

Comparison of Two Milkweed (*Asclepias*) Sampling Techniques on Eastern Nebraska Grasslands

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Abstract - In recent years, interest in estimating the number of *Asclepias* Linnaeus (Milkweeds) on the landscape has grown, because the plants serve as hosts for the larvae of *Danaus plexippus* Linnaeus (Monarch Butterflies). Monarchs are a species of high conservation concern, whose status for federal listing under the Endangered Species Act is currently warranted but precluded by work on higher-priority listing actions. The widespread loss of milkweed throughout the Monarch's range is one of the primary factors implicated in the butterfly's decline. In 2017 and 2018, we led a study to compare estimates of milkweed abundance and richness from two sampling methods, belt-transects and plot sampling, in four land categories on 48 grasslands in eastern Nebraska. The four site types included: 1) high-diversity local ecotype plantings, 2) privately owned agricultural lands with grassland enhancement, 3) public wildlife management area grassland rehabilitations, and 4) unmanaged pastures (i.e., with no specific conservation management activities or plans). Overall, twice as many milkweed species and 31.80 times as many stems of milkweed were detected by the belt-transect method than by the plot method. More distinct differentiation was indicated by the transect method that was not captured by plot sampling with similar time and effort expended in the field. High-diversity planting sites and wildlife management areas had more milkweed species per hectare than unmanaged pastures and privately owned agricultural lands with grassland enhancement. Variance was much higher for counts obtained via plot sampling relative to counts from transect surveys. The findings from this study suggest that the belt-transect method is more effective than the plot method for predicting milkweed stems per hectare as well as milkweed species richness while expending similar effort in the field. The ability to choose the most useful methods for identifying and quantifying milkweed on a variety of land management types is paramount for implementing a conservation plan for the Monarch butterfly not only in Nebraska but throughout the midwestern United States.

Introduction

Over the past two decades, it is estimated that the eastern population of *Danaus plexippus* Linnaeus (Monarch Butterfly) has declined by as much as 84% (Brower et al. 2012, Semmens et al. 2016, Vidal and Rendón-Salinas 2014). Because Monarch caterpillars forage solely on *Asclepias* Linnaeus (milkweeds) in the Apocynaceae family, the abundance of milkweeds in the Monarch's upper midwestern breeding grounds is thought to be critical for the species' survival (Brower 1984, Pleasants and Oberhauser 2012). Several studies have indicated a strong correlation between the amount of milkweed in breeding grounds in the midwestern United States and overwintering Monarch populations in the central Mexican Highlands (Brower et al. 2012, Flockhart et al. 2014, Pleasants and Oberhauser 2012, Stenoien et al. 2016). Reductions in the abundance of milkweed within the Monarch's breeding range have been attributed to land-use change (Pleasants 2017). Restoring eastern Monarch butterfly populations will require establishing, rehabilitating, and managing

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habitat by using techniques that increase the abundance of milkweed across many land-management types within grassland ecosystems.

In 2016, the Nebraska Monarch and Pollinator Initiative set a goal of establishing 125 million milkweed stems across the state. In order to reach this goal, it is necessary to understand how different land-management types affect the establishment of milkweeds on a variety of grassland landscapes. *Asclepias* is a genus of herbaceous perennial, dicotyledonous plants native throughout a large portion of North America, excluding Alaska and parts of the Pacific Northwest (Borders and Lee-Mäder 2014); at least 72 milkweed species, not including subspecies, are native to the continental United States and Canada (Borders and Lee-Mäder 2014). Some milkweed species occur as scattered stems, others form colonies in dense stands, and some form clumps from a thick woody base. In the midwestern US, milkweeds grow in a variety of habitats including prairies, woodlands, wetlands, road rights-of-way, and croplands (Hartzler and Buhler 2000).

It is estimated that milkweed abundance declined 58% over the midwestern landscape between 1999 and 2010 (Pleasants and Oberhauser 2012). This represents a loss of 861 million milkweed stems, with nearly two million additional milkweeds being lost annually because of continued grassland conversion to other uses (Pleasants 2017). It has been stated that to slow or halt the decline of Monarchs, significant habitat restoration must occur (Nabhan et al. 2015). New populations of milkweed must be established and existing populations enhanced within the grassland regions of the Monarch's eastern breeding grounds. The Mid-America Monarch Conservation Strategy (MAFWA 2018), for example, seeks to add over one billion stems of milkweed in various habitats within the Monarch's midwestern breeding range. In the future, as conservationists, farmers, utility and roadside managers, rural communities, and individuals restore Monarch habitat, there will be a need to document changes in populations of milkweed to determine if conservation objectives are met. Thus, it is necessary to evaluate different methodologies to assess milkweed abundance and species richness.

The two objectives of this study were to compare two methods of estimating the abundance and richness of milkweeds to determine their relative usefulness on sites in eastern Nebraska, and to compare milkweed density across land categories. Understanding how milkweed richness, abundance, and density change over time in response to habitat manipulation is necessary for effective conservation decision-making.

Thogmartin et al. (2017) states the need for accurate estimates of milkweed based on field studies across land cover types. The results from our study can better inform objectives and actions in existing Monarch conservation plans in Nebraska (Panella 2017) and the Midwest (e.g., Mid-America Monarch Conservation Strategy [MAFWA 2018]), as well as guide updates to those plans and development of new conservation strategies.

Methods

The study area included the Monarch's primary breeding range in Nebraska, which corresponds to the tallgrass prairie ecoregion as described in the Nebraska Natural Legacy Project (Schneider et al. 2011). Our field sampling occurred between 26 June to 6 October 2017, and from 29 May to 3 October 2018. We surveyed 48 grassland areas in four site types by using two sampling methods, plots and belt-transects. The site types were distinguished by the land-management practices that had been applied. These included high-diversity, local-ecotype prairie plantings; wildlife management areas; Pheasants Forever restored agricultural properties; and unmanaged pastures.

Site Types

Four different site types were surveyed during the summers (June to August) of the study in a quasi-random order based on their geographic location and to accommodate for efficient surveyor travel time among sites.

High-diversity, Local-Ecotype Plantings. Prairie Plains Resource Institute, Inc. facilitated access to survey high-diversity sites that had been planted previously (2007–2014) with native grasses and forbs, including *Asclepias* species. Seventeen properties were sampled with an average site size of 11.50 ha. Prior to seeding, all of the properties had been cropland. Most of these properties were on private land, and management differed depending on the landowner. However, on most of the properties, land management involved a combination of burning, grazing, or resting, and in some cases tree removal or spraying for noxious weeds. Additionally, these sites were located in varying soils and across a moisture gradient. All properties were planted with a high diversity of prairie plant species and a milkweed mix, which included *A. syriaca* Linnaeus, *A. sullivantii* Engelman ex A. Gray, *A. verticillata* L., and *A. speciosa* Torrey, in addition to *A. incarnata* L. and *A. arenaria* Torr depending on the site specifics. The seed mixes were local ecotypes and consisted of up to 100 different species of native grasses, sedges, legumes, composites, and other forbs.

Pheasants Forever Restored Agricultural Properties. Access to 13 privately owned properties was facilitated by the Nebraska Pheasants Forever conservation program. These properties were restored agricultural land that included corn and soybean field pivot corners as well as more extensive tracts of land bordering agricultural production areas. These sites had been planted between 2013 and 2014 with a seed mix that included two *Asclepias* species, *A. syriaca* with either *A. incarnata* or *A. arenaria*. The average site size that we sampled for these properties was 5.45 ha.

Unmanaged Pastures. Eleven unmanaged grassland pastures were included to provide information regarding the “standard condition” of grazed, native pastures in Nebraska’s tallgrass prairie ecoregion. These properties include land that may have supported crops previously and currently have no specific conservation management activities or plans. Data were collected from Common Land Units from the US Department of Agriculture’s Farm Service Agency database (USDA FSA 2021). “Pasture” land type was randomly selected, and then aerial imagery was used to select sites that were currently pasture. The average site size of unmanaged pastures was 10.11 ha.

Wildlife Management Areas. Seven wildlife management areas were surveyed. These state-owned properties are rehabilitated grasslands managed by the Nebraska Game and Parks Commission. The properties selected were chosen because they had been managed for wildlife habitat by using prescribed burning, noxious weed control, and grazing. The average size of wildlife management areas was 59.6 ha.

Sampling Methods

Two sampling methods (i.e., belt transect and plot) were implemented on each site for comparison of their effectiveness in detecting milkweeds. Both sampling methods occurred in each field on the same day so as to avoid variations resulting from sampling date as new stems continue to emerge and become more visible. Belt-transect sampling was conducted first, followed by the plot method. Prior to field sampling, we used aerial photographs and ArcMap 10.5 (Esri, Redlands, California, USA) to randomly place 10 or more evenly spaced transects across a site; transects were aligned in a north-to-south or east-to-west orientation and at least 10 m (32.8 ft) apart. The length of each transect varied from 100 m (0.06 mi) to a maximum of 2,000 m (1.2 mi) because of the irregular shapes of some

sites. Transects were started at least 5 m (16.4 ft) from the property border to minimize edge effects that may be caused by woodlands, roads, or other edge habitats frequently associated with grassland boundaries. To avoid sampling in non-target habitats (e.g., woodlands, wetlands), it was sometimes necessary to adjust transect length and layout. Following methods described in Oberhauser (2001) and using stems as a metric that represents habitat restoration (MAFWA 2018), we counted milkweeds by their ramet (that is, an individual stem of a clone). The two techniques we assessed were designed to require similar field effort to directly compare the utility of the methods.

Belt-Transect Method. Transect locations for this sampling method were generated in ArcMap 10.5. In order to sample 12–20% of the total accessible area, different widths of transects were implemented depending on the site size. Site boundaries were delineated according to land ownership because of access limitations. For larger areas, transects were wider to maintain consistent percent coverage. The width of the transects ranged from 1 to 4 m (3.28 to 13.12 ft). There were at least 10 transects per site with each transect being at least 10 m (32.8 ft) apart. On larger sites (>40 ha), we sampled more than 10 transects. A surveyor walked with a length of PVC pipe of the corresponding measurement in order to visually demarcate the width of each transect. The surveyor walked alertly between the start and end points of each transect, while using GPS points as a guide. We recorded the total number of milkweed species and the number of stems for each type.

Plot Method. For the plot method, we evenly placed points every 10 m (32.8 ft) along the same transects that we had generated with ArcMap 10.5. We then used a random number table to determine 100 of the plots to include in our sampling. For example, if the number five was randomly generated, then we would sample the fifth point as the center of a plot and continue in this manner. On site, we placed a 1-m² quadrat of PVC pipe on the ground to delineate the borders of our plot. Therefore, the area surveyed was 100 m² (1,076 ft²) per site. The total number of milkweed stems was counted within each plot, and stems were differentiated by species.

Statistical Analysis

For statistical analysis, we conducted negative binomial regression by using R Foundation for Statistical Computing (R Core Team 2018) to model the over-dispersed milkweed stem count data obtained by the plot sampling method and the belt-transect sampling method in package MASS (Modern Applied Statistics with S; Venables and Ripley 2002). The response variable was the number of stems per hectare for each site. This was calculated for each site by counting all stems in the belt-transect and dividing by the area covered by the belt transect. For the plot method, it was calculated by counting all stems in the plots and dividing by the area covered by all plots at the site. The observational unit is the site for both methods, which is necessary to avoid pseudo-replication when assessing differences in site type. Land-management type was included in the models as a predictor. To test for differences in stem density among land-management types for each sampling method, we used a likelihood ratio test to compare the full model to a null model without the land-management type predictor. Post-hoc multiple comparisons among land-management types were then calculated with a Tukey's test and family-wise error controlled by applying a Bonferroni correction. We then evaluated each regression function (the regression function for stem counts obtained via plot sampling and that for counts obtained via belt-transect sampling) to obtain predicted stem counts per hectare for each land-management type, using parameter estimates from the models.

In addition, we tested for differences in the modeled stem counts obtained through plot sampling versus those obtained through belt-transect sampling by combining the data from both methods and using a negative binomial regression with both site type and sampling type as predictors. We used a likelihood ratio test to compare this model to a reduced model with only land-management type as a predictor to determine if modeled counts differed between the two sampling methods. We also compared the milkweed richness detected by plot and transect methods with a Poisson model; statistically, the richness data were not over-dispersed. Richness (the number of milkweed species) was the response variable, and sampling method was the predictor.

Results

Milkweed Summary

We surveyed a total of 48 properties and encountered over 25,000 milkweed stems across the study. Species were comprised of *Asclepias syriaca* Linnaeus (Common Milkweed), *A. sullivantii* Engelman ex A. Gray (Sullivant’s Milkweed), *A. verticillata* Linnaeus (Whorled Milkweed), *A. incarnata* Linnaeus (Swamp Milkweed), *A. speciosa* Torrey (Showy Milkweed), *A. viridis* Walter (Green antelopehorn), *A. viridiflora* Raf. (Green Comet Milkweed), and *A. tuberosa* Linnaeus (Butterfly Weed) (Table 1). *A. syriaca* was the milkweed species most often encountered, and *A. tuberosa* was least often encountered.

Transect Sampling Method

The negative binomial model was a good fit for milkweed stems per hectare obtained by the belt-transect method. The expected 95% χ^2 deviance on 44 degrees of freedom was 53.4, and the model residual deviance was 56.3. The likelihood ratio test for the belt-transect sampled model indicated that there was a difference among site types ($\chi^2 = 56.3, P < 0.001$). Multiple comparisons showed that high-density planting sites had more milkweed stems per hectare than wildlife management areas and the Pheasants Forever sites. We found that unmanaged pastures had fewer milkweed stems per hectare than all other site types when we used the transect method (Fig. 1, Table 2, Predicted counts Table 3). Predicted counts of milkweed stems per hectare averaged across all site types was 324. Predicted richness was 2.08 milkweed species.

Plot Sampling Method

The negative binomial model was a good fit for milkweed stems per hectare obtained by the plot method. The expected 95% χ^2 deviance on 44 degrees of freedom was 53.4 and the model

Table 1. Species of milkweed detected on four different land management types by using two survey methods in the eastern tallgrass prairie ecoregion of Nebraska. AsSy = *Asclepias syriaca*, AsSu = *A. sullivantii*, AsVe = *A. verticillata*, AsIn = *A. incarnata*, AsSp = *A. speciosa*, AsViri = *A. viridis*, AsVir = *A. viridiflora*, AsTu = *A. tuberosa*

Land Management Type	Milkweed Species Detected via Plot Method	Milkweed Species Detected via Belt-transect Method
High-diversity plantings	AsSy, AsSu, AsVe, AsIn	AsSy, AsSu, AsVe, AsIn, AsSp, AsViri
Pheasants Forever sites	AsSy, AsVe	AsSy, AsSu, AsVe, AsTu
Unmanaged pastures	AsSy	AsSy, AsSu, AsVe, AsIn, AsViri
Wildlife Management Areas	AsSy, AsSu, AsVe, AsIn	AsSy, AsSu, AsVe, AsIn, AsVir, AsTu

residual deviances was 55.3. The likelihood ratio test for the plot-sampled model indicated that there was a difference among site types ($\chi^2 = 56.3, P < 0.001$), and multiple comparisons revealed that high-density planting sites and wildlife management areas had more milkweed stems per hectare than did unmanaged pastures and the Pheasants Forever sites during our study (Fig. 2, Table 2, Predicted counts Table 3). Predicted counts of milkweed stems per hectare, averaged across all site types, was 1675. Predicted richness was 1.08 milkweed species.

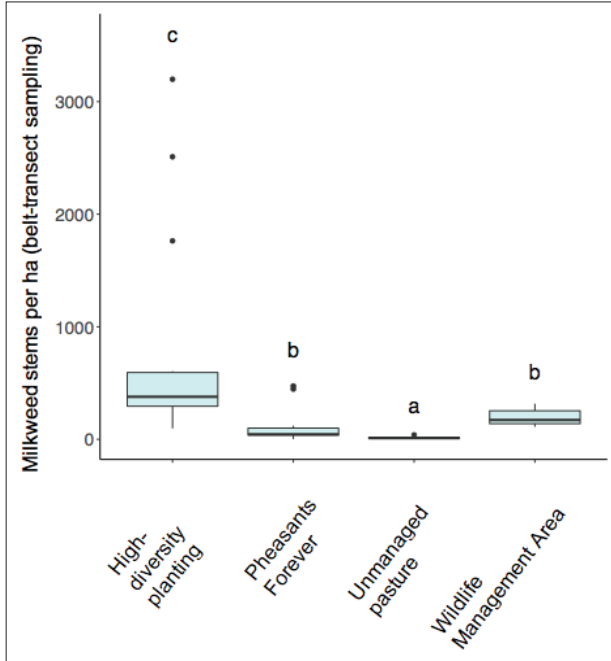


Figure 1. Milkweed stems per hectare estimated using a belt-transect sampling method comparing densities across four land-management types. Different letters denote significant differences among sites.

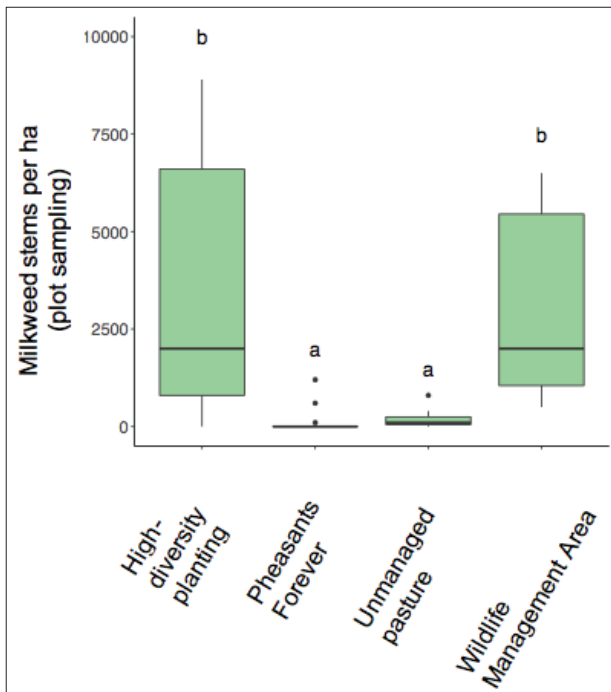


Figure 2. Stems of milkweed per hectare estimated using a plot sampling method comparing densities across four land-management types. Different letters denote significant differences among sites.

Sampling Method Comparison

We found differences between the two survey methods that we employed in their ability to detect milkweeds. Across the 48 sites, we detected a sum of 804 milkweed stems when using the plot method, whereas we detected a total well over 25,000 stems when using the transect method. The count when using the transect method would of course have included some of the same plants detected when using the plot method on the same sites on the same day. While both plot and belt-transect models showed differences in milkweed stem density across site types, the plot method appeared to be less effective at detecting differences among sites than the belt-transect method. The negative binomial model was a good fit for the combined plot and transect data, with sampling type as predictor for stems per hectare.

Table 2. Bonferroni corrected multiple comparisons for plot stem counts and belt-transect stem counts of species of *Asclepias*. PF= Pheasants Forever, HDP = high-diversity plantings, UP = unmanaged pastures, and WMA = Wildlife Management Areas. The 0 in PF-HDP=0 indicates that our regression was testing the null hypothesis that the number of stems in the PF sites minus the number of stems in the HDP sites equals 0. The estimates are differences in log odds of expected counts among the site types compared, reported here to two decimal places.

Linear Hypothesis	Estimate	Standard Deviation	Z-score	P-value
Plots				
PF-HDP=0	-3.08	0.75	-4.12	<0.001
UP-HDP=0	-2.77	0.79	-3.52	0.003
WMA-HDP=0	-0.02	0.91	-0.02	1.0
UP-PF=0	0.31	0.83	0.38	1.0
WMA-PF=0	3.07	0.95	3.22	0.008
WMA-UP=0	2.75	0.98	2.80	0.30
Belt-transects				
PF-HDP=0	-1.86	0.33	-5.64	<0.001
UP-HDP=0	-3.92	0.35	-11.00	<0.001
WMA-HDP=0	-1.31	0.40	-3.27	0.006
UP-PF=0	-2.06	0.37	-5.49	<0.001
WMA-PF=0	0.54	0.42	1.30	1.0
WMA-UP=0	2.60	0.44	5.93	<0.001

Table 3. Predictions of milkweed densities (stems per hectare) from two sampling methods (i.e., plot and transect) for four different site types.

Land Management Type	Predictions for Plot Models (milkweed stems per ha)	Predictions for Belt-transect Models (milkweed stems per ha)
High-diversity plantings	3,194	736
Pheasants Forever sites	146	115
Unmanaged pastures	200	15
Wildlife management areas	3,143	198

The expected 95% χ^2 deviance on 91 degrees of freedom is 114.3, and the model residual deviance was 118.0. The likelihood ratio test to compare models with and without sampling type included as a predictor shows that milkweed stems per hectare differed by sampling type ($\chi^2 = 18.43$, $P < 0.001$). The differences in log expected counts between transects and plots was -1.6 ± 0.31 ($Z = -5.1$, $P < 0.001$). The numbers of predicted stems per hectare were higher for plots than transects for all site types (Table 3).

The variance was much higher for stem counts obtained via plot sampling relative to counts obtained via transects (Fig. 3). In fact, the standard deviation for the differences in log odds of expected stem counts for plots was almost double that for transects (Table 2). Milkweed richness was lower for plots than transects. The difference in log odds of milkweed richness between transects and plots was 0.65 ± 0.17 ($Z = 3.83$, $P < 0.001$).

We did find similarities in the amount of survey effort expended using the belt-transect method and the plot method. Each technique required a comparable amount of time and energy to walk the site. On average, a site that was 40–60 ha would take about three hours to survey 12–20% of a study area via transect or to sample 100 random plots.

Discussion

Detecting and sampling *Asclepias* species will likely remain an important objective to describe host plant availability in the Monarch's breeding habitat. Conservation programs aim for a substantial increase in the number of milkweed stems (e.g., MAFWA 2018), while grassland conversion continues to reduce available habitat (Thogmartin et al. 2017). The resulting fluctuations in milkweed abundance necessitates periodic monitoring on lands under diverse ownership and management to maintain a basic understanding of overall milkweed availability and to make relevant recommendations for Monarch conservation actions.

As expected, we found differences in milkweed density and richness at sites according to their management types. The high-diversity, local-ecotype plantings had the most species of *Asclepias*, indicating survival of seeded milkweed and success for meeting Monarch management objectives. The privately owned agricultural lands with grassland enhancement (i.e., Nebraska Pheasants Forever land management) and the public wildlife management area grassland rehabilitations (i.e., Nebraska Game and Parks Commission land management) fared similarly in terms of species of milkweed per ha as indicated under belt-transect

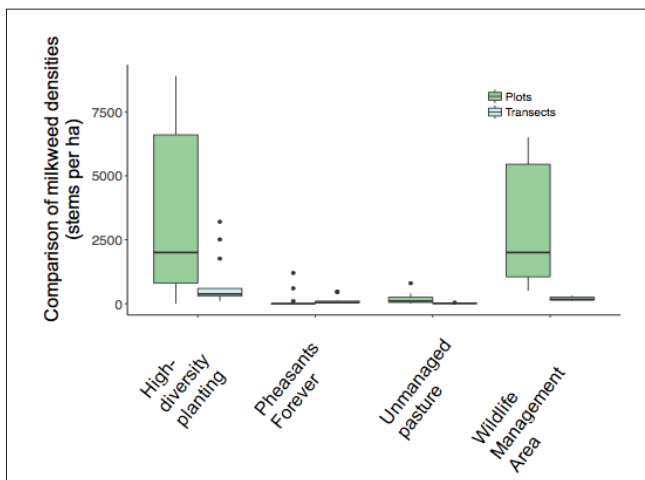


Figure 3. Comparison of milkweed densities (milkweed stems per hectare) by site type using the plot method and the belt-transect method.

sampling. Richness was higher in all seeded site types, and milkweed species that were not part of the original seeding mixtures were even occasionally detected at the previously planted sites. Unsurprisingly, pastures that were not managed specifically for milkweed, or for pollinator resources in general, had lower diversity and abundance of milkweeds.

Our results suggest that by choosing a diverse seed mix of native plants with a milkweed component and undertaking conservation-based management practices, a land manager increases the chances of providing Monarch breeding habitat. Similarly, Zaya et al. (2017) reported that agricultural areas that were not managed for pollinators had fewer milkweeds than natural areas (e.g., grasslands, wetlands, and forests) in Illinois. They also found that sites planted with low-diversity seed mixes or those frequently mowed or sprayed with herbicides, as well as those that are overgrazed, are poor Monarch habitat. They promoted management practices that support plant diversity at various successional states to improve habitat for *A. syriaca*. In Iowa, Kaul and Wilsey (2019) found higher densities of milkweed species in remnant native prairies that had higher forb diversity. In our study area, pastures that were not managed for pollinator benefits had fewer milkweeds, and thus cannot be expected to support much Monarch breeding without some degree of management. This finding is likely associated with grazing regimes and other land management practices that may reduce native plant diversity, and results could differ in other locations as these variables change.

Monitoring efforts in the Midwest to count milkweeds and assess Monarch breeding habitat have employed a variety of methods, highlighting the myriad options available to managers. For example, the Integrated Monarch Monitoring Protocol (IMMP) and Monarch Larva Monitoring Project (MLMP) (Monarch Joint Venture 2021a, 2021b) include well-known community science programs. Lukens et al. (2020) used the IMMP in Minnesota, Wisconsin, and Iowa. Kasten et al. (2016) used a modified interrupted belt-transect sampling method where they randomly generated starting points for milkweed roadside surveys within a 402.34 m (250 mi) radius of Minneapolis, Minnesota. Kaul and Wilsey (2019) chose a randomly located 3.14-m² round plot method in Iowa. Thogmartin et al. (2017) used spatial information from the 2014 Cropland Data Layer (CDL; USDA NASS 2021) and other sources in order to develop a land-cover map that was used to estimate milkweed density in all or portions of several mid-western states. The US Geological Survey Upper Midwest Environmental Sciences Center (UMESC 2021) considers multiple methods to meet the challenges of Monarch conservation.

With limited resources, conservation program managers want to select, and surveyors want to use, the most appropriate and effective method for their circumstances. Individuals charged with the task of milkweed monitoring must take into consideration the pros and cons of their survey choices. Land managers voice common concerns regarding the amount of time, training, human resources, and funding required to conduct milkweed counts on a variety of land management types. They also express challenges related to working during the peak of summer (e.g., hotter temperatures, higher ultraviolet radiation exposure), while maximizing outcomes to get accurate measurements during periods of peak milkweed blooming to aid in its detection and identification. Assessing the different methods available as a pilot to the development of a monitoring approach can help maximize efficiency in the face of these concerns.

We assessed only two methods on four different types of properties in the tallgrass prairies of eastern Nebraska, and it was clear that the methods did not perform equally. Our results indicated that the belt-transect technique allowed us to survey more area and exhibited lower variation; therefore, it seems more useful in describing species abundance

and richness at our sites than the plot sampling method. We were also able to implement the belt-transect method with a similar amount of time and effort in the field, compared to the plot method. Thus, it was not costlier or more difficult to implement while at the site, although a bit more time was needed in making planning preparations to visit a particular site. A biologist needed to review aerial imagery of the survey sites to first find the total area of a site, and then digitally plan placement of the transect coordinates in order to effectively survey at least 12% of a total site. As this was part of the biologist's other concurrent responsibilities, we did not track the time or cost per hour associated with this milkweed survey planning activity as it was being completed. This would make for an interesting analysis for future work to further compare the relative benefits of the two types of survey methods.

Perhaps, our findings also reflect the biological characteristics of *Asclepias* species. Milkweed seeds are often dispersed by wind, but several milkweed species can reproduce asexually by vegetative cloning and spreading rhizomes. For example, Common Milkweed and Showy Milkweed are rhizomatous species (Luna and Dumroese 2013) that allow for spreading growth in a "clumpy" (i.e., patchy) manner. Likewise, Kasten et al. (2016) noted the patchy distribution of milkweeds across the landscape during their multi-state roadside surveys. This tendency for clumping growth habit is not dependent on seed dispersal by wind and could help explain why the belt-transect method was more effective than the plot method at detection of milkweeds at our sites. Therefore, managers should also consider the biology of the milkweed species likely to be found in their region in selecting monitoring methods.

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