Running head: Disturbance enhances monarch butterfly survival

Grassland disturbance effects on first-instar monarch butterfly survival, floral resources, and flower-visiting insects

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Abstract

Many species of conservation concern are disturbance-dependent, relying on periodic ecosystem disruptions to maintain habitat quality. Mounting evidence suggests monarch butterflies are one such organism: they can benefit from growing-season disturbance to grassland habitats in their breeding range, with regenerating stems of milkweed host plants supporting more oviposition and lower densities of arthropod predators. Here we address three questions that were raised by previous work in this system. First, we tested if survival of neonate monarch larvae is enhanced on milkweed stems that regrow after disturbance. Second, we tested if disturbance affects spore densities of the parasite *Ophryocystis elektroscirrha* (OE) on milkweed leaves. Finally, we documented effects of disturbance on the abundance of floral resources and flower-visiting insects. We found that first-instar monarch survival over 48h periods was 2.3-2.5 times higher on regenerating milkweed stems than on undisturbed controls. OE spores were not detected on any of the milkweed stems in our study. Disturbance reduced floral resource abundance and floral visits for 3-5 weeks, although some species that were initially suppressed bloomed later in the season with the net effect of extending the bloom period. Our results show grassland disturbance can enhance survival of immature monarchs and could be used strategically to help stabilize the eastern monarch population. More work is needed to understand how disturbance in this system affects resources for pollinators and to optimize habitat management for monarchs and the broader pollinator community.

Introduction

Organisms often depend on disturbance to maintain their habitat (Sousa 1984). This is particularly true for imperiled butterflies, which often occupy rare early-successional habitats shaped by disturbances like fire (Haddad 2018, Thomas 1980, Schultz and Crone 1998, Thomas et al. 2009, Schultz and Crone, 2015, Schultz et al. 2011, Dunwiddie et al. 2016). However, disturbance also characterizes human-dominated environments and therefore may be important for maintaining insect populations in anthropogenic landscapes. Recent evidence suggests this could be the case for the monarch butterfly (*Danaus plexippus* L., Nymphalidae; Haan and Landis 2019a), a flagship species in agricultural and urban landscapes (Guiney and Oberhauser 2008).

Adults of the Eastern monarch population overwinter in the highlands of central Mexico, but breed in the Midwest and Eastern US and Canada, where eggs are laid on milkweeds (Gentianales: Apocynaceae), especially common milkweed, *Asclepias syriaca* L. (Malcolm et al. 1993). This iconic population has declined in recent decades and is being reviewed for listing under the US Endangered Species Act (Center for Biological Diversity 2014), prompting conservation scientists to investigate causes of the decline and determine what could be done to reverse it (Inamine et al. 2016, Zaya et al. 2017, Stenoien et al. 2018, Malcolm 2018). The overwintering population rebounded somewhat since 2017, but models suggest it is too small in most years and at risk of quasi-extinction (Semmens et al. 2016). The monarch's decline probably has multiple causes, but the most prominent hypothesis is loss of milkweed host plants from breeding habitats in the US Midwest (Pleasants and Oberhauser 2013). Since the late 1990s nearly all corn and soybean farmers have adopted herbicide-resistant varieties and treat fields with broad-spectrum herbicides. As a result, an estimated 40% of milkweed stems have been lost from the region (Pleasants 2017, Pleasants et al. 2017), and monarchs now rely on milkweed stems in non-crop habitats, mostly perennial grasslands.

Although monarchs are not always perceived as disturbance-dependent organisms, mounting evidence suggests they benefit from perturbations to their breeding habitat during the growing season. Common milkweed is a modular and resilient perennial species, and when aboveground growth is removed or damaged it quickly produces new tissue from axillary and/or belowground buds. Milkweed that regrows after disturbance is very attractive to ovipositing monarchs; several studies have documented large numbers of eggs and larvae on regenerating stems (Fischer et al. 2015, Alcock et al. 2016, Haan and Landis 2019b, Knight et al. 2019). These stems also contain fewer predatory arthropods for the first several weeks of regeneration (Haan and Landis 2019b). Since predation rates on early-stage monarchs are quite high (Prysby 2004, De Anda & Oberhauser 2015, Myers et al. 2019), the reduction in predators following disturbance could provide them with enemy-free space.

Taken together, this evidence suggests growing season disturbance could potentially be used to enhance monarch breeding habitat and help stabilize the population. However, a number of questions need to be addressed before any specific recommendations are made regarding vegetation management practices to benefit monarch butterflies in the Midwest US.

First, disturbance strongly reduces arthropod predator densities on milkweed stems, but it is unclear whether this translates to higher survival of immature monarchs. In recent work we found a non-significant trend toward higher survival of eggs and neonates after disturbance in June (citation redacted for double-blind review). However, our ability to detect a potential difference statistically was limited by low sample size, and since we assessed survival of one individual egg or larva per treatment at each study site, the response was binary and data precision was low.

Second, monarch fitness can be limited by the protozoan parasite *Ophryocystis elektroscirrha* (OE). Larvae ingest OE spores, which proliferate and eventually coat the exteriors of adults which serve as dispersal vectors while having reduced longevity and fecundity. OE is spread vertically when spores are transferred onto eggs during oviposition, and environmentally if spores are scattered onto milkweed

leaves which are eaten by larvae (McLaughlin & Myers 1970, Altizer & Oberhauser 1999). Disturbance during the growing season could conceivably increase or decrease the risk of OE transmission: if spores have accumulated on older milkweed stems, removing them and stimulating fresh tissue production could reduce environmental spore densities. However, regenerating stems also attract more adults; if infected adults contaminate these sites with spores, risk of environmental transmission to larvae could increase.

Finally, grassland habitats are important for diverse taxa beyond monarchs. One area of concern is how disturbance affects floral resources for pollinating insects. In the immediate aftermath of most forms of disturbance, floral resources are likely to be absent or reduced. However, for plant species that regenerate and (re)bloom later in the season, disturbance could also serve to diversify blooming phenology, broadening the window of time when floral resources are available or filling gaps in floral availability, which have been shown to occur in late summer in our region (Wood et al. 2018, Dolezal et al. 2019).

We carried out three experiments to address these questions. First, we tested whether disturbance influenced 48h survival rates of neonate monarch larvae. We predicted survival rates on stems that regenerate after disturbance would be higher than on undisturbed stems. Second, we tested if OE spore densities on milkweed leaves differed between leaves of regenerating stems and those we left undisturbed. Third, we tested for effects of disturbance on floral abundance and phenology, and on the flower-visiting insects. We expected floral abundance to decrease in disturbed plots after disturbance, but also that some regenerating species would flower later in the summer, extending the bloom period. We expected flower-visiting insects to mirror this trend, with initial reductions following disturbance but resurgences later in summer.

Methods

Site selection and treatments

We conducted this study in 2019 using 13 patches of common milkweed (hereafter, 'sites') located in and around East Lansing, MI, USA (Table S1.1). We used the same experimental layout as described in (citation redacted). Five of the sites were used for our study the previous year, while eight were in new locations. Sites contained at least 100 milkweed stems and were located in agricultural field margins, old fields, and other disturbed areas dominated by cool-season grasses. Each site was divided into three plots of approximately equal area (each containing >30 stems) and randomly assigned to be disturbed in mid-June, mid-July, or left undisturbed. We disturbed the plots by mowing with gasoline-powered trimmers equipped with brush cutting blades (Stihl™ chisel tooth circular saw blade 4112_713_4203) and cut vegetation in the plot to a height of 5-20 cm. The mowing treatments occurred on June 17-18 and July 15-16.

Monarch colony procedures

We kept monarchs in colony in the laboratory, where they laid eggs on common milkweed. The colony originated from eggs collected in the field in May 2019 in and around East Lansing, MI. All adults were screened for OE using transparent tape using methods adapted from Altizer et al. (2000). Milkweed was harvested from the field and returned to the lab; all stems were soaked for 20 minutes in 5% bleach solution before being rinsed and provided to larvae. Milkweed stems were searched daily for eggs, which were transferred to petri dishes lined with moist paper towel in a growth chamber (25°C, 50% RH) until hatching.

Experiment 1: disturbance effects on survival

We deployed first instar larvae on milkweed stems from all three treatments to test whether survival rates differed. In total we assessed the fates of 1373 larvae. Larvae were used within 24h of hatching and transported to the field in a cooler to minimize temperature fluctuations. After mowing in June, we placed larvae on milkweed stems in all 13 June-mowed and control plots for 48h periods beginning July 1, 3, 8, and 10. This was the period of time after stems had regrown and were in a pre-

128 flowering stage, when oviposition rates are especially high. After mowing in July, we deployed larvae to
129 all three plots at each site for 48h periods beginning August 1, 3, 7, 8, 12, and 14. During the first four
130 dates in August our colony did not produce enough individuals to deploy at all sites simultaneously;
131 therefore on August 1 and 3 we deployed larvae to five sites (different sites each time), on August 7 we
132 deployed to ten sites with remaining sites receiving larvae the following day, and for the remaining two
133 trials we deployed to all sites. In August, regenerating milkweeds at one site were repeatedly eaten by
134 mammals, so we dropped this site from the July analysis.
135 When deploying larvae we selected five milkweed stems in each plot by choosing the stem
136 closest to the center of the plot plus four additional stems, each ~2 m from the first one, in each of the
137 cardinal directions. Then for subsequent trials in the same plot we selected the closest adjacent stem to
138 the one that had been used previously, shifting systematically clockwise so each trial occurred on a
139 different stem until all stems in the plot had been used, at which point stems were re-used if necessary.
140 At one site on one date only three stems had emerged, so we deployed larvae on three stems instead of
141 five. When enough neonates were available we placed two on each of the five milkweed stems per plot;
142 otherwise we placed one individual per stem. The number of individuals per stem was always equal
143 among plots within a site. Neonates were transferred with a paintbrush and placed on the top surface of
144 the most apical leaf that was at an angle <45° from horizontal, so they could establish without falling off.
145 In each case, 48 h after deployment we searched the entire milkweed stem for the larva. First instar
146 larvae are restricted to a single stem and have a low chance of survival if they are dislodged from it or
147 disperse for some reason (Zalucki et al. 2001), so if they were absent from the stem we assumed they
148 were dead.
149 We tested for differences in survival among treatments with binomial generalized linear mixed
150 models (GLMMs) using the package *lme4* (Bates et al. 2015) in R 3.4.4 (R Development Core Team,
151 2018). We built separate models for the larvae deployed after mowing in June and the those after

mowing in July, as they had different numbers of treatments. Within each subset we used survival data from each plot as a replicate, meaning each replicate described survival of between 15 and 30 larvae (thus N = 13 sites after disturbance in June and N = 12 sites after disturbance in July). We modeled survival as a function of mowing treatment plus a random effect for site and compared these to equivalent models with only the random effect using a likelihood ratio test. Since the model for the second time period included three treatments, we examined significant results with pairwise contrasts using the package *emmeans* (Lenth 2016).

Experiment 2: disturbance effects on risk of environmental OE transmission

We sampled milkweed stems from each treatment for OE spores on two dates: July 12 (control and June-mowed plots) and August 12 (all three plots at each site). We chose these dates because new stems had regenerated and allowed time for adult monarchs to visit them and potentially contaminate leaves with spores. We tested 5 stems from each plot. We used a 15cm piece of mailing tape and pressed it to the upper surface of the 8 leaves closest to the apex of each stem, as monarchs usually oviposit here. We pressed the length of tape to the upper surface of all eight leaves, then affixed it to a clear acetate sheet to avoid contamination. During the second round of sampling we also tested leaf undersides to increase chances of detection. We judged this adjustment to be acceptable because we were interested in comparing OE densities among treatments but not between the two sampling periods. We attached the clear sheets to paper with 9x22cm grids made of 0.5x0.5cm cells, centered on the tape samples. To search for OE spores, we initially assessed all grid cells under a microscope at 40x, increasing magnification as needed to closely examine any objects that were present in a grid cell.

To our knowledge OE spores have not been sampled on milkweed leaves in the field. Therefore, to verify that our methods could detect OE spores, we created a positive control by brushing an OE-infected monarch very lightly against several milkweed leaves. Spores were easily found, suggesting if an infected butterfly contaminated milkweed in the field, spores would be detectable using our methods.

During field sampling we also detected spores or sporelike structures belonging to a number of other taxa, and frequently encountered monarch wing scales which had fallen on the leaves. These lines of evidence suggest if there were OE spores present, our methods would be able to detect them.

Experiment 3: Disturbance effects on floral resources and pollinators

We recorded the identity and number of insect-pollinated flowers in each plot approximately weekly from mid-June until the end of September. For plants with small flowers grouped in inflorescences or capitula (e.g., *Daucus*, *Solidago*), we considered the inflorescence or capitulum to be a single floral unit. For plants with very large numbers of flowers in a plot, we visually estimated abundance by counting the floral units in a small area then extrapolating to the rest of the plot. Thus, our estimates were sometimes rounded to the nearest 5, 10, or 100 depending on the density of flowers.

During each visit we also conducted a 2-minute pollinator survey for flower-visiting bees, flies, and wasps in each plot using methods adjusted from Ward et al. (2014). Surveys occurred between 9:30a and 4:30p in partial to full sun. In each plot the surveyor slowly walked a diagonal transect and recorded all insects that visited flowers within 2m. A visit was defined as touching a flower and/or hovering or pausing in its vicinity, since we were interested in quantifying disturbance effects on flower-visiting insects rather than pollination *per se*. If clumps of floral resources occurred >2m from the transect, the surveyor visited these as well, never spending more than 1 minute in any single location.

Floral abundance was summed for each plot on each survey week and adjusted to account for differences in plot area. We tested whether floral abundance differed among the three treatments during each week using Linear Mixed Models (LMMs). Floral abundance was ln(x+1) transformed prior to analysis. We ran models separately for the time periods before and after disturbance in July as the number of treatments differed. We modeled floral abundance as a function of treatment, week, and their interaction as main effects, plus a random effect for site. To assess overall significance, we

compared this model to a null model without a term for treatment using a likelihood ratio test and calculated pairwise differences among treatments. We also filtered the dataset to include only the species that flowered in multiple plots at five or more sites in order to gain additional inference at the species level. Seven species met these criteria, and for each of these we conducted the same statistical procedure as before for overall floral abundance. Finally, we tested if the number of flower-visiting insects differed among the treatments, again using LMMs with the same transformations and model structure as described for plants.

Results

Experiment 1: Disturbance effects on survival

Disturbance treatments strongly affected neonate survival, with larvae on regenerating stems surviving at higher rates than those on stems that were left undisturbed (LR-test for June treatment: $\chi^2_{[1]} = 49.854$, $p < 0.001$; for July treatment: $\chi^2_{[2]} = 41.800$, $p < 0.001$; Fig. 1). During early July when stems were regenerating after disturbance in June, survival of larvae in disturbed plots was substantially higher than those in plots we left undisturbed (mean% ± SEM = 41.5% ±4.8 in disturbed plots; 16.7% ±3.1 in undisturbed). We observed the same trend in early August when stems were generating after disturbance in July (43.8% ±6.2 in July-disturbed plots; 18.9% ±3.8 in undisturbed). By this time the stems that were disturbed in June had regrown for more than a month and survival rates were similar to those of the undisturbed stems (22.3% ±4.1). In general, when larvae survived we found they had advanced to the second instar. When they died, in some cases we found desiccated remains of larvae, but it was impossible to tell whether they had been predated by organisms that leave the exoskeleton behind (e.g., hemipterans), or died for some other reason. We did not observe any larvae that had obviously died from being mired in latex.

Experiment 2: disturbance effects on risk of environmental OE transmission

No OE spores were detected on milkweed in any of the plots (see Discussion).

Experiment 3: Disturbance effects on floral resources and pollinators

Disturbance treatments had strong overall effects on floral resource abundance (LR-test after June disturbance $\chi^2_{[8]} = 80.330$, $p < 0.001$; after July disturbance $\chi^2_{[18]} = 169.890$, $p < 0.001$). Floral abundance was very low immediately after disturbance but in both treatments recovered within five weeks after disturbance. From late August onwards, the June-disturbed plots tended to have greater resource abundance than control plots, but this trend was not statistically significant (Fig 2).

In total there were 57 plant taxa for which we recorded floral abundance (Table S1.2). The floral community at our study sites was comprised of weedy and mostly exotic species characteristic of cool-season grasslands in disturbed sites. When we examined individual taxa that bloomed in multiple plots at five or more sites, species-specific patterns emerged (statistical results in Fig 3). Some species produced fewer flowers immediately following disturbance, but recovered and flowered later in the summer, ultimately extending the bloom period for that species. For example, disturbance in June reduced and shifted the bloom period of *A. syriaca* to late July and August, after undisturbed plants had senesced. Similarly, flowering of undisturbed *Berteroa incana* (L.) DC. peaked in July and then steadily declined, but if disturbed in either June or July, it continued blooming through September. Other species' flowering periods were not strongly affected by disturbance (e.g., *Silene vulgaris* (Moench) Garcke), were delayed somewhat (*Daucus carota* L.) or were reduced without producing later-season blooms (e.g., *Cirsium arvense* (L.) Scop.). Finally, *Solidago* spp., which bloom in the fall, were delayed about one month if disturbed in June, and strongly suppressed if disturbed in July.

Flower-visiting insects were also initially suppressed by disturbance; after disturbance in June their abundance was lower for three weeks, while after disturbance in July it was lower for five weeks (LR-test after June disturbance $\chi^2_{[3]} = 42.319$, $p < 0.001$; after July disturbance $\chi^2_{[18]} = 105.19$, $p < 0.001$; Fig 4). A taxonomic breakdown of flower-visiting insects can be found in Table S1.3.

Discussion

Our results add to a growing body of evidence that monarch butterflies can benefit from strategically timed disturbance during the growing season. Multiple studies have found that ovipositing butterflies favor new milkweed stems that emerge after disturbance (Fischer et al. 2015, Alcock et al. 2016, Haan & Landis 2019, Knight et al. 2019), and during this window of time arthropod predators are suppressed (Haan & Landis 2019). Here we found that in addition to increased oviposition and reduced predator densities, survival of neonate larvae on regenerating stems was 2.3-2.5 times higher than on undisturbed stems.

Among Lepidoptera, early instars are a critical period when mortality rates are often high. It has been estimated that 54% of first instar Lepidopteran caterpillars die on average, but the rate often ranges from 25 to 75% (Zalucki et al. 2002), and monarch egg and early-instar mortality often exceeds 80% (Prysby 2004, De Anda and Oberhauser 2015, Myers et al. 2019). Here we found disturbance reduced average mortality over a two-day period from c.80% to <60%. We did not test effects of disturbance on egg survival, but diverse taxa consume both eggs and first instars (Hermann et al. 2019), so we expect effects on eggs to be similar.

It is not known whether increasing first instar survival ultimately produces more adults, as density-dependent predation or pathogen transmission could limit survival at later stages. One potential mechanism for this which we tested was the potential for increased OE transmission, but we did not find any OE spores during the study. Three lines of evidence suggest our methods would have detected spores if they were present: first, they were readily detected when we used an OE-infected butterfly as a positive control; second, we found monarch wing scales on sample slides, meaning residues were left behind by ovipositing monarchs and detected in our samples; third, we frequently detected spores or spore-like structures belonging to other taxa (i.e., similar to OE but different shape or size). OE did not appear to figure heavily into the landscapes where we conducted this study but could be important in

271 other years or elsewhere in the breeding range, particularly further south where infections are more
272 prevalent (Altizer et al. 2000).
273 *Floral resources and pollinators*
274 Disturbance produced a temporary gap in availability of flowers to pollinators. Overall floral
275 abundance was reduced for 4 weeks after disturbance, after which time it was similar to control plots
276 (Fig 2); pollinator abundance exhibited a similar pattern (Fig 4). The individual plant species we
277 examined more closely were idiosyncratic, with disturbance extending the bloom window for some,
278 delaying or truncating it for others, or having little effect (Fig 3).
279 At landscape scales, strategic disturbance of milkweeds and surrounding vegetation could in
280 some cases benefit pollinator communities by increasing phenologic heterogeneity of floral resources. If
281 disturbance reduces resources during peak bloom (when they are not limiting) but supplements them
282 later in the season during a resource gap, the net effect on pollinators could be positive even if floral
283 resources are reduced overall. While we did not observe increased late-summer visitation in
284 regenerating plots (Fig 4) our ability to do so may have been hampered by summer drought (July-August
285 precipitation = 78mm, compare to 162mm average for previous four years; EnviroWeather 2020).
286 Diversity in disturbance regimes could also enhance arthropod diversity in general by increasing
287 variation in vegetation structure, composition, and thermal conditions which in turn influence arthropod
288 community structure (e.g., Schaffers et al. 2008, Prather & Kaspari 2019). More work is needed to
289 understand effects of growing-season disturbance on pollinators in these habitats.
290 *Management implications*
291 Growing season disturbance could be used as a management strategy to help stabilize the
292 Eastern monarch population. We suggest that managers could disturb subsets of milkweed patches at
293 different times in the summer (e.g., June and July as in this study) while leaving some stems within a
294 patch undisturbed to maximize phenologic diversity. Effects of disturbance will likely vary

295 geographically, and future work could shed light on the optimal timing of disturbance in different parts
296 of the breeding range.
297 We need more information on how growing-season disturbance affects pollinator communities.
298 If disturbance is found to be generally beneficial, disturbance regimes region-wide could be diversified
299 to promote heterogeneity in cool-season grassland community composition, structure, and bloom
300 phenology. If it is concluded to be ultimately negative to pollinators, habitat management for monarchs
301 and pollinators will need to be balanced. This could be achieved by focusing disturbance specifically
302 within milkweed patches, rather than grasslands in general, as milkweed patches often occupy a small
303 percentage of the landscape.
304 Finally, we perceive that current disturbance regimes in Midwestern grasslands may frequently
305 constitute ecological traps for monarchs. For example, when mowing occurs more than once per
306 growing season in (e.g.) rights of way or hay fields, our results suggest the initial disturbance event
307 attracts ovipositing butterflies and can result in high densities of larvae, but if subsequent disturbances
308 occur before adults emerge, large numbers of monarchs could be killed. Dismantling ecological traps like
309 this by reducing mowing frequency could be a straightforward opportunity to boost monarch habitat
310 productivity.

Acknowledgements

E. D'Auria, S. Christensen, L. Stiffler, A. Conley, A. McClear, and C. Johnston contributed to monarch colony care and field data collection. Site access was provided by Michigan Department of Natural Resources, Department of Technology, Management and Budget, and L. Hughes. Support for this research was provided by USDA National Institute for Food and Agriculture (2017-68004-26323), the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research (Award Number DE-FC02-07ER64494), by the National Science Foundation Long-term Ecological Research Program (DEB 1832042) at the Kellogg Biological Station, The North-Central IPM Center, and MSU AgBioResearch.

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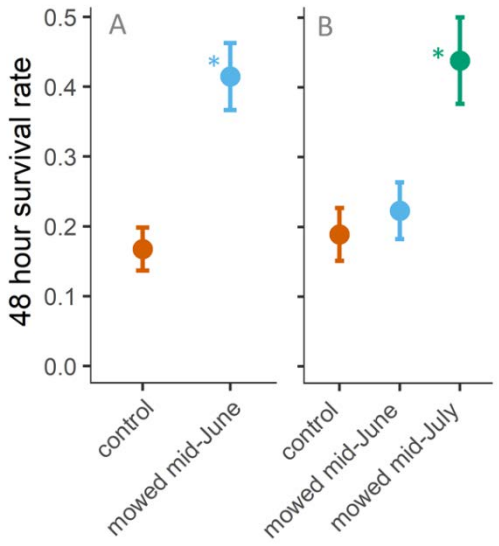
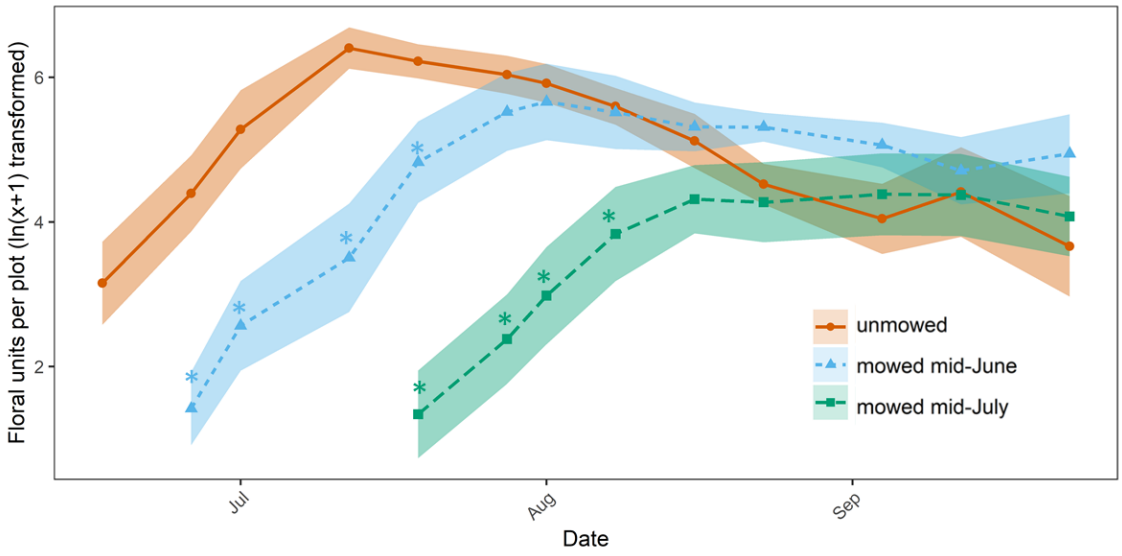


Figure 1. Survival of first instar larvae was higher on newly regenerating milkweed stems. Points represent means, error bars denote ±1 SEM, and asterisks denote treatments that differed significantly from undisturbed controls. A) Larvae deployed between July 1 and July 10 as milkweeds regrew after disturbance in mid-June were approximately 2.5 times more likely to survive than their counterparts on undisturbed stems. B) Larvae deployed between August 1 and August 14 as milkweeds regrew after disturbance in mid-July were on average 2.3 times more likely to survive than larvae placed on undisturbed stems. At this point in the season survival on stems that regenerated after June disturbance did not differ from the undisturbed control.



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435 Figure 2. Overall floral resource abundance was reduced for four weeks following disturbance. Plots that
436 were disturbed in mid-June tended to contain more floral resources in late summer, but the trend was
437 not significant. Points represent means for each treatment and ribbons show ±1 SEM. Asterisks indicate
438 when treatments differed significantly from undisturbed controls. The June- and July-mowed plots were
439 disturbed one week before beginning data collection. Note that data are ln(x+1) transformed.

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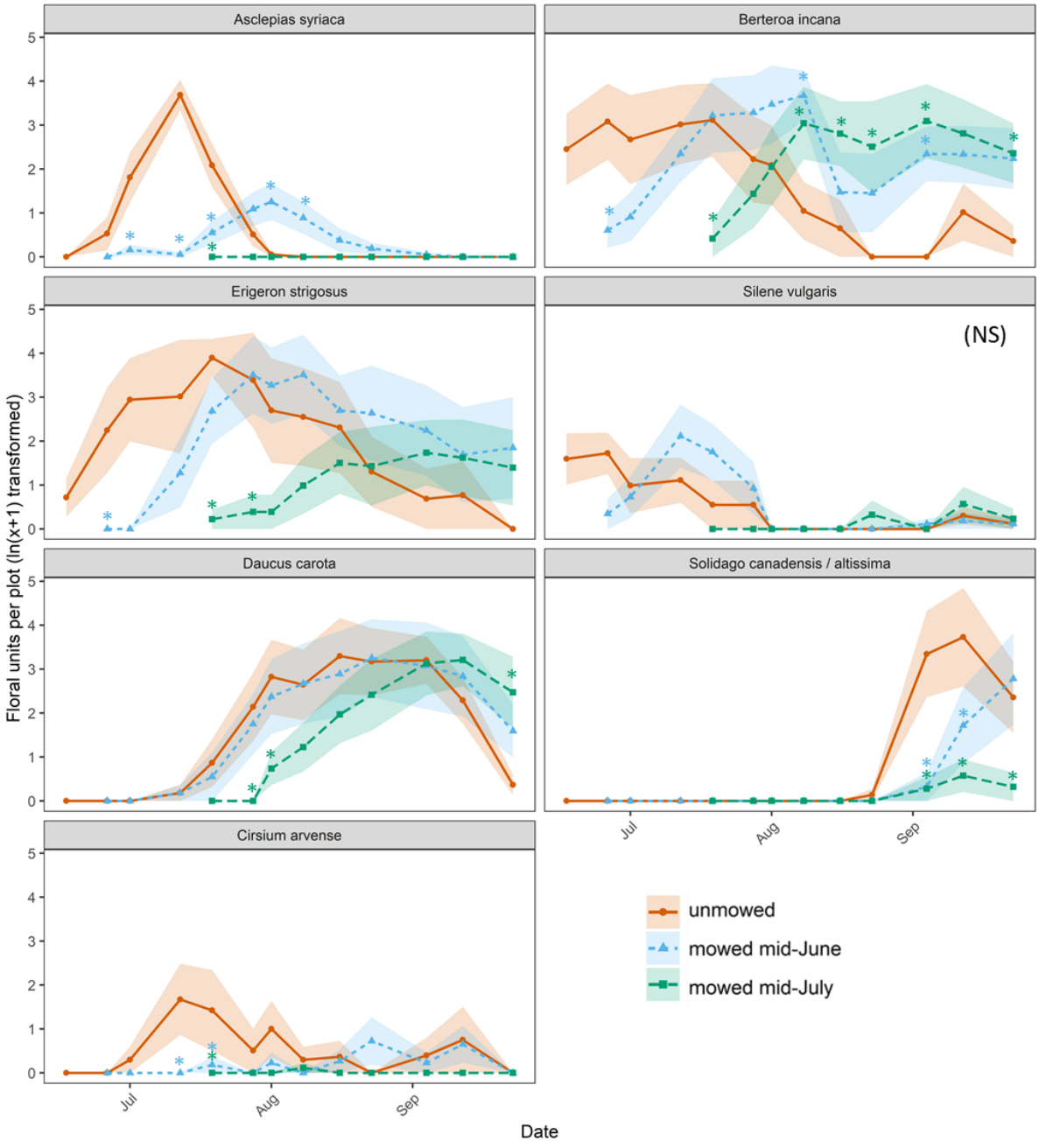

Figure 3. Effects of growing-season disturbance on the bloom periods of seven focal plant species that occurred in multiple plots in at least five study sites. Lines represent means, and ribbons show ±1 SEM. Asterisks show weeks when disturbance treatments differed significantly from the undisturbed control.

446 Disturbed plots were mowed one week prior to beginning data collection. Note that data are ln(x+1)-
447 transformed.
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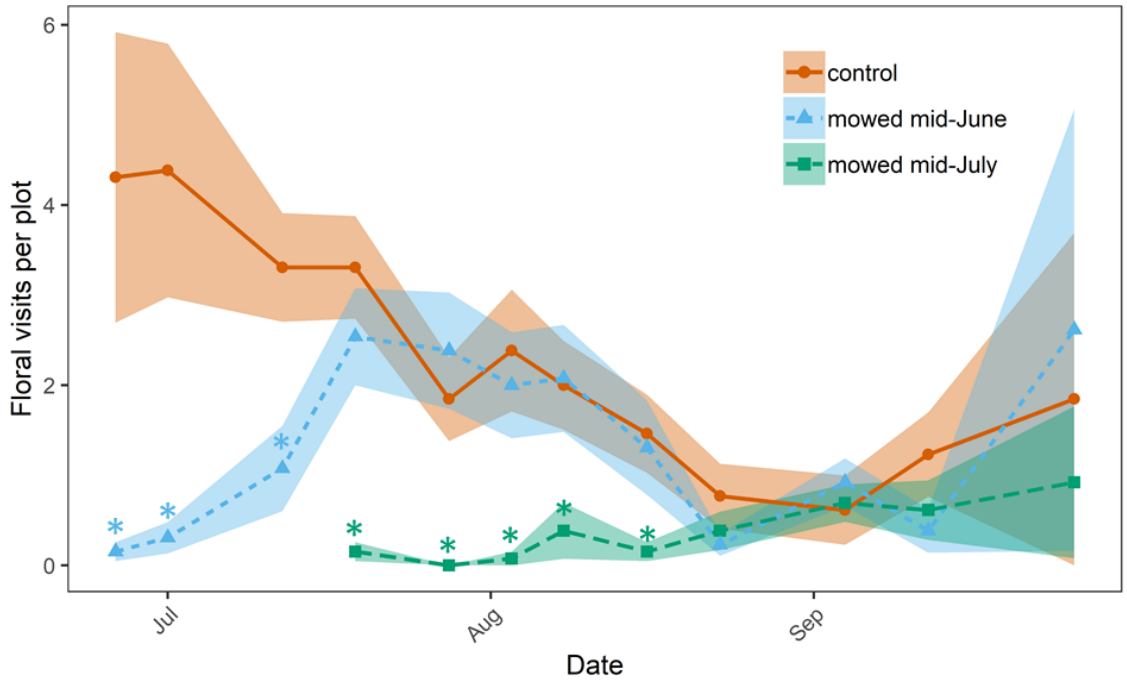

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450 Figure 4. Effects of disturbance on the abundance of flower-visiting bees, flies, and wasps. After
451 disturbance in June, visitors were suppressed for three weeks, and after disturbance in July they were
452 suppressed for five. Points show mean values for each week and ribbons denote ±1 SEM. Asterisks show
453 weeks when treatments differed significantly from the undisturbed control.

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplement:

Grassland disturbance effects on first-instar monarch butterfly survival, floral resources, and flower-visiting insects

S1. Supplemental information on site locations, nectar-producing plant species composition, and flower-visiting insects.

Table S1.1 Study site locations, located in and around East Lansing, Michigan, USA.

Site	Latitude	Longitude
1	42.69521	-84.50009
2	42.96230	-84.49147
3	42.68888	-84.47259
4	42.68041	-84.48642
5	42.67396	-84.47881
6	42.66969	-84.46526
7	42.73585	-84.28893
8	42.73645	-84.28922
9	42.73687	-84.29029
10	42.67128	-84.66112
11	42.67036	-84.67134
12	42.67136	-84.67539
13	42.67290	-84.67474

Table S1.2. Flowering plant species recorded in this study.

Species	Authority	Family	Total floral units (season-long)	Number of sites occupied
Abutilon theophrasti	Medik.	Malvaceae	1	1
Achillea millefolium	L.	Asteraceae	231	2
Anemone canadensis	L.	Ranunculaceae	858	1
Arctium lappa	L.	Asteraceae	57	1
Asclepias syriaca	L.	Apocynaceae	3546	13
Asclepias verticillata	L.	Apocynaceae	1	1
Berteroa incana	(L.) DC.	Brassicaceae	8199	5
Centaurea maculosa	Lam.	Asteraceae	1771	4
Cichorium intybus	L.	Asteraceae	749	1
Cirsium arvense	(L.) Scop.	Asteraceae	867	7
Cirsium vulgare	(Savi) Ten.	Asteraceae	740	4
Convolvulus arvensis	L.	Convolvulaceae	2	1
Cornus foemina	Mill.	Cornaceae	38	1
Daucus carota	L.	Apiaceae	5588	9

Erigeron strigosus	Muhl. Ex Willd.	Asteraceae	7336	8
Eupatorium perfoliatum	L.	Asteraceae	25	1
Euthamia gramnifolia	(L.) Nutt.	Asteraceae	30	2
Gnaphalium uliginosum	L.	Asteraceae	108	1
Heliopsis helianthoides	(L.) Sweet	Asteraceae	265	1
Hieracium sp.		Asteraceae	141	2
Hypericum perforatum	L.	Hypericaceae	364	3
Leucanthemum vulgare	Lam.	Asteraceae	226	1
Linaria vulgaris	Mill.	Plantaginaceae	175	1
Lobelia siphilitica	L.	Campanulaceae	70	1
Lotus corniculatus	L.	Fabaceae	17623	3
Medicago sativa	L.	Fabaceae	37	1
Melilotus albus	Medik.	Fabaceae	6707	4
Melilotus officinalis	(L.) Pall.	Fabaceae	463	3
Myrrhis odorata	(L.) Scop.	Apiaceae	89	3
Origanum vulgare	L.	Lamiaceae	484	1
Oxalis stricta	L.	Oxalidaceae	10	4
Polygonum virginianum	(L.) Gaertn.	Polygonaceae	100	1
Potentilla recta	L.	Rosaceae	29	3
Prunella vulgaris	L.	Lamiaceae	2	1
Ratibida pinnata	(Vent.) Barnhart	Asteraceae	240	1
Rudbeckia hirta	L.	Asteraceae	11	1
Scrophularia nodosa	L.	Scrophulariaceae	17	1
Securigera varia	(L.) Lassen	Fabaceae	5518	1
Silene vulgaris	(Moench) Garcke	Caryophyllaceae	775	6
Solanum carolinense	L.	Solanaceae	1607	2
Solanum dulcamara	L.	Solanaceae	53	1
Solidago canadensis / altissima		Asteraceae	2425	7
Solidago rugosa	Mill.	Asteraceae	17	1
Sonchus oleraceus	L.	Asteraceae	1382	5
Spiraea alba	Du Roi	Rosaceae	4	1
Symphiotrichum novae-angliae	(L.) G.L. Nesom	Asteraceae	225	1
Symphiotrichum pilosum	(Willd.) G.L. Nesom	Asteraceae	6619	4
Taraxacum officinale	(L.) Weber ex. F.H. Wigg	Asteraceae	5	3
Tragopogon sp.		Asteraceae	1	1
Trifolium campestre	Schreb.	Fabaceae	199	2
Trifolium pratense	L.	Fabaceae	1677	3
Trifolium repens	L.	Fabaceae	2	1

Verbascum thapsus	L.	Scrophulariaceae	4	2
Viola sp.		Violaceae	12	1
Unknown Asteraceae sp.		Asteraceae	378	2
Unknown Brassicaceae sp. 1		Brassicaeae	17	2
Unknown Brassicaceae sp. 2		Brassicaceae	189	1

Table S1.3. Flower-visiting insect groups recorded during the study. The rough taxonomic categories used here follow Ward et al. (2014).

Group	Proportion of observations
Apis mellifera L.	0.29
Bombus spp.	0.19
Xylocopa spp.	0.01
Small black bee (<1cm)	0.07
Large black bee (≤1cm)	0.05
Small green bee (<1cm)	0.02
Large green bee (≥1cm)	0.01
Syrphidae	0.28
Other flies	0.06
Wasps	0.01

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