaves) of various milkweed species. We used young milkweed plants in this study as females will more readily lay eggs on young plants compared to mature plants (Zalucki and Kitching 1982), but young plants do not resemble mature plants in the field, as they often have fewer stems, and no buds or flowers. In the field, differing chemical concentrations among clones, differing modularity, and differing phenology among milkweed species also contribute to oviposition preference. We acknowledge that females used in this experiment encountered a simplified array of milkweeds compared to nature. Understanding how females respond to mature plants in the context of oviposition will allow scientists and managers to even more specifically gauge their potential value in habitat restoration.

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