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Research

Plant-Insect Interactions

Habitat Type Influences *Danaus plexippus* (Lepidoptera: Nymphalidae) Oviposition and Egg Survival on *Asclepias syriaca* (Gentianales: Apocynaceae)

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Abstract

As agricultural practices intensify, species once common in agricultural landscapes are declining in abundance. One such species is the monarch butterfly (*Danaus plexippus* L.), whose eastern North American population has decreased approximately 80% during the past 20 yr. One hypothesis explaining the monarch's decline is reduced breeding habitat via loss of common milkweed (*Asclepias syriaca* L.) from agricultural landscapes in the north central United States due to the adoption of herbicide-tolerant row crops. Current efforts to enhance monarch breeding habitat primarily involve restoring milkweed in perennial grasslands. However, prior surveys found fewer monarch eggs on common milkweed in grassland versus crop habitats, indicating potential preference for oviposition in row crop habitats, or alternatively, greater egg loss to predation in grasslands. We tested these alternative mechanisms by measuring oviposition and egg predation on potted *A. syriaca* host plants. Our study revealed that habitat context influences both monarch oviposition preference and egg predation rates and that these patterns vary by year. We found higher monarch egg predation rates during the first 24 h after exposure and that much of the predation occurs at night. Overall, we documented up to 90% egg mortality over 72 h in perennial grasslands, while predation rates in corn were lower (10–30% mortality) and more consistent between years. These findings demonstrate that weekly monarch egg surveys are too infrequent to distinguish oviposition habitat preferences from losses due to egg predation and suggest that monarch restoration efforts need to provide both attractive and safe habitats for monarch reproduction.

Key words: habitat, restoration, oviposition, predation, Lepidoptera

Agricultural expansion and intensification are among the greatest threats to global biodiversity (Tilman et al. 2001, Tscharntke et al. 2005), and developing cropping systems that simultaneously support biological diversity and other ecosystem services remains a significant challenge (Tilman et al. 2002, Foley et al. 2011, Glamann et al. 2017). As agricultural practices intensify, even species that were historically abundant in agroecosystems can be affected (Van Dyck et al. 2009, Stanton et al. 2018). One such case is the migratory eastern North American population of the monarch butterfly (*Danaus plexippus* L.), which has declined precipitously during the past two decades in its Mexico overwintering range (Taylor 2010, Brower et al. 2012) and by some estimates in also its summer breeding range (Stenoien et al. 2018, Pleasants et al. 2017). Although the reasons for monarch declines are probably multifactorial (Thogmartin et al. 2017b, Belsky and Joshi 2018), one hypothesis suggests that declining abundance of common milkweed (*Asclepias syriaca* L.) in the U.S. Midwest is a key driver (Pleasants and Oberhauser 2013, Thogmartin et al. 2017b; but see Inamine et al. 2016). Common milkweed (hereafter milkweed) historically occurred in natural prairie habitats throughout the region and later as a common weed in row crops (Bhowmik and Bandeen 1976, Pleasants 2015). However, primarily due to widespread adoption of herbicide-tolerant corn and soybean and associated herbicide use, milkweed has declined in agricultural crops. Recent losses of cropland milkweed in Midwestern region of the United States are well documented (Hartzler 2010, Zaya et al. 2017). A synthesis of milkweed surveys by Pleasants (2017) estimated that nearly 40% of Midwest milkweed disappeared between 1999 and 2014, which includes primarily plants lost from herbicide application in row crops but also a smaller portion lost from grasslands converted to agriculture.

In eastern North America, monarch butterflies oviposit on milkweed in both natural and agricultural habitats, and studies have noted consistent trends in egg density in these habitats (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pitman et al. 2018).

Monarch egg surveys in the Midwestern United States found greater numbers of monarch eggs per milkweed stem in agricultural fields than in adjacent nonagricultural grasslands in July and August (Oberhauser et al. 2001, Pleasants and Oberhauser 2013). Pitman et al. (2018) found similar patterns of more monarch eggs on milkweed in corn and soybean versus roadside and other nonagricultural habitat in southern Ontario. This pattern implies that milkweed loss from crop fields may have a disproportionately large effect on landscape-level monarch breeding productivity. Based on the numbers of eggs/stem in different habitats, Pleasants (2017) estimated that milkweed declines from Midwestern row crops resulted in a 76% reduction of monarch milkweed resources in the region. This hypothesis has served as the impetus to increase milkweed stems in the monarch breeding range (Thogmartin et al. 2017a). However, questions remain regarding the influence of habitat type and habitat configuration on monarch productivity and mechanisms underlying egg density, and conservation efforts would benefit greatly from more knowledge in these areas (Landis 2017, Grant et al. 2018).

Greater monarch egg densities on milkweed in agricultural versus nonagricultural habitats have been largely interpreted as habitat-related differences in attractiveness for oviposition preference (Pleasants and Oberhauser 2013, Pitman et al. 2018). Proposed mechanisms of increased attraction to agricultural milkweed include higher plant quality, smaller patch size, and greater apparency against a monoculture background (Pleasants 2015, Pitman et al. 2018). However, because egg surveys in these studies were conducted on a weekly basis, reported egg densities could reflect differential predation rates as well as oviposition preference (Pleasants 2015). Indeed, monarch eggs and larvae are known to experience high rates of predation in grasslands (Borkin 1982, Prysby 2004, De Anda and Oberhauser 2015, Oberhauser et al. 2015). And survival estimates based on population age structure suggest that monarchs experience lower survival in nonagricultural grasslands versus agricultural fields in the Midwest breeding region (Oberhauser et al. 2001). It is therefore plausible that the observed pattern of more eggs in corn could be due to reduced predation in addition to, or instead of, increased oviposition (Supp Fig. 1 [online only]). Furthermore, if predation is the primary driver of egg mortality in these systems, this could mean that grassland areas where milkweed restoration efforts are common (Thogmartin et al. 2017a) represent higher risk habitat for monarchs. The research presented here focuses on understanding the relationships among habitat type, monarch oviposition, and predation risk in agroecosystems to enhance the effectiveness of monarch habitat restoration and conservation efforts.

During the summers of 2016 and 2017, we conducted experiments in southwestern Michigan to quantify monarch butterfly oviposition and egg predation on potted common milkweed plants placed in corn, soybean, bare ground, and grassland habitat treatments. Our goal was to determine whether monarch egg densities differ, and if so, if this a function of oviposition habitat preference, differential predation, or a combination of these non-mutually exclusive causes (Supp Fig. 1 [online only]). We hypothesized that if oviposition was the major driver of this pattern, we would observe greater numbers of eggs laid by wild monarchs in agricultural versus grassland habitats when sampled at a high frequency (one to two times daily) as this would limit time for predation losses. Alternatively, if predation was the dominant cause of monarch egg densities, we would observe greater rates of predation on monarch eggs within grasslands versus agricultural habitats. We interpret our results in the context of monarch conservation efforts and recommend further work to advance knowledge about monarch breeding biology and habitat management.

Materials and Methods

Study Site Description

All experiments were conducted at the Michigan State University Kellogg Biological Station Long Term Ecological Research Site (KBS LTER) Cellulosic Biofuels Diversity Experiment (CBE) located in Hickory Corners, MI (42.407 N, 85.374 W). The CBE was established in 2008 and includes 12 biofuel cropping system treatments arrayed as 12 adjoining 9.1 m by 27 m plots each replicated within four randomized blocks (Fig. 1). Individual plots are immediately adjacent, and between the blocks and surrounding the entire experiment are 12.2-m-wide alleyways of mowed turf grass. Our experiments used four of these treatments: corn, soybean, bare ground, and prairie. Corn and soybean were grown in rotation with both crops present in each year. The bare ground treatment was formerly continuous corn, but during 2016 and 2017 treated with glyphosate to prevent plant colonization. The prairie plots were a species mixture comprising six grass and four forb species native to Michigan. A meteorological station 200 m north of the CBE measures a variety of weather variables including hourly temperature 3 m above ground and rainfall to the nearest millimeter (https://lter.kbs.msu.edu/datatables/13). For detailed information on agronomic practices, prairie planting seed mixes, and experimental site history, see Dickson and Gross (2015).

Host Plants

None of the treatments used for the study contained naturally growing milkweed. Because plant age, nutrition, and other plant condition variables could be influenced by habitat and affect attractiveness to ovipositioning monarchs (Pleasants 2015), we used potted *A. syriaca* plants for all experiments to control for these factors. We sourced plants from native Michigan genotype seeds grown for us at Wildtype Nursery, Mason, MI. Plants were grown in three separate cohorts each year corresponding to our experimental periods. Seeds were planted in a nursery greenhouse in Fafard 3B Mix (Sun Gro Horticulture, Agawam, MA), fertilized with Osmocote Plus 15:9:12 NPK slow release fertilizer (Everris NA, Inc., Dublin, OH), and watered ad libitum. One week prior to deployment, each plant was transferred to a single plastic 1-liter pot and acclimatized in an outdoor courtyard on the Michigan State University campus. Plants were 14–19 wk old and approximately 50 cm tall at deployment and remained in a vegetative (i.e., pre-flowering) stage throughout the experiments. Occasionally, a plant would be damaged or die in the field, in which case it was replaced using a spare plant from the same cohort.

In July and August of 2017, plants were colonized by the oleander aphid *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) while growing in the greenhouse. Although this species occurs naturally and concurrently with monarchs under field conditions in our region, we removed them to provide consistency with previous deployments of plants. To remove aphids, every plant used in 2017 experiments was rinsed under running water and brushed with a small paint brush 3–5 d before deployment. We then submersed each individual plant in a solution of short-lived Safer Insect Killing Soap (Woodstream Corporation, Lititz, PA) to kill any remaining aphids. On the morning of each deployment, plants were rinsed in fresh water to remove any remaining soap.

Plant Deployment

Common milkweed is a clonal species and typically grows in patches (Bhowmik and Bandeen 1976). We mimicked this patchiness by arranging potted plants in three groups of three adjoined pots, with

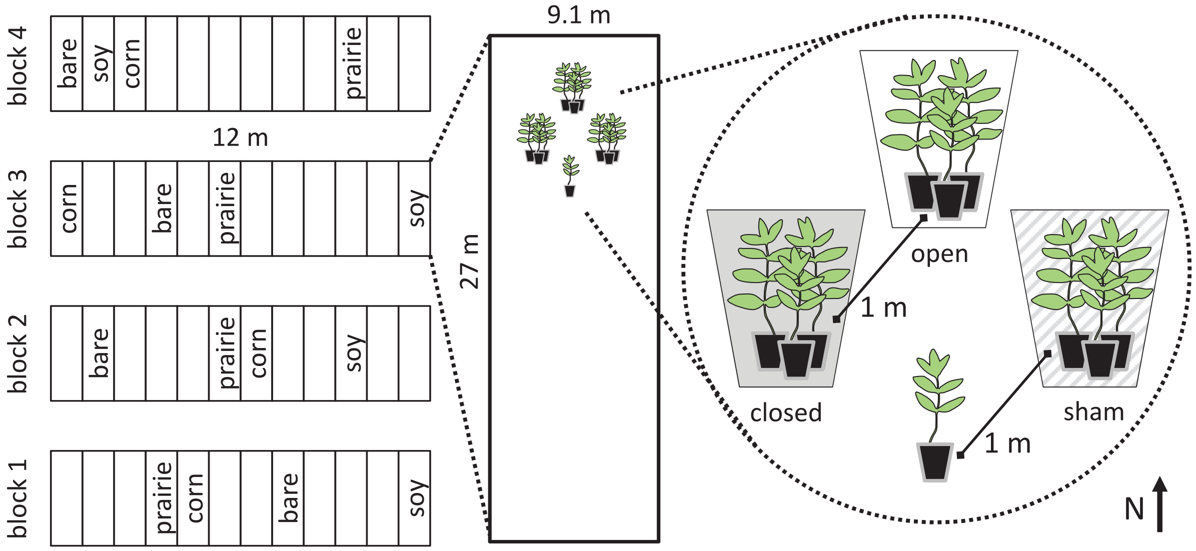

Fig. 1. Experimental plot arrangement (left) and configuration of potted *Asclepias syriaca* patches within one experimental plot for monarch oviposition and survival experiments in 2016 and 2017. Each plant patch comprised three adjoining pots, patches were arranged in an equilateral triangle 1 m apart, and one of three exclosure treatments was randomly assigned to each patch (for survival experiments). The single plant on the south side of the triangle was included during the oviposition experiments in 2017 only.

each pot containing a single milkweed stem (hereafter each group of three pots is called a 'patch') in each treatment plot. Individual pots were 10 × 10 cm, so the plant stems within each patch were approximately 14 cm apart. The three patches were positioned 1 m apart arranged in a north-pointing equilateral triangle within each experimental plot for a total of nine plants per plot in 2016 (Fig. 1) and a total of 144 plants (nine plants × four treatments × four replicates). In 2016, the plants were randomly placed near the centroid of either the north or south half of the plot. In 2017, plants were always placed at the north centroid to accommodate an unrelated experiment. In 2017, a tenth plant was added south of the triangle such that it was 1 m from the two southern patches, for a total of 160 plants (10 plants × 4 treatments × 4 replicates). The purpose of adding this plant was to perform a parallel experiment not presented here; however, eggs laid on this plant were counted and included in oviposition checks (see Oviposition Experiment section of Methods). Each pot was anchored to the ground with a 1 m bamboo stake, and the three stakes of each patch were tied at the top for added stability. Plants were hand watered as needed every 1–2 d.

Oviposition Experiment

To determine whether habitat context influences monarch oviposition choice, we conducted an oviposition experiment during three separate time periods representing the early, mid, and late summer breeding generations. Egg checks began the day immediately following plant deployment. To minimize the potential effects of predation on egg counts, we checked plants one to two times per day for eggs laid by wild monarchs (except when prevented by weather or travel logistics; see Supp Fig. 2 [online only]). During the 2016 field season, we conducted one egg check from 10:00 to 12:30 and a second egg check from 13:00 to 15:30 on a typical day. In 2017, we performed daily egg checks from 10:00 to 12:30 and then from 17:00 to 19:30 to better sample eggs laid in the afternoon hours. Egg checks were performed by visually inspecting stems and both upper and lower surfaces of all leaves. We removed eggs as they were detected to prevent double counting and damage to the plants by subsequent larvae and to minimize the influence of con-specific eggs on oviposition. We also recorded each observation of an adult monarch butterfly within the study plot or immediate grassy border. Each oviposition check took approximately 2.5 h, and we standardized adult butterfly observations to number of individuals observed per h.

Oviposition Data Analysis

For each day in each plot, we calculated the number of eggs laid per stem by summing all the eggs observed and dividing by the number of plants present in the plot that day. In cases where the number of plants varied within a single day (e.g., a dead plant was replaced in the morning and then checked in the afternoon), we used the mean number of plants present for the day. Because oviposition events were generally rare, we averaged all daily values of eggs per stem to generate a single mean eggs per stem per day value for each of the 16 plots within each of the two study years for analysis.

All statistical analyses were performed in R version 3.4.3 (R Core Team 2017). We tested the effect of the habitat treatment on numbers of eggs observed with negative binomial generalized linear mixed effects models using the glmer.nb function in the MASS package (Venables and Ripley 2002). Habitat treatment was included as a fixed effect, block as a random effect, and the number of plants present as an offset variable. We used an analysis of deviance in base R (R Core Team 2017) to perform a χ^2 likelihood ratio test comparing the full model to a null model to determine whether habitat treatment had a significant effect on oviposition. Pairwise contrasts of modeled treatment means were performed using the emmeans function with a Holm adjustment for multiple comparisons (Lenth et al. 2018).

Survival Experiment

To determine the impact of habitat context on 72-h monarch egg survival, we performed three separate experiments (23 August 2016, 17 July 2017, and 23 August 2017; Supp Fig. 2 [online only]) that followed the fates of monarch eggs placed on milkweed plants. Each survival experiment immediately followed the previously described oviposition experiments using the same plants (Fig. 1). Using the same plants as the oviposition experiment ensured that plants were colonized by each habitat's local predator community, as all plants were deployed for at least 8 d before the start of each survival experiment. Within each plot, the three plant patches were randomly assigned to the three exclosure treatments, with the exclosures covering the three adjoining plants: a full exclosure (hereafter 'closed' treatment) intended to exclude all predators; an open treatment allowing access by all predators; and a sham treatment designed to allow entry by predators while controlling for cage effects on abiotic conditions. For the closed treatment, we removed any predators on plants before applying the exclosure.

We constructed predator exclosures using No-see-um Netting (Skeeta Inc., Bradenton, FL) supported by 142-cm-tall, 46-cm-diameter steel tomato cages. To prevent entry by ground predators, the bottom of the no-see-um barrier was sewed closed into a bag shape with tomato cage tines pierced through the netting into the ground. Sham treatments were identical to the full exclosures, but with the addition of three 75-cm-long vertical slits in the netting to allow entry by arthropod predator and the bottom left open to ground predators. Open treatments received a tomato cage, but no netting (Supp Fig. 3 [online only]).

All monarch eggs used in experiments were produced by a colony housed at Michigan State University in East Lansing, MI. Approximately 60 h before egg deployment, a potted *A. syriaca* plant was introduced to the colony butterflies for oviposition. Approximately 12 h before the experiment, eggs were gently removed from the plant and placed onto moistened paper towels in large plastic Petri dishes and stored overnight at approximately 7°C to arrest development. The following morning eggs were transported to the field in a cooler with ice packs. We attached eggs to leaves by gluing one egg per leaf to the undersides of the four uppermost fully emerged leaves where monarchs naturally lay most eggs (Zalucki and Kitching 1982). To attach eggs, a tiny droplet of Elmer's Glue-All (Elmer's Products, Columbus, OH) was placed on the end of a fine paint brush, which was subsequently used to gently pick up and attach eggs onto plants. Laboratory feeding trials (Herman et al., unpublished data) and field video recording (A.M. and D.A.L., unpublished data) demonstrated that a variety of arthropods (including members of Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera, Dermaptera, Neuroptera, and Araneae) will feed on monarch eggs glued to plants using this method and that eggs hatch normally. Video surveillance also demonstrated that very few glued eggs fall off plants; of 152 eggs monitored for an average of 60 h, three or fewer fell off the plant (A.M. and D.A.L., unpublished data). Furthermore, by day 3 of the 2017 survival experiments, several eggs began to hatch, demonstrating that egg development was unaffected by our gluing method.

Four eggs were affixed to each plant in August 2016 and July 2017 deployments (for a total of 576 eggs per experiment), and three eggs per plant were used in August 2017 (for a total of 432 eggs). To compare survival among treatments, we checked egg survival every 2–3 h for 24 h and again at 48 and 72 h. At each survival check, we identified and counted arthropod predators on plants in the open and closed exclosure treatments to the lowest taxonomic level possible in the field. We categorized arthropods as monarch predators if they met one or more of the following criteria: 1) the species or family is commonly considered a predatory taxon; 2) no-choice lab trials indicate that the taxon consumes monarch eggs (Herman et al., unpublished data); and/or 3) we directly observed predators consuming monarch eggs during experiments. Because we only recorded the presence/absence of ants for August of 2016, all predator data presented from August 2016 exclude ants, whereas for July and August 2017, we include ant abundance data.

Survival and Predator Data Analysis

Egg survival at 72 h was assessed separately for each of the three deployments using binomial generalized linear mixed models using the glmer function in the R package lme4 (Bates et al. 2015). In all models, the individual patch was considered a random effect, and depending on the model, either exclosure treatment or habitat was considered fixed effects. To test whether predators were the cause of egg mortality, we first evaluated the effect of exclosure treatment on survival response. We predicted that survival would be higher in predator exclusion treatments than in sham or fully open ones. We used an analysis of deviance in base R (R Core Team 2017) to perform a χ^2 likelihood ratio test comparing the model that included both the fixed effect of exclosure type and the random effect of individual patch to a null model, which only included the random effect of patch. We then performed post hoc comparisons in the same manner as for the oviposition analysis described in Oviposition Data Analysis section of Methods.

After confirming that predators reduced survival of monarch eggs (see Results), we used the same approach used to determine the effect of exclosure to test the effect of habitat treatment on survival of monarch eggs in the open exclosures only. We used a binomial generalized linear mixed effects model with survival as the response variable, habitat treatment as a fixed effect predictor variable, and individual patch as a random effect, and each deployment date was assessed separately. The full model was compared with the null, which included the random effect of patch only. We performed this analysis for each of the three experimental periods. For periods in which survival was significantly influenced by habitat treatment, we proceeded to post hoc, pairwise comparisons of survival within all four treatments using the R package emmeans (Lenth et al. 2018).

We analyzed predator abundance separately from survival data. For analyses, we summed the total number of predators across all surveys repeated during the first 72 h (9, 13, and 14 surveys for August 2016, July 2017, and August 2017 experimental periods, respectively) within habitat treatment and exclosure treatment (i.e., summed across dates within each plot). Because predators were rarely found in exclosure cages (Table 1), we only performed statistical analysis using surveys from the plants in the open exclosure treatments. To determine whether habitat treatment significantly affected total predator numbers, we modeled total predator numbers as a function of habitat treatment using negative binomial generalized linear models using the glmer.nb function in the R package MASS (Venables and Ripley 2002). As with the survival data, we performed this analysis for each of the three experimental periods separately. In experiments where habitat significantly influenced total predator numbers, we proceeded to post hoc Holm-adjusted pairwise comparisons among habitats using the R package emmeans. Because ants were often the most abundant arthropods we observed and are considered important monarch predators (Calvert 2004, Prysby 2004, Mooney and Agrawal 2008), we repeated the same analysis for ants separately for the July 2017 and August 2017 experiments when ant numbers were recorded.

Results

Oviposition Experiment

We observed a low but generally steady rate of adult monarch butterfly visits to our study sites (Table 2). Visitation rates ranged between 0.09 and 0.14 adults observed per hour (i.e., one individual for every 7–11 h of observation), apart from July 2017, when we observed an

Table 1. Results of predator surveys conducted during three monarch butterfly survival experiments in the summers of 2016 and 2017

Order	Family	Mean number of predators/stem/survey					
		Aug. 2016		July 2017		Aug. 2017	
		Closed	Open	Closed	Open	Closed	Open
Orthoptera	Acrididae	0	0	0	0.001	0	0.010
	Tettigoniidae	0	0	0	0	0	0.008
	Gryllidae (Nemobiinae)	0	0	0	0	0	0.008
	Gryllidae (Oecanthinae)	0	0	0	0	0	0.002
Dermatpera	Forficulidae	0.002	0.009	0	0.033	0	0.027
Hemiptera	Lygaeidae (sp. *Lygaeus kalmii*)	0	0.028	0	0.115	0	0.002
	Miridae	0	0	0	0	0	0.016
	Nabidae and Reduviidae	0	0.002	0	0.027	0	0.006
	Pentatomidae (nymph and adult)	0	0	0.001	0.004	0	0.008
	Anthocoridae	0	0	0	0.001	0	0.002
Neuroptera	Chrysopidae and Hemerobiidae	0	0.002	0	0	0	0
Coleoptera	Coccinellidae (larva and adult)	0.005	0.014	0	0.007	0	0.002
	Cantharidae (larva)	0	0.002	0	0	0	0
Hymenoptera	Formicidae	NA	NA	0	0.244	0	0.171
Araneae	(All families)	0	0.007	0.003	0.016	0	0.011
Opiliones	(All families)	0	0	0	0	0	0.048
Gastropoda	(All families)	0	0	0	0.004	0	0.002
	Total	0.007	0.065	0.004	0.454	0	0.322

Numbers are mean predators observed per stem per survey pooled across habitat treatments within closed and open exclosure treatments. Gray cells indicate instances of predators observed in closed exclosure treatments. NA (not applicable).

Table 2. Total numbers of monarch adults and eggs observed during 2016 and 2017 monarch oviposition monitoring experiments

Year	Deployment date range	Days checked	Adults observed	Adults observed/ hour ± SEM	Total eggs observed	Eggs observed/ stem/day ± SEM
2016	7–24 June	16	6	0.088 ± 0.048	1	0.0004 ± 0.0004
	1 July–3 Aug.	13	4	0.092 ± 0.066	18	0.0102 ± 0.0047
	9–23 Aug.	10	6	0.140 ± 0.073	149	0.0965 ± 0.0293
2017	13–30 June	8	7	0.125 ± 0.075	28	0.0220 ± 0.0049
	12–16 July	5	16	0.520 ± 0.162	29	0.0374 ± 0.0284
	15–21 Aug.	6	3	0.100 ± 0.045	42	0.0448 ± 0.0173

Adult numbers are observations noted during egg-counting surveys. Egg numbers are the total numbers of eggs observed on potted milkweed plants during egg checks.

average of 0.5 monarch per h (i.e., one individual for every 2 h of observation). Butterflies were typically observed flying over plots, but we also observed them nectaring within prairie plots, and on one occasion ovipositing on milkweed plants in a bare treatment.

Overall monarch oviposition differed between 2016 and 2017. First examining all habitat treatments and deployments combined, in 2016 oviposition increased exponentially over the course of the breeding season, ranging from 0.0004 to 0.10 eggs/stem/d laid from June to August in contrast to 0.022–0.04 eggs/stem/day from June to August 2017, respectively (Table 2).

Oviposition habitat preference patterns also differed notably between the two study years. In both 2016 and 2017, habitat treatment had a significant effect on monarch oviposition (2016, $\chi^2 = 20.98$, df = 3, $P < 0.001$; 2017, $\chi^2 = 9.46$, df = 3, $P = 0.024$). In 2016, monarch butterflies laid significantly more eggs on *A. syriaca* in the corn versus the other treatments (Fig. 2). Oviposition did not differ between prairie and bare treatments, but oviposition was significantly lower in soybean versus prairie and bare treatments. In 2017, egg laying was greater in prairie versus soybean habitat treatments, but no other treatment pairs differed.

Survival Experiment

For each of the three experimental periods, survival of eggs differed among exclosure treatments (August 2016, $\chi^2 = 9.93$, df = 3, $P = 0.019$; July 2017, $\chi^2 = 9.40$, df = 3, $P = 0.024$; August 2017, $\chi^2 = 13.41$, df = 3, $P = 0.004$). In August of both years, survival in the closed treatment was significantly greater than in the open and sham treatments, and survival in open versus sham did not significantly differ (Fig. 3A and C). In July 2017, survival patterns were similar to the August studies, although in this period, survival in the sham did not differ statistically from that of the closed treatment (Fig. 3B). These results combined with our observations of near-complete exclusion of predators in the closed treatments (Table 1) indicate that our exclosure treatments were effective at increasing survival via reduced predation levels and that the cage effect on survival was small. Therefore, we investigated the effect of habitat treatment on survival using only the open exclosure treatments.

Survival varied with habitat treatment for the August 2016 and 2017 experimental periods, but was similar across all habitats in July 2017 (August 2016, $\chi^2 = 43.98$, df = 3, $P < 0.001$; July

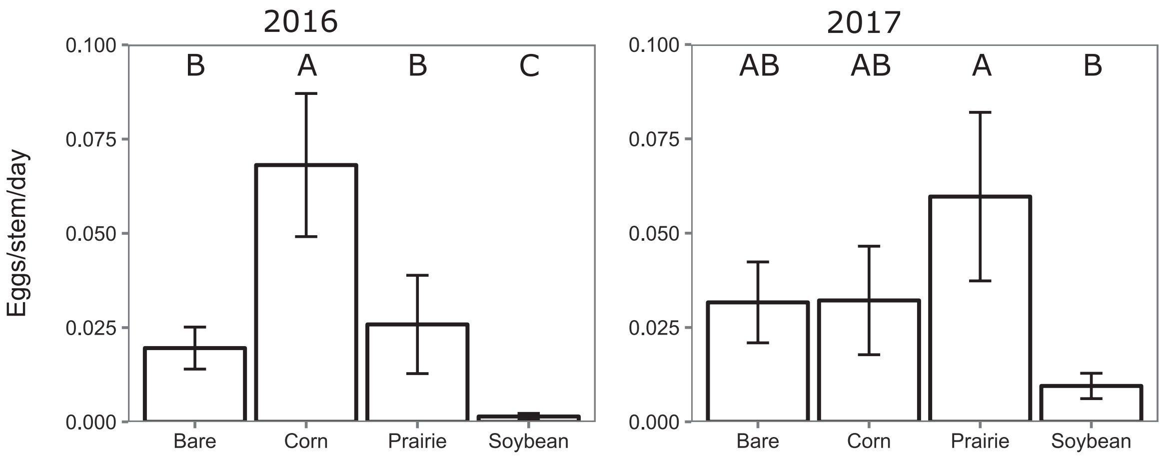

Fig. 2. Monarch oviposition expressed as mean ± SEM eggs observed per milkweed stem per day (*n* = 4 replicates per treatment). Grouping letters represent post hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from negative binomial generalized linear mixed model, α = 0.05.

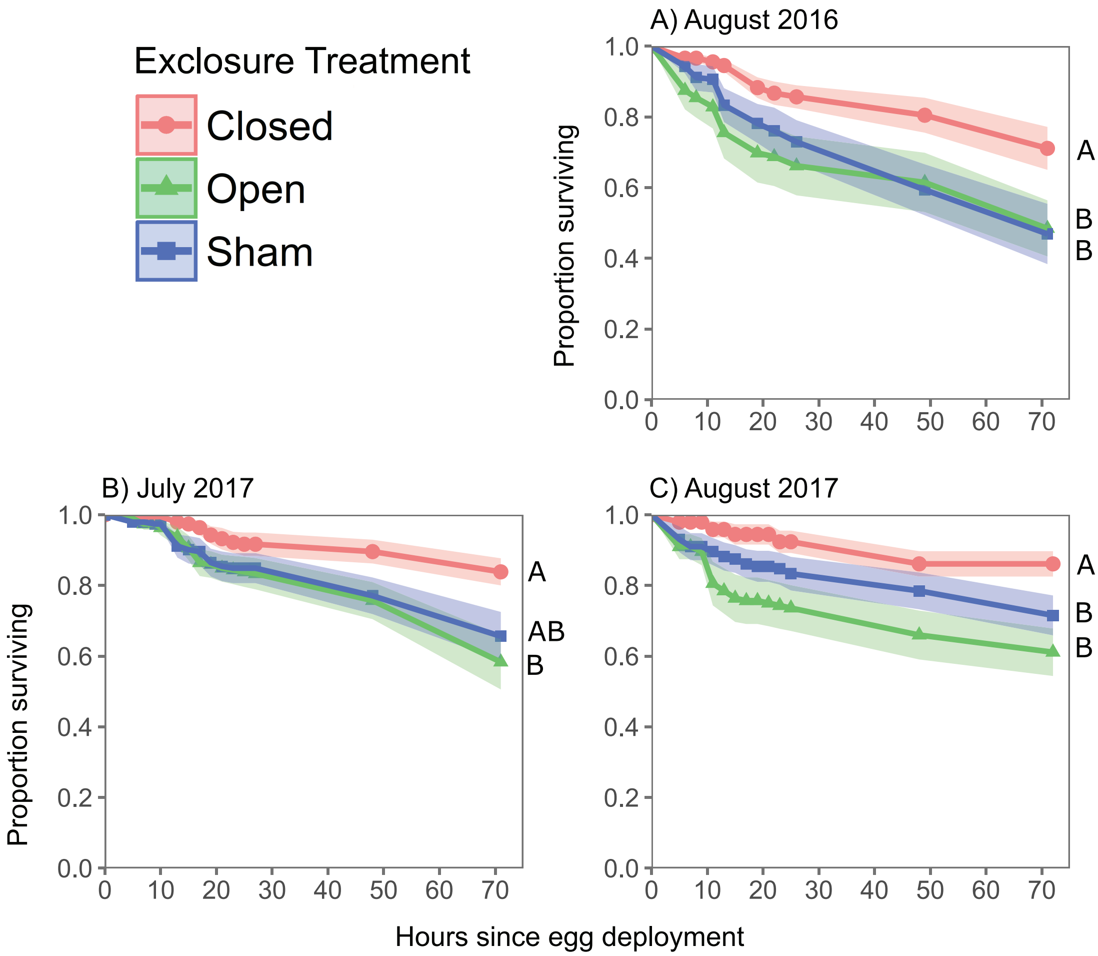

Fig. 3. Monarch egg and first-instar survival to 72 h under three exclosure treatments for three experimental periods combined across all habitat treatments: (A) August 2016; (B) July 2017; (C) August 2017. Points represent arithmetic means of survival across the four experimental blocks, and colored areas are SEM. Grouping letters represent post hoc pairwise contrast groupings of estimated marginal means from binomial generalized linear mixed model, α = 0.05.

2017, χ^2 = 4.34, df = 3, *P* = 0.227; August 2017, χ^2 = 15.02, df = 3, *P* = 0.002). In August 2016, overall survival to 72 h was highest in bare soil and corn treatments, intermediate in soybean, and lowest in prairie (Fig. 4A). In August 2017, survival was highest on milkweeds in corn and lowest on those in soybean, with intermediate survival in bare and prairie treatments (Fig. 4B). Repeated

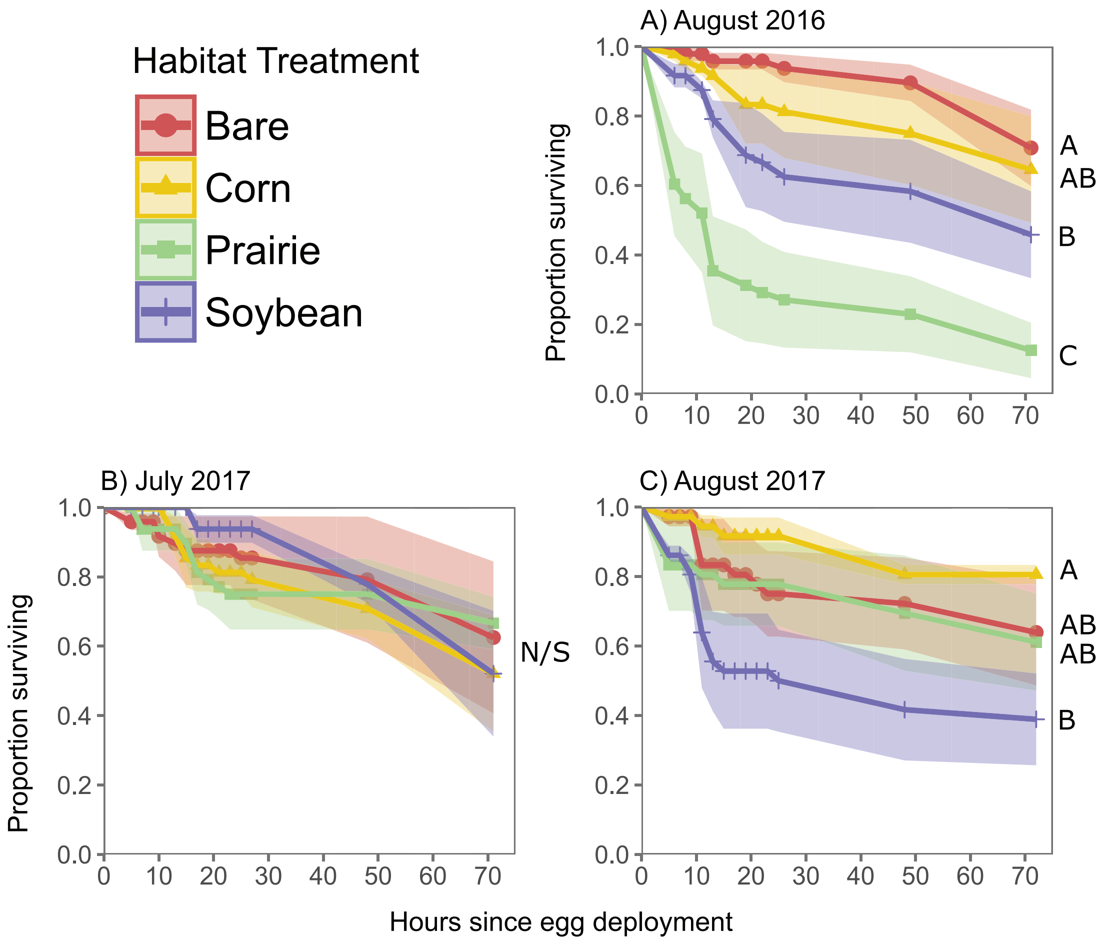

Fig. 4. Monarch egg and first-instar survival to 72 h in four habitat treatments for three experimental periods: (A) August 2016; (B) July 2017; (C) August 2017. Points represent arithmetic means of survival across the four experimental blocks, and colored areas are SEM. Grouping letters represent post hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from binomial generalized linear mixed model, $\alpha = 0.05$. Shaded columns represent nighttime hours from 20:00 to 6:00.

egg survival checks (every 2–3 h) during the initial 24 h revealed that up to 50% of egg predation occurred during the first night following egg deployment. We reran the same survival analysis at 24 h and found the same effects of exclosure and habitat treatments on egg survival as at 72 h.

Predators on Experimental Plants

Predators rarely breached exclosures, with no predators found in the exclosure cages in August 2017, and 10–100× more predators per stem in open versus closed exclosure treatments in August 2016 and July 2017 (Table 1). We observed five arthropod families feeding on sentinel monarch eggs: Coleoptera: Cantharidae (larva, likely *Chauliognathus pensylvanicus* DeGeer); Coleoptera: Coccinellidae (adult, *Harmonia axyridis* Pallas); Hemiptera: Nabidae (adult, unknown sp.); Hemiptera: Miridae (adult, unknown sp.); and Dermaptera: Forficulidae (adult, *Forficula auricularia* L.). Total predator numbers varied by habitat for the August 2017 experiment (August 2017, $\chi^2 = 8.06$, df = 3, $P = 0.045$), but not for the August 2016 or July 2017 survival experiments (August 2016, $\chi^2 = 1.72$, df = 3, $P = 0.63$; July 2017, $\chi^2 = 4.45$, df = 3, $P = 0.22$; Supp Fig. 4 [online only]). When ants were examined in isolation they exhibited a similar pattern; ant numbers varied by habitat in August 2017 ($\chi^2 = 13.72$, df = 3, $P = 0.003$), but not in July 2017 ($\chi^2 = 5.74$, df = 3, $P = 0.12$). In the August 2017 sampling, we observed significantly more predators on plants in the bare treatment primarily driven by ants.

Discussion

Both the differential oviposition and predation-influenced egg loss hypotheses of monarch egg abundance among different habitats. We found habitat context influenced monarch oviposition patterns, with corn as the most attractive egg-laying habitat in 2016 and prairie the most attractive in 2017. Eggs also experienced differential predation across habitats and over time. Egg predation was greatest in August of both years with bare and corn treatments consistently providing the safest habitats for egg survival. In combination, these findings demonstrate that weekly egg surveys of oviposition often performed in monarch population monitoring programs reflect the combined effects of oviposition preference and predation losses in different habitats.

In 2016, we observed the most eggs laid within corn plots. This pattern is consistent with observations by Pleasants and Oberhauser (2013) and Pitman et al. (2018) and supports the hypothesis that monarchs prefer *A. syriaca* growing in corn as oviposition habitat. In 2017, however, oviposition differences among treatments were less pronounced, with the most eggs laid in the prairie treatment and the only significant pairwise treatment difference between prairie and soybean. Contrary to our prediction and the findings of egg survey studies (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pitman et al. 2018), soybean was consistently and significantly the least selected oviposition habitat in both years.

We found that predation on monarchs during the first 72 h varied strongly by habitat type and in one instance reached 90% (prairie in 2016). However, we note that habitat effects on predation rates varied through time. In August 2016 survival was greatest in bare and corn, intermediate in soybean, and lowest in prairie. In July of 2017, survival was equivalent among all four habitat treatments. In August of 2017, we observed the greatest survival rate in corn, the lowest in soybean, and intermediate in bare and prairie. The highest monarch egg predation rates observed in the prairie treatment indicate that perennial grasslands can be relatively risky habitats for monarch eggs.

Several nonexclusive mechanisms could explain why monarchs exhibited oviposition habitat preference and experienced differential predation by habitat and why these patterns differed among years. Pleasants (2015) hypothesized that milkweed growing in corn is more attractive for oviposition due to higher nitrogen from agricultural fertilization, younger vegetation from frequent disturbance, and greater apparency against a monoculture background. Because our study held host plant age, fertilization, and density constant while still detecting differential oviposition, we do not find support for plant quality variables as key drivers of oviposition preference. We cannot rule out the possibility that host plant apparency affected oviposition preference. However, if this were the primary driver it seems likely that plants in the bare plots would receive the most eggs, especially considering the milkweed plants were always beneath the height of the surrounding vegetation in the other three treatments, with the exception of those in the soybean and corn treatments during the month of June. We interpret this to indicate that other factors besides apparency still contribute to oviposition. Another possible explanation also put forth by Pleasants (2015) is that monarchs are attracted to the shaded milkweed plants growing under corn. Indeed, Agrawal et al. (2012) found that *A. syriaca* grown in shade had lower concentrations of defensive compounds, lower leaf toughness, and higher growth rates of monarch larvae than *A. syriaca* plants exposed to full sun.

Differences between the two study years in preferred oviposition habitat indicate that habitat preference may be mediated by other factors that vary from year to year. The most obvious difference between 2016 and 2017 was weather. During 2017, southwestern Michigan experienced a significant midsummer drought, with exceptionally warm temperatures and rainfall 50% below average (https://lter.kbs.msu.edu/datatables/7). These conditions resulted in visibly water-stressed corn plants in our treatment plots compared with 2016. Monarchs seeking more humid habitats for oviposition during this dry period may have targeted prairie plots, whose vegetation stayed significantly greener and less wilted than the corn plants. Finally, it is possible that monarchs could adjust oviposition preference based on some signal of predation risk, which could vary by both habitat and year. However, in a post hoc analysis, we found no evidence for a positive correlation between oviposition preference and survival (Supp Fig. 5 [online only]). Further studies into the mechanisms driving monarch oviposition habitat preference would be useful in helping to design or manage breeding habitat for monarchs.

Overall mean monarch egg density in our study was similar in 2016 and 2017 and was similar to those found in other monarch egg surveys in the region that reported eggs/milkweed stem. We found mean ranges of 0.001–0.069 (soybean and corn) and 0.003–0.022 (soybean and prairie) eggs/stem/d in 2016 and 2017, respectively. In 2000 from June to August, Oberhauser et al. (2001) observed means of 0.0134 and 0.0612 egg/stem/survey in nonagricultural and corn sites, respectively, in their weekly upper Midwest surveys. In July–August of 2015 and 2016, Pitman et al. (2018) observed an overall mean of 0.1 eggs/stem/weekly survey in southern Ontario. At 14 sites across Iowa, on *A. syriaca*, Pocius et al. (2018) found approximately 0.001, 0.006, and 0.006 eggs/stem/weekly survey in June–August of 2015, 2016, and 2017, respectively. Finally, Blader (2018) found a range of 0.064–1.2 mean eggs/stem/week at four prairies in Iowa.

Patterns of oviposition across each breeding period differed between 2016 and 2017. The summer of 2016 started with very low egg numbers in early June followed by an exponential increase in oviposition in July and August (Table 2). In 2017, however, oviposition was intermediate in June and slowly increased through the summer. Low early summer oviposition could be explained by very few monarchs returning in the spring of 2016 from Mexico, as monarch numbers are correlated between sequential stages in the northerly migration (Pleasants and Oberhauser 2013, Inamine et al. 2016). Although monarch overwintering aggregations preceding the summer of 2016 were larger than in 2017 (4.01 vs 2.91 Ha), a snowstorm in March 2016 killed 30–40% of the overwintering population of monarchs in at least two of the major overwintering colonies before they departed Mexico (Brower et al. 2017), which could have resulted in the low oviposition we observed in early 2016. Monarch egg numbers, however, did not reflect the number of adult monarchs observed at our field sites, which was overall consistent between months and years with the exception of July 2017 when we saw 5-fold higher numbers. It is possible that most of these observations were male butterflies patrolling their territories, and the numbers of males observed in one small area could be more limited by the size of the study area rather than the number of individual butterflies on the landscape.

Higher egg survival in the closed exclosure treatments compared with the open and sham treatments indicated that predators were responsible for much of the mortality we observed in the survival experiment. However, eggs within full exclosures did experience some mortality (14–28% average over 72 h). This level of background mortality was likely due to a combination of predators sometimes breaching the exclosures (Table 1) and eggs occasionally falling off plants due to disturbance from observers frequently removing netting to check egg status. Low survival rates in prairie in 2016 supported our prediction that monarch eggs experience greater predation rates on milkweed in prairie versus crop habitats. However, this pattern did not hold for the following year. July 2017 was characterized by relatively moderate 72 h monarch egg survival (50–60% mean treatment survival) with no significant differences among habitat treatments. In August 2017, survival rates in all treatments matched those in August 2016 except in prairie where survival was considerably higher (10% vs 60% mean prairie survival in August 2016 vs 2017). In July, egg survival rates were moderate and not significantly different among the habitat treatments. It is plausible that one or more important grassland predator population does not reach meaningful abundance until late summer. Indeed, many generalist predators frequenting grasslands in our study area reach their highest abundances in late summer (Fiedler and Landis 2007). Nevertheless, the annual variability in August predation rate in prairie remains unexplained. Although abiotic conditions, such as the 2017 drought, may affect predation rates of monarch eggs, it seems unlikely that the abiotic conditions would affect our habitat treatments so unevenly. Perhaps more probable is that a key predator group in prairie underwent a decline in its population cycle between the 2 yr. Generalist predator numbers can vary greatly from year to year as part of normal population cycling, or in response to alternate prey available in a given habitat (Bahlai et al. 2013). Indeed, long-term research of insect communities at our study site shows large year-to-year variation in abundance and biocontrol service of the ladybeetle (Coleoptera: Coccinellidae) community, and at times, these population cycles diverge between annual crop and perennial habitats within a given year (Bahlai et al. 2013).

Although exclosure cages provided strong evidence that predators were responsible for much of the egg mortality, we did not detect an effect of mean predator numbers per milkweed stem on monarch egg survival rate. In our experiment, the bare habitat was the only treatment to have significantly more total predators and ants per milkweed stem than other treatments, and bare plots were among the consistently safer habitats for monarch eggs. Notably, ants were the most numerically abundant predators on milkweed plants when their numbers were recorded in the 2017 experiments. Although ants are considered important predators of monarch eggs and larvae (Calvert 2004, Prysby 2004, Mooney and Agrawal 2008), we never directly observed ants consuming monarch eggs. These results align with those of Pitman et al. (2018), who found that milkweed predator abundance was not a predictor of monarch egg density. We speculate that, aside from ants, many monarch egg predators could be highly mobile (e.g., Miridae and Coccinellidae) or visit plants at night and may be underrepresented in predator surveys. Furthermore, some predators could be more voracious than others, making overall predator numbers less meaningful to egg disappearance rates. More research regarding the identities, natural history, and population dynamics of monarch egg and larva grassland predators is crucial to develop schemes to increase monarch production in U.S. agricultural landscapes.

Our results indicate that variation in monarch productivity among different habitats is a function of both oviposition habitat preference and differential survival due to predation. This has important implications for monarch population monitoring and conservation efforts. Because much of the predation we recorded occurred at night and during the first 24 h following oviposition, weekly egg surveys are not adequate to determine monarch oviposition habitat preferences in habitats with different levels of predation. This would be particularly true in years like 2016, when rapid predation between survey periods could inflate estimates of oviposition difference between grassland and crop habitats. We recommend that monarch egg surveys seeking to elucidate predictors of oviposition habitat section either be conducted more frequently or be combined with egg predation studies.

Finally, our study investigated monarch oviposition habitat preference and predation rates at a relatively fine spatial scale considering the long-distance dispersal capabilities of monarch butterflies and the much larger habitat patches occurring in agricultural landscapes. Although our experimental design was useful in that it operated as a 'choice test' for monarchs that could easily move among the study plots, we recommend further oviposition habitat experiments at the landscape level to complement existing observational studies and to validate recent agent-based monarch movement models by Grant et al. (2018). It would also be useful to determine whether egg density is more strongly related to the number of egg-laying monarchs in a given area or the number of eggs laid per individual. It is also possible that some predators could move among our small habitat patches (Blitzer et al. 2012). We expect that similar future experiments in large-scale agricultural landscapes with larger habitat patches may have less predator community spillover and would show stronger predation differences among habitats.

The potential for high predation rates in grassland habitats is an important consideration for monarch conservation, as monarch breeding habitat restoration efforts are primarily proposed to increase milkweed plantings in perennial grasslands occurring on conservation lands, roadsides, and other rights-of-way (Thogmartin et al. 2017a). Developing management strategies to enhance monarch egg and larva survival in grasslands could make restoration more tenable and efficient in terms of land use. Indeed, a recent rangewide monarch population model estimated that increasing monarch survival and fecundity rates each by 2% in the northcentral breeding range would stabilize the eastern North American monarch population (Oberhauser et al. 2017). Consideration of egg and larva survival in monarch habitat management strategies could greatly increase chances of a successful monarch restoration effort. One promising possibility is the reintroduction of disturbance regimes to grasslands supporting monarch habitat. Recent work by Haan and Landis 2019 demonstrated that late summer mowing of milkweed patches both generates new milkweed growth that is highly attractive for monarch oviposition, but also increases egg and larvae survival before predators fully recolonize plants. Recent work has also highlighted how milkweed species plays a role in influencing monarch oviposition and larval survival and performance (Pocius et al. 2017a,b, 2018). Monarch conservation would benefit from a better understanding of how grassland type (e.g., prairies, roadsides, old fields), milkweed species, and disturbance regimes interact to influence monarch oviposition and predation risk.

During much of the 20th-century monarchs probably benefited from agricultural practices in the north central United States through the creation of better *A. syriaca* growing conditions with few monarch predators. Now relegated to breeding in relatively small areas of perennial grasslands, monarchs are left with fewer host plants in more risky habitats. As conventional agricultural practices attempt to deliver ever more primary productivity from limited lands, they will inevitably result in more simplified habitats at both the field and landscape scales (Landis et al. 2000, Rusch et al. 2016). Consequently, even species such as monarch butterflies that once thrived in agricultural landscapes could be at risk. Conserving global biodiversity will require intentional efforts to determine both the underlying causes of species declines and management practices, which will allow for biodiversity to exist alongside productive agricultural systems.

Supplementary Material

Supplementary data are available at *Environmental Entomology* online.

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Data Availability

Data from this study are available from the Dryad Digital Repository: doi:10.5061/dryad.k9f1p42.

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