

# Milkweed Matters: Monarch Butterfly (Lepidoptera: Nymphalidae) Survival and Development on Nine Midwestern Milkweed Species

V. M. Pocius,<sup>1,4</sup> D. M. Debinski,<sup>1,5</sup> J. M. Pleasants,<sup>1</sup> K. G. Bidne,<sup>2</sup> R. L. Hellmich,<sup>2</sup> and L. P. Brower<sup>3</sup>

<sup>1</sup>Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, <sup>2</sup>United States Department of Agriculture, Agricultural Research Station, Corn Insects and Crop Genetics Research Unit, and Department of Entomology, Iowa State University, Ames, IA 50011, <sup>3</sup>Department of Biology, Sweet Briar College, Sweet Briar, VA 24595, <sup>4</sup>Corresponding author, e-mail: [pociusv@iastate.edu](mailto:pociusv@iastate.edu), and <sup>5</sup>Department of Ecology, Montana State University, Bozeman MT 59717

Subject Editor: Yasmin Cardoza

Received 13 May 2017; Editorial decision 24 July 2017

## Abstract

The population of monarch butterflies east of the Rocky Mountains has experienced a significant decline over the past 20 yr. In order to increase monarch numbers in the breeding range, habitat restoration that includes planting milkweed plants is essential. Milkweeds in the genus *Asclepias* and *Cynanchum* are the only host plants for larval monarch butterflies in North America, but larval performance and survival across nine milkweeds native to the Midwest is not well documented. We examined development and survival of monarchs from first-instar larval stages to adulthood on nine milkweed species native to Iowa. The milkweeds included *Asclepias exaltata* (poke milkweed) (Gentianales: Apocynaceae), *Asclepias hirtella* (tall green milkweed) (Gentianales: Apocynaceae), *Asclepias incarnata* (swamp milkweed) (Gentianales: Apocynaceae), *Asclepias speciosa* (showy milkweed) (Gentianales: Apocynaceae), *Asclepias sullivantii* (prairie milkweed) (Gentianales: Apocynaceae), *Asclepias syriaca* (common milkweed) (Gentianales: Apocynaceae), *Asclepias tuberosa* (butterfly milkweed) (Gentianales: Apocynaceae), *Asclepias verticillata* (whorled milkweed) (Gentianales: Apocynaceae), and *Cynanchum laeve* (honey vine milkweed) (Gentianales: Apocynaceae). In greenhouse experiments, fewer larvae that fed on *Asclepias hirtella* and *Asclepias sullivantii* reached adulthood compared with larvae that fed on the other milkweed species. Monarch pupal width and adult dry mass differed among milkweeds, but larval duration (days), pupal duration (days), pupal mass, pupal length, and adult wet mass were not significantly different. Both the absolute and relative adult lipids were different among milkweed treatments; these differences are not fully explained by differences in adult dry mass. Monarch butterflies can survive on all nine milkweed species, but the expected survival probability varied from 30 to 75% among the nine milkweed species.

**Key words:** monarch butterfly, *Asclepias*, milkweed, conservation, larval survival

The populations of monarch butterflies east and west of the Rocky Mountains have experienced a significant decline in overwintering numbers over the past 20 yr (Brower et al. 2012, Espeset et al. 2016, Stenoien et al. 2016). Although this decline may not be representative of the monarch population size during other times of the year (Davis 2012, Davis and Dyer 2015), this decline has been attributed to multiple factors including the loss of milkweed (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pleasants 2017, Zaya et al. 2017) and nectar sources (Inamine et al. 2016) within the breeding range. Recent modeling work has 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). A large proportion of the

monarchs that overwintered in Mexico originated from the Midwest (Wassenaar and Hobson 1998, Flockhart et al. 2017) and fed on common milkweed, *Asclepias syriaca* (*Asclepiadaceae*), as larvae (Seiber et al. 1986, Malcolm et al. 1989). Restoration of monarch habitat in this region is essential to increase population numbers (Oberhauser et al. 2016) and federal, state, and non-profit groups have undertaken efforts to establish monarch habitat. These projects have focused on adding milkweed plants, the only host plants of monarch larvae, to the landscape.

Traditionally, row crop agriculture in the Midwest was a significant source of common milkweed (*A. syriaca*), among the most heavily used host plants by monarchs in the North American breeding range (Oberhauser 2001, Pleasants and Oberhauser 2013). Virtually all

habitat restoration recommendations are based on *A. syriaca*, whereas the historic Midwestern grassland and wetland habitats contained several milkweed species (Hayden 1919, Woodson 1954, Pleasants 2015). These other milkweed species could potentially provide a broader base of resources adapted to a wider range of sites and weather for a more sustainable approach to habitat restorations. More information is needed about monarch larval survival and performance on these milkweeds to understand how they contribute to population growth.

Several prior studies have addressed various aspects of monarch survival from larvae to adults, but few include comparative work on multiple milkweed species. Comparative studies on North American monarchs include Schroeder's (1976) energy budget for larvae that fed on *A. syriaca*, larval performance and nutrition on four milkweed species (Erickson 1973), and growth differences between monarchs collected from eastern and western North America on widely distributed milkweed species (Ladner and Altizer 2005). Other studies have examined growth differences of larvae that fed on *A. syriaca* and *Cynanchum laeve* (Yeargan and Allard 2005) and on milkweeds native to Southern California (Zalucki et al. 2012) throughout development. Additional work has focused on the survival of early-instar larvae on a range of North American species native to Florida (Zalucki and Brower 1992), the Midwest (Pocius et al. 2017), and across the Eastern United States (Zalucki and Malcolm 1999). Furthermore, Robertson et al. (2015) investigated larval preferences among four milkweeds native to the California desert, while Agrawal et al. (2015) compared larval performance on a wide variety of milkweed species 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 were: *A. syriaca* (common milkweed), *Asclepias incarnata* (swamp milkweed), *Asclepias tuberosa* (butterfly milkweed), *Asclepias verticillata* (whorled milkweed), *Asclepias speciosa* (showy milkweed), *Asclepias exaltata* (poke milkweed), *Asclepias sullivantii* (prairie milkweed), *Asclepias hirtella* (tall green milkweed), and *Cynanchum laeve* (honeysuckle milkweed). These species have overlapping ranges (Woodson 1954), varying concentrations of cardenolides (Woodson 1954, Roeske et al. 1976, Malcolm 1991, Agrawal et al. 2009, Rasmann and Agrawal 2011), quercetin glycosides (Haribal and Renwick 1996, Agrawal et al. 2009), and adaptation to different habitats (Woodson 1954, Kaul et al. 1991, Eilers and Roosa 1994). We examined larval performance and survival on young plants of the nine species listed earlier to determine any differences in the resulting adults including mass, forewing length, and hindwing length, or development time (days) in the larval and pupal stages relative to the milkweed species on which the larvae fed. Our prior work suggested that there were differences in both mass and lipid content in young larvae, second and third instars, that fed on both leaves and young plants of different milkweed species (Pocius et al. 2017), although there were no differences in survival. We suspected that these differences could change as the monarch larvae develop to adulthood because there were no significant differences in pupal weight and development time among larvae that fed on *A. syriaca* and *C. laeve* (Yeargan and Allard 2005), although larval growth rates differed based on the host plant species (Ladner and Altizer 2005, Yeargan and Allard 2005). Understanding how milkweed species influence monarch development and survival will be critical in choosing milkweed species for monarch habitat restoration, and given the large number of acres that are being planted, this knowledge could also have significant economic implications.

## Materials and Methods

### Monarch Larvae Used in Experiments

A monarch butterfly colony was started by collecting 253 monarch eggs and young larvae from 21 May to 9 June 2014 from Boone and Story Counties in Iowa. Larvae were reared on *A. syriaca* through the summer growing season and *A. curassavica*, a tropical milkweed, from greenhouse-grown plants through the fall and winter. Upon eclosion, adults were tested for *Ophryocystis elektroscirrha* (OE). Adults that tested negative for OE 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; inbreeding should not affect monarch preferences as colony breeding for multiple generations did not influence monarch growth or performance on different milkweeds (Ladner and Altizer 2005).

### Milkweed Feeding Assay

Milkweeds of all nine species were grown from seed without the use of chemical pesticides in a greenhouse (21.1–35°C, 16 h photophase, and 56% RH) at Iowa State University. Growing conditions represent a middle ground among the nine species tested. Seeds were sown into 128-cell plug trays (Landmark Plastics, Akron, OH) and then at approximately 6 wk following germination were transplanted into 8.9 cm square deep perennial pots (Kord, Ontario, Canada). Plants ranged from 10 to 30 cm in height depending on milkweed species. Milkweeds were 8 wk old when used in each trial; all plants were healthy with undamaged leaves at the start of 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. The experiment was arranged in a randomized complete block design with the block including one plant of each of the nine milkweed species growing in each pop up cage. Each trial (six blocks) was replicated six times for a total of 36 blocks.

All blocks were kept on the same bench in the greenhouse (21.1–35°C, 16 h photophase, and 56% RH) positioned in a randomized complete block design (six trials of six blocks). Greenhouse temperature was recorded hourly via Thermochron sensors (Embedded Data Systems, iButton, New South Wales, Australia). Larvae were monitored for survivorship on Days 5, 10, and 14, when the larvae ranged from second to fifth instar. Beginning at Day 10, we monitored each cage for pupae in order to record the most accurate pupation date; we did not monitor young larvae daily in order to reduce stress on the larvae and young milkweed plants. Milkweed plants were watered daily, and additional milkweed plants were added on Days 6 and 10 to provide adequate food for each larva. No larvae ran out of food over the course of this experiment. Larvae were monitored daily for pupation starting at Day 12.

Following pupation, chrysalids were allowed to sclerotize in the greenhouse for 24 h after which they were removed from each cage and transported to the laboratory. Hardened pupae were weighed to the nearest hundredth of a milligram on an AND GR-202 balance (A&D Company, Limited, Toshima-ku, Tokyo, Japan); pupal length and width were measured to the nearest hundredth of a millimeter with digital calipers (Neiko Tools, USA). Individual pupae were attached to wooden applicators with small beads of hot glue (AdTech Detailer Glue Gun), and hung inside individual plastic cups (227 ml, WNAT8T) for eclosion.

Upon eclosion, adult emergence date and sex were recorded. Live adults were weighed to the nearest hundredth of a milligram after allowing their wings to harden for 24 h. Adult forewing length and hindwing length were measured to the nearest hundredth of a millimeter using digital calipers (Neiko Tools); adults were then frozen for subsequent lipid extraction.

### Adult Lipid Assay

Lipid content was quantified for half of the resulting adults at Sweet Briar College in July 2016. Lipids were extracted following the procedure outlined by Brower (2006), which includes drying the butterflies, weighing them, extracting the lipid in petroleum ether, evaporating the petroleum ether, and then weighing the extracted lipid (Alonso-Mejía et al. 1997, Brower 2006, Brower et al. 2015). Because there were no significant differences in lipid content between the sexes, lipids from males and females were pooled for analysis (Alonso-Mejía et al. 1997, Brower 2006, Brower et al. 2015). Data are presented both as average milligrams of lipid and lipid as a percentage of butterfly mass for butterflies that fed on each milkweed species.

### Statistical Analysis

Data were analyzed using R version 3.1.2 (R Core Team 2014). Within each experiment, data were combined across trials (36 blocks total), 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 that fed on each milkweed species. Pairwise log-rank tests were used to compare species (Jokela et al. 2016) as this analysis allowed us to include individuals that spent different amounts of time as larvae and pupae; a Bonferroni correction was used to adjust the significance level for pairwise comparisons (adjusted  $\alpha = 0.0014$ , Thielges 2005). A one-way ANOVA was used to assess differences in pupal and adult responses (mass, pupal length, pupal width, forewing length, and hindwing length) among milkweed species. A Tukey HSD test was used to assess pairwise differences in larval development time among milkweed species. A one-way ANOVA was used to assess differences in total percent of lipids between groups relative to the milkweed species they were fed. A Tukey HSD test was used to assess pairwise differences in lipid percentages. Sexes were pooled for all analyses, as there were no significant differences when males and females were analyzed separately.

## Results

### Milkweed Feeding Assay

Survivorship from first instar to adult varied from 30 to 70% across milkweed species, averaging 58% across all milkweeds species. Survivorship differed among milkweed species ( $\chi^2 = 32.8$ ,  $df = 8$ ,  $P < 0.001$ , Figs. 1 and 2D). Fewer monarchs that fed on *A. hirtella* survived than those that fed on *A. tuberosa* ( $P < 0.001$ ), or *A. exaltata* ( $P < 0.001$ ). Fewer monarchs that fed on *A. sullivantii* survived than those that fed on *A. exaltata* ( $P < 0.001$ ). No other pairwise differences in survival were significant. When survival was analyzed in 5-d increments, there were no differences in the proportion of larvae that survived on each milkweed species (Fig. 2A and B), although there was lower survival on *C. laeve* during the first 5 d (Fig. 2A), on *A. sullivantii* for the first 10 d (Fig. 2B), and both *A. hirtella* and *A. sullivantii* during the first 14 d (Fig. 2C). Between pupation and eclosion, there was high mortality in both *A. hirtella* and *A. sullivantii* (Fig. 2D). There were no differences in larval or pupal duration,

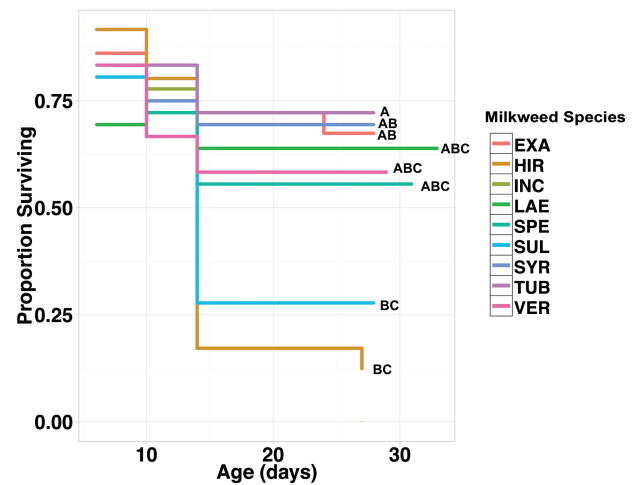


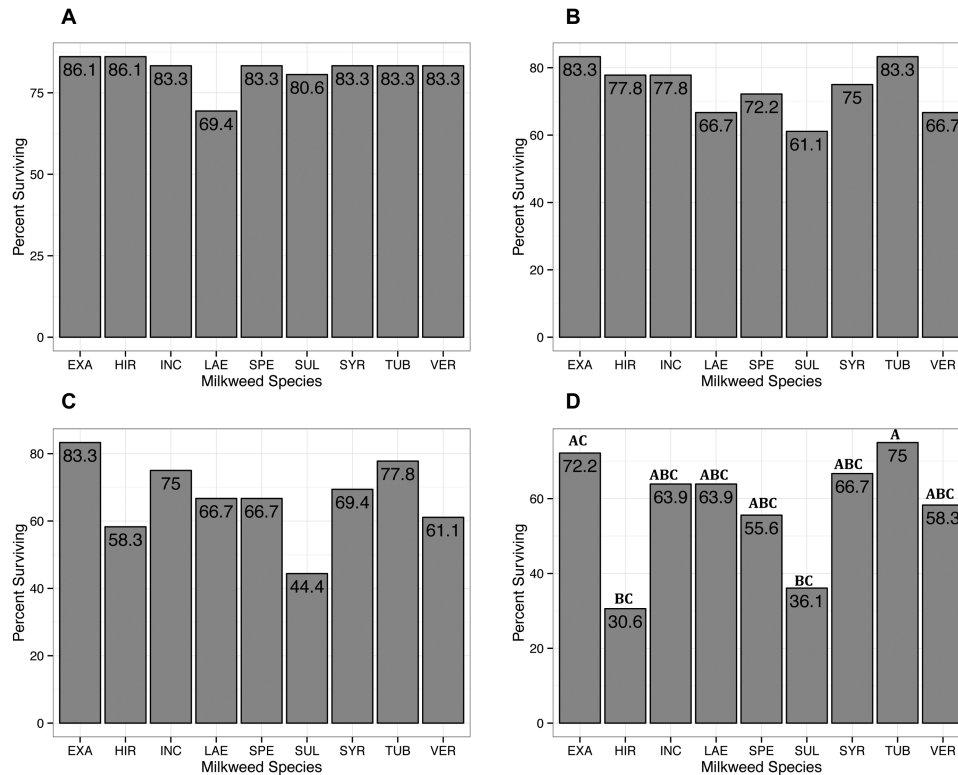
Fig. 1. Visualization of Kaplan–Meier survival probability over time (days) of monarch butterflies from larvae to adults that fed on nine different milkweed species (EXA = *A. exaltata*, HIR = *A. hirtella*, INC = *A. incarnata*, LAE = *C. laeve*, SPE = *A. speciosa*, SUL = *A. sullivantii*, SYR = *A. syriaca*, TUB = *A. tuberosa*). At the beginning of the experiment,  $n = 36$  larvae for each milkweed species. Each line represents one milkweed species. Fewer monarchs that fed on *A. hirtella* survived than those that fed on *A. tuberosa* or *A. exaltata*; fewer monarchs that consumed *A. sullivantii* survived than those that consumed *A. exaltata*. Lines that do not share a letter are significantly different from each other at  $P < 0.001$ .

defined by number of days as a larva (all instars combined), or as a pupa, among feeding treatments. Monarchs spent 14–15 d as larvae and 9–11 d as pupae across treatments. There were no differences in adult wet mass or hindwing lengths, but forewing length ( $F = 4.12$ ,  $df = 8$ ,  $P < 0.001$ , Table 1) and adult dry mass were significantly different among the resulting adults ( $F = 4.17$ ,  $df = 8$ ,  $P < 0.001$ , Table 1). When adults were dried before lipid analysis, adults that fed on *A. hirtella* weighed less than adults that fed on *A. incarnata* ( $P < 0.001$ ). Adults that fed on *A. exaltata* ( $P < 0.01$ ), *A. incarnata* ( $P < 0.01$ ), *A. speciosa* ( $P < 0.01$ ), *A. syriaca* ( $P < 0.001$ ), *A. tuberosa* ( $P < 0.001$ ), *A. verticillata* ( $P < 0.01$ ), and *C. laeve* ( $P < 0.05$ ) as larvae had longer forewings than those that fed on *A. hirtella* (Table 1). No other species showed difference in pairwise comparisons in forewing length.

Pupal mass was significantly different across milkweed treatments ( $F = 4.04$ ,  $df = 8$ ,  $P < 0.001$ , Table 2). Pupae that consumed *A. hirtella* as larvae weighed less than those that fed on *A. exaltata* ( $P < 0.001$ ), *A. incarnata* ( $P < 0.001$ ), *A. speciosa* ( $P < 0.01$ ), *A. syriaca* ( $P < 0.001$ ), *A. tuberosa* ( $P < 0.001$ ), *A. verticillata* ( $P < 0.01$ ), and *C. laeve* ( $P < 0.01$ ). Pupal length was not different among milkweed treatments, but pupal width ( $F = 3.08$ ,  $df = 8$ ,  $P < 0.01$ , Table 2) was different among milkweed treatments. Pupae that consumed *A. exaltata* ( $P < 0.05$ ), *C. laeve* ( $P < 0.05$ ), *A. speciosa* ( $P < 0.01$ ), *A. tuberosa* ( $P < 0.05$ ), and *A. verticillata* ( $P < 0.05$ ) as larvae were wider than those that fed on *A. hirtella*.

### Lipid assay

The total amount of lipid (milligrams) was significantly different among adults that fed on the nine different milkweed species ( $F = 3.36$ ,  $df = 8$ ,  $P < 0.01$ , Table 1). Adults that fed on *A. exaltata* ( $P < 0.01$ ), *A. incarnata* ( $P < 0.01$ ), and *A. syriaca* ( $P < 0.01$ ) had higher lipid content than those that fed on *A. hirtella* as larvae. Adults contained between 1.9 and 25.5 mg of lipid across species (for species averages, see Table 1). Lipid concentration (lipids



**Fig. 2.** Percent of monarchs surviving from 0 to 5 d as larvae (A), 0–14 d as larvae (B), 0–14 d during pupation (C, pupation), and from neonate to adulthood (D). There are no significant differences among milkweed species when survivorship is examined at 5, 10, or 14 d. Survival is different among milkweed treatments from neonate to adulthood (D). More monarchs survived on *A. exaltata* and *A. tuberosa* than on *A. hirtella* ( $P < 0.05$ ); more monarchs survived on *A. tuberosa* than on *A. sullivantii* ( $P < 0.05$ ).

**Table 1.** Mean adult measurements ( $\pm 95\%$  confidence intervals) from six trials ( $n = 168$  butterflies total)

Milkweed species	Milkweed common name	No. of adults measured ( $n$ )	Mean adult wet mass (mg)	Mean adult dry mass*	Mean forewing length (mm)**	Mean hindwing length (mm)	Mean lipid content (mg)***
<i>A. exaltata</i> (EXA)	Poke milkweed	22; 13 for lipids	718.8 $\pm$ 181.3	177.3 $\pm$ 21.1 <sup>A</sup>	49.7 $\pm$ 0.75 <sup>A</sup>	33.9 $\pm$ 0.61	13.0 $\pm$ 1.69 <sup>A</sup>
<i>A. hirtella</i> (HIR)	Tall green milkweed	6; 3 for lipids	307.2 $\pm$ 33.4	87.0 $\pm$ 30.4 <sup>B</sup>	43.8 $\pm$ 1.4 <sup>B</sup>	30.4 $\pm$ 1.0	2.2 $\pm$ 0.30 <sup>B</sup>
<i>A. incarnata</i> (INC)	Swamp milkweed	25; 12 for lipids	543.8 $\pm$ 12.3	193.6 $\pm$ 9.2 <sup>A</sup>	50.6 $\pm$ 0.44 <sup>A</sup>	33.7 $\pm$ 0.45	15.9 $\pm$ 5.8 <sup>AB</sup>
<i>C. laeve</i> (LAE)	Honeyvine milkweed	18; 11 for lipids	502.7 $\pm$ 18.2	152.4 $\pm$ 19.8 <sup>AB</sup>	49.8 $\pm$ 0.52 <sup>A</sup>	33.6 $\pm$ 0.45	6.3 $\pm$ 0.75 <sup>AB</sup>
<i>A. speciosa</i> (SPE)	Showy milkweed	18; 10 for lipids	529.3 $\pm$ 16.9	174.4 $\pm$ 8.6 <sup>A</sup>	50.7 $\pm$ 0.69 <sup>A</sup>	37.5 $\pm$ 3.6	7.2 $\pm$ 1.6 <sup>AB</sup>
<i>A. sullivantii</i> (SUL)	Prairie milkweed	9; 4 for lipids	456.1 $\pm$ 59.1	167.4 $\pm$ 104.6 <sup>A</sup>	46.1 $\pm$ 2.9 <sup>AB</sup>	31.7 $\pm$ 1.8	8.3 $\pm$ 1.9 <sup>AB</sup>
<i>A. syriaca</i> (SYR)	Common milkweed	22; 13 for lipids	552.4 $\pm$ 10.5	174.3 $\pm$ 22.8 <sup>A</sup>	50.8 $\pm$ 0.41 <sup>A</sup>	33.9 $\pm$ 0.29	12.5 $\pm$ 1.4 <sup>A</sup>
<i>A. tuberosa</i> (TUB)	Butterfly milkweed	25; 12 for lipids	529.2 $\pm$ 14.7	161.9 $\pm$ 20.2 <sup>A</sup>	50.9 $\pm$ 0.60 <sup>A</sup>	34.8 $\pm$ 0.41	16.7 $\pm$ 8.2 <sup>A</sup>
<i>A. verticillata</i> (VER)	Whorled milkweed	23; 11 for lipids	513.6 $\pm$ 16.4	171.0 $\pm$ 14.1 <sup>A</sup>	49.9 $\pm$ 0.89 <sup>A</sup>	34.4 $\pm$ 0.78	6.9 $\pm$ 2.5 <sup>AB</sup>

Each measurement represents mean  $\pm$  standard error. Adult mass and hindwing length were not different across milkweed species. Adult dry mass was significantly different across milkweed species at a significance level of  $P < 0.001$ . \*\*Forewing length was significantly different across treatments at a significance level of  $P < 0.01$ . \*\*\*Milligrams of lipid were significantly different across treatments at a significance level of  $P < 0.05$ . Log-transformed lipids were used for analysis; untransformed lipid values are reported here. Cells within columns that do not share a letter are significantly different from each other

as a percentage of total adult mass) was also significantly different among milkweed treatments ( $F = 5.35$ ,  $df = 8$ ,  $P < 0.0001$ , Table 1). Adults that fed on *A. exaltata* as larvae had higher lipid concentrations than those that fed on *A. hirtella* ( $P < 0.05$ ), *A. speciosa* ( $P < 0.05$ ), or *A. verticillata* ( $P < 0.001$ ). Adults that fed on *A. syriaca* as larvae had higher lipid concentrations than those that fed on *A. hirtella* ( $P < 0.05$ ), *A. speciosa* ( $P < 0.05$ ), *A. tuberosa* ( $P < 0.001$ ), and *A. verticillata* ( $P < 0.05$ ). All other comparisons were not significantly different.

## Discussion

Monarchs can survive on and will consume all nine milkweed species tested, but survivorship throughout development is higher on some species compared with others (Figs. 1 and 2). Seven 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. Our findings suggest that *A. hirtella* and *A. sullivantii* are not the best choice for these plantings because monarchs had a lower probability of reaching adulthood when fed



**Table 2.** Mean pupal measurements ( $\pm 95\%$  confidence intervals) from six trials ( $n = 188$  pupae total)

Milkweed species	Number of pupae measured ( $n$ )	Pupal mass (mg)*	Pupal length (mm)	Pupal width (mm)**
EXA	28	1386.3 $\pm$ 41.6 <sup>A</sup>	23.7 $\pm$ 0.29	10.9 $\pm$ 0.17 <sup>A</sup>
HIR	6	903.2 $\pm$ 88.6 <sup>B</sup>	21.5 $\pm$ 1.0	9.5 $\pm$ 0.31 <sup>B</sup>
INC	27	1417.1 $\pm$ 48.5 <sup>A</sup>	24.2 $\pm$ 0.27	10.6 $\pm$ 0.13 <sup>AB</sup>
LAE	18	1330.2 $\pm$ 41.1 <sup>A</sup>	23.7 $\pm$ 0.36	10.7 $\pm$ 0.12 <sup>A</sup>
SPE	21	1395.7 $\pm$ 36.0 <sup>A</sup>	24.2 $\pm$ 0.30	11.0 $\pm$ 0.13 <sup>A</sup>
SUL	13	1167.1 $\pm$ 133.3 <sup>A</sup>	22.3 $\pm$ 1.2	10.3 $\pm$ 0.39 <sup>AB</sup>
SYR	25	1379.2 $\pm$ 37.5 <sup>A</sup>	24.5 $\pm$ 0.37	10.6 $\pm$ 0.13 <sup>AB</sup>
TUB	26	1365.1 $\pm$ 43.5 <sup>A</sup>	24.1 $\pm$ 0.42	10.9 $\pm$ 0.17 <sup>A</sup>
VER	24	1313.1 $\pm$ 40.4 <sup>A</sup>	23.5 $\pm$ 0.29	10.8 $\pm$ 0.16 <sup>A</sup>

Milkweed abbreviations are the same as in Table 1. \*Pupal mass was significantly different across milkweed treatments at a significance level of  $P < 0.001$ . \*\*Pupal width was different among milkweed treatments at a significance level of  $P < 0.01$ . Cells within columns that do not share a letter are significantly different from each other.

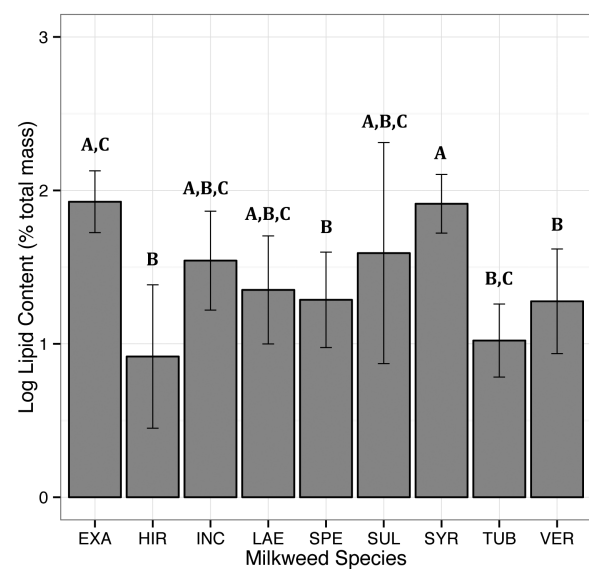
young plants of these milkweed species. Only 30% of larvae that fed on *A. hirtella* and 36% that fed on *A. sullivantii* reached adulthood compared with 75% that fed on *A. tuberosa* and 72% that fed on *A. exaltata*.

On average, larval survival was above 50% for the entirety of the study when larvae fed on young plants, higher than larval survival recorded in the field (Oberhauser and Solensky 2004, Nail et al. 2015). Handling the larvae during plant replacements or increased larval stress due to feeding on fresh milkweeds with intact plant defenses such as latex may have contributed to mortality rates. Unlike Ladner and Altizer (2005), we found no difference in larval survival among *A. incarnata*, *A. speciosa*, and *A. syriaca* (Fig. 1), but they recorded larval survival to fifth instar on milkweed leaf cuttings, not plants. *A. exaltata* and *A. tuberosa* had the highest survivorship in our study, but these species were not tested by Ladner and Altizer (2005). We did not see highest larval mortality during early instars as Ladner and Altizer (2005) did, but rather during pupation and eclosion (Fig. 2). We did see increased early instar mortality on *C. laeve* as in Pocius et al. (2017), but this difference was not significant (Fig. 2). Unlike our previous work, there were no developmental lags in larvae that fed on *C. laeve* plants. Larvae that fed on *C. laeve* progressed through both larval and pupal stages in the same amount of time as larvae that fed on other species.

### Differing Water Content in Live Butterflies Most Likely Masked the Differences in Dry Tissue Weight When Each Adult was Measured Initially

Our prior work suggested that *A. hirtella* produced lighter larvae after Day 5 than other milkweed plants (Pocius et al. 2017); this difference in mass was evident in the pupal stage (Table 2), but not when wet mass was compared in live adults. When adults were dried, those that fed on *A. hirtella* had a lower dry mass than adults that fed on other milkweed species (Table 1).

Given that larval development is driven by temperature, the similarities in development time across species were not surprising (Zalucki and Kitching 1982) although development can vary with food quality (Lavoie and Oberhauser 2004). Monarchs spent 14–15 d as larvae and 9–11 d as pupae across treatments. Unlike Yeargan and Allard (2005), we did not see any growth differences between larvae, pupae, and adults that fed on *C. laeve* versus *A. syriaca*. We did see differences in pupal mass, as did Yeargan and Allard (2005), but only *A. hirtella* pupae were significantly lighter than pupae that fed on other milkweeds as larvae (Fig. 3). Fewer early instars reared on *C. laeve* plants survived during the first 5 d of this study, but those



**Fig. 3.** Differences in relative adult lipid content (% total mass) among nine native milkweed species. This graph represents the lipid content from half of the resulting adults from eight trials ( $n = 89$  total). Error bars depict 95% confidence intervals. EXA = *A. exaltata* ( $n = 13$  butterflies), HIR = *A. hirtella* ( $n = 3$  butterflies), INC = *A. incarnata* ( $n = 12$  butterflies), LAE = *C. laeve* ( $n = 11$  butterflies), SPE = *A. speciosa* ( $n = 10$  butterflies), SUL = *A. sullivantii* ( $n = 4$  butterflies), SYR = *A. syriaca* ( $n = 13$  butterflies), TUB = *A. tuberosa* ( $n = 12$  butterflies), and VER = *A. verticillata* ( $n = 11$  butterflies). Bars that do not share a letter are significantly different from each other at  $P < 0.05$ .

that did survive were the same size as other pupae and adults; this indicates that any early differences in mass, as in Pocius et al. (2017), can be overcome during later developmental stages (Table 1). In prior work, young larvae that fed on *A. verticillata*, a milkweed species that tends to have low cardenolide levels produced the heaviest larvae (Pocius et al. 2017); however, this difference in mass did not carry into subsequent developmental stages (Table 1).

### Cardenolide Content is Only one Factor That Could Contribute to the Variation in Survival That we Observed

Although we did not measure cardenolide content in our milkweed plants, *A. hirtella* has higher average foliar cardenolides when compared with other milkweed species in prior studies (Woodson 1954, Roeske et al. 1976, Agrawal et al. 2009, Rasmann and Agrawal

2011). This difference in cardenolide content may influence monarch survival (Malcolm 1994, Malcolm and Zalucki 1996) and persists whether cardenolides are induced or remain at constitutive levels (Rasmann and Agrawal 2011). Plants grown inside the greenhouse in smaller pots may not respond to larval feeding by inducing higher cardenolide concentrations (Baldwin 1987, 1988), but differences in constitutive cardenolide levels may have influenced larval performance in our experiment. *A. hirtella* had higher average published cardenolide content compared with other species tested and those larvae struggled to pupate, but larvae that fed *A. speciosa*, a milkweed with published cardenolide content higher than most of the species tested (Roeske et al. 1976, Agrawal et al. 2009, Rasmann and Agrawal 2011), pupated without difficulty (Fig. 2C). Other factors such as differing latex 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 monarch survival (Zalucki and Brower 1992, Malcolm and Zalucki 1996, Zalucki and Malcolm 1999, Zalucki et al. 2001a,b). We observed differences in adult forewing length among milkweed species, but these measures are within the range observed in wild monarchs (Altizer and Davis 2010). We do not know if there is an advantage of larger forewings for a breeding monarch, but autumn migrants usually have longer forewings (Altizer and Davis 2010).

We observed differences in pupal mass and length (Table 2). Some of these differences in mass did carry over to the adult stage, but only when the adults were dried (Tables 1 and 2). Although these data are noteworthy, we do not know how the measured parameters may influence monarch success.

The lipid content of freshly eclosed monarchs was similar to previous studies in which monarchs were collected in the field and reared in the laboratory (Beall 1948, James 1984, Cohen 1985, Brower et al. 2006, Brower et al. 2015). Lipids ranged from 2 to 50 mg across treatments; importantly, differences in dry adult mass do not entirely explain the differences in lipid content (Table 1). Like Cookman et al. (1984), we observed differences in lipid concentration among larvae reared on different host plants. Our results suggest that *A. exaltata*, *A. incarnata*, and *A. syriaca* may be more lipid-rich food sources for monarch larvae, and that other milkweeds, such as *A. hirtella*, may not be as good a food source for lipid content (Table 1, Fig. 3). Alternatively, monarchs may be able to process toxins from *A. exaltata*, *A. incarnata*, and *A. syriaca* more effectively, leading to higher lipid storage (Roeske et al. 1976). Lipid content is only one potential indicator of host plant quality for monarch larvae; larvae that fed on *A. tuberosa* eclosed with lower lipid stores than larvae that fed on other milkweeds (Fig. 3), but more larvae survived on *A. tuberosa* than any other milkweed in this experiment (Figs. 1 and 2D). Although lipid stores are an important energy source for monarchs (Brower 2006), we do not know how these differences may affect breeding adults.

Although survivorship was highest on *A. exaltata* and *A. tuberosa*, monarch habitat should include milkweed species with habitat needs that best match the potential restoration site. Growing conditions used in this study represent middle ground for the nine species tested; some species may have grown better in more specialized conditions such as *A. incarnata* in a moist environment. All nine milkweeds 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 2017). While *A. exaltata* had the second highest survival, this species tends to favor woodland edges and is rare across the state (Woodson 1954, Eilers and Roosa 1994, USDA-NRCS 2017).

Future research should investigate adult female egg load and potential fecundity for individuals that have fed on different milkweed species in order to further assess the value of different milkweeds on the landscape. These trials should use mature, hardened milkweed plants so that monarchs encounter both buds and blooms. We acknowledge that our experiment was conducted under artificial conditions; feeding choices made by monarchs in the wild may differ from the results presented here. More information is needed about how monarchs respond to milkweeds grown in conditions mirroring native habitat and both the oviposition response and preference of female monarchs for different milkweed species to gauge their potential value in habitat restoration.

## Acknowledgments

This work was partially funded by the USDA National Institute of Food and Agriculture, Hatch project number 1009926 (IOW05478) and by the USDA, Natural Resources Conservation Service's Conservation Innovation Grant program under Agreement Number 69-3A75-16-006. Additional support was provided by Prairie Biotics Inc. and the Iowa Monarch Conservation Consortium. Mention of a proprietary product does not constitute an endorsement or a recommendation by Iowa State University or USDA for its use. We would like to thank Nicholas Oppedal, Jaclyn Appelhans, Cory Haggard, and Nancy Shryock for their help with experimental set up and collecting measurements. We thank the staff of the ISU Forestry Greenhouse for their help with plant care, as well as Dean Adams, Sue Blodgett, and two anonymous reviewers for their feedback, which improved this manuscript.

## References Cited

- Agrawal, A. A., J. P. Salminen, and M. Fishbein. 2009. Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution* 63: 663–673.
- Agrawal, A. A., J. G. Ali, S. Rasmann, and M. Fishbein. 2015. Macroevolutionary trends in the defense of milkweeds against monarchs: latex, cardenolides, and tolerance of herbivory, pp. 47–59. *In* K. S. Oberhauser, S. Altizer, and K. Nail (eds.), *Monarchs in a changing world: biology and conservation of an iconic insect*. Cornell University Press, Ithaca, NY.
- Alonso-Mejia, A., E. Rendon-Salinas, E. Montesinos-Patino, and L. P. Brower. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecol. Appl.* 7: 934–947.
- Altizer, S. and A. K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. *Evolution* 64: 1018–1028.
- Baldwin, I. T. 1987. Damage-induced alkaloids in tobacco: pot-bound plants are not inducible. *J. Chem. Ecol.* 14: 1113–1120.
- Baldwin, I. T. 1988. Short-term damage-induced increases in tobacco alkaloids protect plants. *Oecologia* 75: 367–370.
- Beall, G. 1948. The fat content of a butterfly, *Danaus plexippus* Linn., as affected by migration. *Ecology* 29: 80–94.
- Brower, L. P., L. S. Fink, and P. Walford. 2006. Fueling the fall migration of the monarch butterfly. *Integr. Comp. Biol.* 46: 1123–1142.
- Brower, L. P., O. R. Taylor, E. H. Williams, D. A. Slayback, R. R. Zubieta, and M. I. Ramirez. 2012. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *Insect Conserv. Divers.* 5: 95–100.
- Brower, L. P., L. S. Fink, R. J. Kiphart, V. Pocius, R. R. Zubieta, and M. I. Ramirez. 2015. Effect of the 2010–2011 drought on the lipid content of Monarchs migrating through Texas to overwintering sites in Mexico, pp. 117–129. *In* K. S. Oberhauser, S. Altizer, and K. Nail (eds.), *Monarchs in a changing world: biology and conservation of an iconic insect*. Cornell University Press, Ithaca, NY.
- Center for Biological Diversity, The Center for Food Safety, The Xerces Society, and L. P. Brower. 2014. Petition to protect the monarch butterfly (*Danaus plexippus*) under the endangered species act. (<http://www.xerces.org/wp-content/uploads/2014/08/monarch-esa-petition.pdf>).
- Cohen, J. A. 1985. Differences and similarities in cardenolide contents of queen and monarch butterflies in Florida and their ecological and evolutionary implications. *J. Chem. Ecol.* 11: 85–103.

- Cookman, J. E., M. J. Angelo, F. Slansky, and J. L. Nation. 1984. Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, *Anticarsia gemmatalis*, as affected by larval diet. *J. Insect Physiol.* 30: 523–527.
- Davis, A. K. 2012. Are migratory monarchs really declining in eastern North America? Examining evidence from two fall census programs. *Insect Conserv. Divers.* 5: 101–105.
- Davis, A. K. and L. A. Dyer. 2015. Long-term trends in Eastern North American monarch butterflies: a collection of studies focusing on spring, summer, and fall dynamics. *Ann. Entomol. Soc. Am.* 108: 661–663.
- Eilers, L. J., and D. M. Roosa. 1994. The vascular plants of Iowa: an annotated checklist and natural history. University of Iowa Press, Iowa City, IA.
- Erickson, J. M. 1973. The utilization of various *Asclepias* species by the larvae of the monarch butterfly, *Danaus plexippus*. *Psyche* 80: 230–244.
- Espeset, A. E., J. G. Harrison, A. M. Shapiro, C. C. Nice, J. H. Thorne, D. P. Waetjen, J. A. Fordyce, and M. L. Forister. 2016. Understanding a migratory species in a changing world: climatic effects and demographic declines in the western monarch revealed by four decades of intensive monitoring. *Oecologia* 181: 819–830.
- Flockhart, D. T., J. B. Pichancourt, D. R. Norris, and T. G. Martin. 2015. Unravelling the annual cycle in a migratory animal: breeding season habitat loss drives population declines of monarch butterflies. *J. Anim. Ecol.* 84: 155–165.
- Flockhart, D. T., L. P. Brower, M. I. Ramirez, K. A. Hobson, L. I. Wassenaar, S. Altizer, and D. R. Norris. 2017. Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. *Glob. Chang. Biol.* 23: 2565–2576.
- Haribal, M., and J. A. Renwick. 1996. Oviposition stimulants for the monarch butterfly: flavonol glycosides from *Asclepias curassavica*. *Phytochemistry* 41: 139–144.
- Hayden, A. 1919. Notes on the floristic features of a prairie province in central Iowa. *Proc. Iowa Acad. Sci.* 25: 376.
- Inamine, H., S. P. Ellner, J. P. Springer, and A. A. Agrawal. 2016. Linking the continental migratory cycle of the monarch butterfly to understand its population decline. *Oikos* 125: 1081–1091.
- Kaul, R. B., S. B. Rolfsmeier, and J. J. Esch. 1991. The distribution and reproductive phenology of the milkweeds (*Asclepiadaceae*: *Asclepias* and *Cynanchum*) in Nebraska. *Trans. Nebr. Acad. Sci. Affil. Soc. XVIII*: 127–140.
- James, D. G. 1984. Phenology of weight, moisture and energy reserves of Australian monarch butterflies, *Danaus plexippus*. *Ecol. Entomol.* 9: 421–428.
- Jokela, K. J., D. M. Debinski, and R. L. McCulley. 2016. Effects of tall fescue and its fungal endophyte on the development and survival of tawny-edged skippers (Lepidoptera: Hesperidae). *Environ. Entomol.* 45: 142–149.
- Ladner, D. T., and S. M. Altizer. 2005. Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. *Entomol. Exp. Appl.* 116: 9–20.
- Lavoie, B. and K. S. Oberhauser. 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environ. Entomol.* 33: 1062–1069.
- Nail, K. R., C. Stenoien, and K. S. Oberhauser. 2015. Immature monarch survival: effects of site characteristics, density, and time. *Ann. Entomol. Soc. Am.* 108: 680–690.
- Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores, pp. 251–291. *In* G. A. Rosenthal and M. R. Berenbaum (eds.), *Herbivores: their interactions with secondary plant metabolites*, 2nd ed. Academic Press, Inc., San Diego, CA.
- Malcolm, S. B. 1994. Milkweeds, monarch butterflies and the ecological significance of cardenolides. *Chemoecology* 5: 101–117.
- Malcolm, S. B., and M. P. Zalucki. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomol. Exp. App.* 80: 193–196.
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. *J. Chem. Ecol.* 15: 819–853.
- Oberhauser, K. S., and M. J. Solensky. 2004. *The monarch butterfly: biology & conservation*. Cornell University Press, Ithaca, NY.
- Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. K. F. Lam, and R. L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl Acad. Sci. USA* 98: 11913–11918.
- Oberhauser, K. S., R. Wiederholt, J. E. Diffendorfer, D. Semmens, L. Ries, W. E. Thogmartin, L. Lopez-Hoffman, and B. Semmens. 2016. A transnational monarch butterfly population model and implications for regional conservation priorities. *Ecol. Entomol.* 42: 51–60.
- Pleasants, J. M. 2015. Monarch butterflies and agriculture, pp. 169–178. *In* K. S. Oberhauser, S. Altizer, and K. Nail (eds.), *Monarchs in a changing world: biology and conservation of an iconic insect*. Cornell University Press, Ithaca, NY.
- Pleasants, J. M. 2017. Milkweed restoration in the Midwest for monarch butterfly recovery: estimates of milkweeds lost, milkweeds remaining and milkweeds that must be added to increase the monarch population. *Insect Conserv. Divers.* 10: 42–53.
- Pleasants, J. M., and K. S. Oberhauser. 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conserv. Divers.* 6: 135–144.
- Pocius, V. M., D. M. Debinski, K. G. Bidne, R. L. Hellmich, and F. K. Hunter. 2017. Performance of early instar monarch butterflies (*Danaus plexippus* L.) on nine milkweed species native to Iowa. *J. Lepid. Soc.* In press.
- R Core Team. 2014. R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria.
- Rasmann, S., and A. A. Agrawal. 2011. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecol. Lett.* 14: 476–483.
- Robertson, G. F., M. P. Zalucki, and T. D. Paine. 2015. Larval host choice of the monarch butterfly (*Danaus plexippus*) on four native California desert milkweed species. *J. Insect Behav.* 28: 582–592.
- Roeske, C. N., J. N. Seiber, L. P. Brower, and C. M. Moffitt. 1976. Milkweed cardenolides and their comparative processing by monarch butterflies (*Danaus plexippus* L.), pp. 93–167. *In* J. W. Wallace and R. L. Mansell (eds.), *Biochemical interaction between plants and insects*, vol. 10. Springer US, New York, NY.
- Schroeder, L. A. 1976. Energy, matter, and nitrogen utilization by the larvae of the monarch butterfly, *Danaus plexippus*. *Oikos* 27: 259–264.
- Seiber, J. N., L. P. Brower, S. M. Lee, M. M. McChesney, H. T. A. Cheung, C. J. Nelson, and T. R. Watson. 1986. Cardenolide connection between overwintering monarch butterflies from Mexico and their larval food plant, *Asclepias syriaca*. *J. Chem. Ecol.* 12: 1157–1170.
- Stenoien, C., K. Nail, J. M. Zalucki, H. Parry, K. Oberhauser, and M. P. Zalucki. 2016. Monarchs in decline: a collateral landscape level effect of modern agriculture. *Insect Sci.* doi: 10.1111/1744-7917.12404.
- Thielges, D. W. 2005. Impact of an invader: epizootic American slipper limpet *Crepidula fornicata* reduces survival and growth in European mussels. *Mar. Ecol. Prog. Ser.* 286: 13–19.
- USDA, NRCS. 2017. The PLANTS Database (<http://plants.usda.gov>, 3 April 2017). National Plant Data Team, Greensboro, NC.
- Wassenaar, L. I. and K. A. Hobson. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proc. Natl Acad. Sci. USA* 95: 15436–15439.
- Woodson, R. E., Jr. 1954. The North American species of *Asclepias* L. *Ann. Missouri Bot. Gard.* 41: 1–211.
- Yeagan, K. V., and C. M. Allard. 2005. Comparison of common milkweed and honeyvine milkweed (*Asclepiadaceae*) as host plants for monarch larvae (Lepidoptera: Nymphalidae). *J. Kans. Entomol. Soc.* 78: 247–251.
- Zalucki, M. P., and L. P. Brower. 1992. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology* 3: 81–93.
- Zalucki, M. P., and R. L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. Chrysippus* L. Larvae (Lepidoptera: Nymphalidae). *Oecologia* 53: 201–207.
- Zalucki, M. P., and J. H. Lammers. 2010. Dispersal and egg shortfall in monarch butterflies: what happens when the matrix is cleaned up? *Ecol. Entomol.* 35: 84–91.

- Zalucki, M. P., and S. B. Malcolm. 1999. Plant latex and first-instar monarch larval growth and survival on three North American milkweed species. *J. Chem. Ecol.* 25: 1827–1842.
- Zalucki, M. P., L. P. Brower, and A. Alonso. 2001a. Detrimental effects of latex and cardiac glycosides on survival and growth of first instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecol. Entomol.* 26: 212–224.
- Zalucki, M. P., S. B. Malcolm, T. D. Paine, C. C. Hanlon, L. P. Brower, and A. R. Clarke. 2001b. It's the first bites that counts: survival of first-instar monarchs on milkweeds. *Austral Ecol.* 26: 547–555.
- Zalucki, M. P., S. B. Malcolm, C. C. Hanlon, and T. D. Paine. 2012. First-instar monarch larval growth and survival on milkweeds in southern California: effects of latex, leaf hairs, and cardenolides. *Chemoecology* 22: 75–88.
- Zalucki, M. P., H. R. Parry, and J. M. Zalucki. 2016. Movement and egg laying in monarchs: to move or not to move, that is the equation. *Austral Ecol.* 41:154–167.
- Zaya, D. N., I. S. Pearse, and G. Spyreas. 2017. Long-term trends in mid-western milkweed abundances and their relevance to monarch butterfly declines. *BioScience* 67: 343–356.