# Expanding the value of the Conservation Reserve Program for monarch butterfly recovery in the upper Midwest

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## Technical Report

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## Executive Summary

The objective of this project is to improve monarch butterfly habitat in the upper Midwest through the CRP program. Establishing field experiments and assessing vegetation outcomes is a long process, and variability in farms, contract requirements and site conditions presents a challenge. We take a two-pronged approach, assessing approved practices on re-enrolling contracts where monarch habitat enhancement is practicable, and assessing methods to improve monarch habitat in newly established contracts. Studies are conducted on both existing farms and in controlled experiments. This report covers data from four growing seasons, representing efforts from Oct 2020- Dec 2024. Result summaries follow for key project tasks:

*Task 1: Estimate baseline monarch habitat value of common CRP practices*

CP25 Rare and Declining Habitat is an extremely common conservation practice in the Upper Midwest well suited for monarch habitat enhancement. We surveyed seventeen sites across eastern Iowa that were at least 10 years old and planning to re-enroll.

- Milkweed abundance in CP-25 fields is on par with other conservation grasslands in the Corn Belt (less than Iowa roadsides managed with Integrated Roadside Vegetation Management principles, but greater than many government/non-profit managed conservation grasslands).
- Nectar plants comprised less than 45% of plant cover, most of which were likely there only due to ambient seed dispersal from other landscape sources.
- Expiring CP-25 fields provide good monarch habitat and further enhancement of CP-25 fields may prove useful given the current dominance of grass in these sites.

*Task 2: Assess approved methods for enhancing CRP a) on existing farms, and b) experimentally.*

- In an on-farm study, we surveyed 4 re-enrolled CRP plantings in eastern Iowa and found that all enhancement methods farmers employed (paired with re-seeding), opened the canopy and allowed at least some seedlings to emerge- sometimes at very high rates. Successful establishment of seedings varied greatly among sites, and approximately two thirds of sites retained or increased abundance of species found as seedlings.
- We expected enhancement at re-enrollment would target low quality sites, and that entire sites would be enhanced in our on-farm study. Contrary to our expectations, end-of-contract habitat quality was not closely related to the requirement to enhance, and enhancement often occurred only on a small portion of the expiring planting.
- In our field experiment that evaluated the effectiveness of a mid-contract management option, we found that effects of grass-selective herbicide application on plant communities are temporary. Treated plots produced more pollinator resources the first year after treatment, but were indistinguishable from controls after two years. Like with other land management methods, herbicide application may need to be carried out repeatedly to have lasting effects.

*Task 3: Improving the long-term performance of new CRP enrollments for monarch habitat on a) existing farms and b) experimentally*

- In an on-farm study, we confirmed previous observations that dormant season planting improves monarch habitat establishment in new CRP prairie strips. Seed mixes with high graminoid diversity produced more diverse stands for nearly the same cost as a low graminoid diversity mix, though we did not find other differences in monarch habitat between mixes. Prairie strips targeting monarch habitat provision should be seeded in the dormant season using seed mixes with diverse forb and graminoid components.
- We evaluated the effectiveness of monarch habitat enhancement methods in cool-season grasslands using a field experiment, and found that intensive site-prep with seeding (grass stands with herbicide applied at least 2x prior to seeding) was essential for establishment success. Plots sprayed twice were fifty times more cost effective than those that were seeded without site prep. Twice sprayed plots had much higher native species abundance richness, and cover of high value monarch species than 1x sprayed or no-herbicide plots.

***Task 1: Estimate baseline monarch habitat value of common CRP practices.***

***Monarch habitat quality in expiring CP-25 fields***

*Background*

The eastern migratory population of the monarch butterfly has been in decline since the mid-2000s, in part due to habitat loss in the upper Midwest, its primary breeding grounds and central migratory path. It is currently under consideration for listing under the Endangered Species Act. CRP has the potential to play a critical role in avoiding a Threatened designation and in key states would be responsible for over 80% of all milkweeds in the agriculture sector (Iowa Monarch Conservation Consortium, 2018). Monarch habitat quality in CRP fields is not well studied. A more systematic approach to studying monarch habitat in CRP is needed to help USDA conservation planners and policy makers understand which practices and approaches to habitat establishment are most effective.

One particularly promising strategy for improving existing conservation lands in the Corn Belt is to enhance the many expiring low-quality CRP acres using interventions (usually reseeding) intended to increase monarch habitat quality through the re-enrollment process. Depending on NRCS staff assessment of field vegetation quality and other administrative considerations, landowners are often required to carry out some kind of enhancement process on their existing fields to qualify for re-enrollment. There is a need to understand the contribution of enhancing existing CRP acres, both to estimate the actual and potential contribution of the strategy to increase the amount and quality of monarch habitat.

Our objective was to characterize monarch habitat provision in typical CRP plantings likely to contribute to future monarch habitat enhancement. These fields were enrolled in the most common practice in Iowa (CP-25) and had contracts expiring in 2021 and were re-enrolling in the CRP program.

*Methods*

*Site Selection*

To identify landowners meeting the study's requirements for baseline conditions in retiring CRP fields, we collaborated with FSA and NRCS staff in several key eastern Iowa counties and sent email and/or letters to several hundred CRP landowners in ten counties within a 90-minute driving radius of Cedar Falls. Of the initial contacts, we identified 18 CP-25 CRP contracts expiring in 2021, as well as 10 non-expiring contracts over 10 years old. We sought landowners intending to re-enroll in the program if allowed. After final site selection we chose 17 expiring CRP fields to survey, located in a 70-mile radius of Cedar Falls, Iowa. All sites were enrolled in the CP-25 practice for 10-15 years. Site size ranged from 0.85 to 38.89 ha with mean of 13.11 ha.

*Data collection and analysis*

We measured plant composition in 100 1 m² quadrats (0.5 m x 2.0 m) placed 5 m apart along a series of parallel transects at each site. The number and length of transects varied depending on site dimensions and size, and start positions obtained were randomly generated using ArcPro (ESRI, 2020). We oriented odd numbered transects by randomly selecting an absolute bearing (0-359°). Even numbered transects had opposite orientation to the previous transect (x + 180°) to provide more complete coverage of sites. We used Garmin Oregon 750t GPS units to navigate to transect start points.

To sample plant composition in each quadrat, we used a modified nested quadrat sampling method described in the Integrated Monarch Monitoring Program (Cariveau et al., 2019). In this method, observers record plant identity and presence of flowering species in a series of nested quadrats (0.25, 0.5, and 1 $m^2$), and record the identity and number of milkweed stems (*Asclepias spp.).* To capture a more complete picture of plant composition at sites, we measured presence of all species rather than flowering forbs. We calculated frequency and species richness metrics using the 1 $m^2$ quadrat measurements. We additionally measured canopy cover of all species in the third nested quadrat (sized 1 x 0.5 $m^2$) at every other full quadrat (50 total quadrats/ site). We used Daubenmire cover classes to assess cover. We used cover class midpoints to estimate total quadrat and individual species cover, then standardized to 100% quadrat cover to assess relative cover.

To characterize vegetation in each site, we also calculated frequency, species richness, and relative cover for a set of important monarch habitat vegetation classes. We summarized sites based on how many species typically comprised each monarch habitat value category, category frequency, and relative cover of each category. We assessed general vegetation type and forb (nectar plant) phenological types.

We categorized general vegetation types based on typical land management objectives of conservation (i.e. prioritizing native perennial plants of high conservation value). We defined the following classifications within this group: 1) native perennial forbs, 2) native perennial grasses, 3) native annual/biennial forbs 4) ruderal weeds (annual or biennial species of any origin with a coefficient of conservatism (CoC) < 1), 5) ruderal native perennials (unsown perennial native species with CoC < 1), 6) perennial weeds (introduced perennial species), 7) woody plants (tree and shrub species of any origin), and 8) other native species (unsown native species with CoC > 1).

To assess monarch nectar plant composition, we categorized species based on potential to provide nectar for monarchs and its season of flowering. We categorized broadleaf plants (forbs, vines, and shrubs, excluding trees) as potential nectar plants, and graminoids and cryptograms (non-seed plants) as non-nectar plants. We further categorized nectar plants based on their blooming phenology (spring- Apr-Jun, summer- Jul-Aug, fall- Sep-Nov) and whether they were commonly found in CP-25 seed mixes.

*Results*

*Milkweed Stems*

Across expiring CP-25 sites, we found relatively high milkweed abundance. When considering all milkweed species combined (*Asclepias spp*.), we found 2497 stems/ ha (SE, 592 stems/ ha). Common milkweed (*Asclepias syriaca*) was the most abundant monarch host plant, with mean density of 1979 stems/ ha (SE, 475 stems/ ha), followed by whorled milkweed (*Asclepias* *verticillata*) which had mean density of 518 stems/ ha (SE, 430 stems/ ha). We found no individuals of other milkweeds such as butterfly milkweed (*Asclepias tuberosa)* or swamp milkweed (*Asclepias incarnata*).

*General Vegetation Characteristics*

Overall, the fields we surveyed were relatively diverse. Mean species richness among sites was 40.1 species (SE, 2.4 species), of which a majority were native species (29.5 species (SE, 2.2 species), and a minority were introduced species (10.6 species (SE, 0.5 species).

On average, expiring CP-25 fields were not strongly dominated by a single vegetation type (Fig. 1.1.1, Appendix 1). Perennial weeds were the most dominant class (primarily smooth brome (*Bromus inermis)* and Kentucky bluegrass (*Poa pratensis*) but comprised of 6.6 species (SE, 0.4 species) on average) with relative cover approaching 35% (SE, 4.6%). Native perennial graminoids were the next most common class (primarily big bluestem (*Andropogon gerardii)* but comprised of 3.8 species (SE, 0.4 species) on average) with relative cover of 24% (SE, 4.4%). Ruderal perennial forbs (primarily canada goldenrod (*Solidago canadensis)* but comprised of 5.0 species (SE, 0.3 species) on average) were the most common class of forbs, with relative cover approaching 20% (SE, 3.3%). Native perennial forbs were also a significant vegetation class, with relative cover near 15% (SE, 2.2%). We found the greatest species diversity (12.3 species (SE, 1.6 species) in this class, with wild bergamot (*Monarda fistulosa*), gray-headed coneflower (*Ratibida pinnata*), and giant goldenrod (*Solidago gigantea*) the most important species. The remaining classes of vegetation (ruderal weeds, native annual/biennial forbs, woody plants, and other native plants) were relatively unimportant by comparison, and made up less than 8% of the overall cover of sites.

*Nectar Plants*

We found that expiring CP-25 fields were primarily comprised of non-nectar plants, though there was strong representation of fall and summer nectar plants (Fig. 1.1.2, Appendix 1). Grass dominated the sites we surveyed, with relative cover of non-nectar plants near 60% (SE, 3.9%). Non-nectar plants were comprised of 9.9 species (SE, .5 species) on average. Among the classes of nectar plants present, fall nectar plants were most abundant with relative cover of 22% (0.8% (SE, 0.03%) commonly seeded, 21.2% (SE, 3.3%) not commonly seeded). While fall nectar plants were abundant, very few were likely planted intentionally- stiff goldenrod *(Solidago rigida*) was the most common of these. By far the most common fall nectar plant was *S. canadensis*, a ruderal species not in commercial seed trade. On average, we found 4.9 uncommonly seeded (SE, 0.7 species) and 1 commonly seeded (SE, 0.1 species) fall nectar plant species. Summer nectar plant species and nectar plants were also somewhat common, with relative cover of around 18%. Commonly seeded and uncommonly seeded species were about equally abundant (9.1% (SE, 1.1%) commonly seeded, 9.3% (SE, 1.5%) not commonly seeded). Wild bergamot (*M. fistulosa*) and gray-headed coneflower (*R. pinnata*) were the most abundant commonly seeded summer nectar plants. Wild parsnip (*Pastinaca sativa*) and wild carrot (*Daucus carota*), both typically considered invasive species, were the most abundant uncommonly seeded summer nectar plants we found. Summer nectar plants were the most diverse, and we found 5.6 commonly seeded (SE, 0.4 species) and 14.9 uncommonly seeded (SE, 1.2 species) summer nectar plant species. We found spring nectar plants at extremely low abundance, with about 2% relative cover (0.7% (SE, 0.05%) commonly seeded, 1.5% (SE, 0.03%) not commonly seeded). Golden alexander (*Zizia aurea*) was the only commonly seeded spring nectar plant, and the most abundant uncommonly seeded species was dandelion (*Taraxacum officinale*). We found few spring nectar plants; there were only 0.4 commonly seeded (SE, 0.1 species) and 3.5 uncommonly seeded (SE, 0.5 species) fall nectar plant species on average.

*Discussion*

In our study, we characterized monarch habitat provision in typical CRP plantings likely to contribute to future monarch habitat enhancement. We found that many expiring CRP plantings already provide high quality monarch habitat. We found relatively high milkweed abundance among sites and nectar plants were well represented. Still, most of the vegetation comprising CP-25 sites is grass and does not produce nectar, suggesting that enhancement with forbs and milkweeds would produce substantial gains in monarch habitat quality over a relatively large area.

Our estimates of milkweed stem density in expiring CP-25 fields were within the range, but on the higher end, of typical land uses of the Corn Belt. Consequential land use types for monarch habitat in the Cornbelt include herbicide tolerant cropland, roadsides (including those seeded with native vegetation), CRP and other conservation grasslands, and remnant tallgrass prairies. Herbicide tolerant cropland density of common milkweed is estimated to support 0.1 to 9 stems/ha (Hartzler and Buhler, 2000; Schutte et al., 2010). Estimates of unseeded roadsides range from 110-141 stems/ha (Hartzler and Buhler, 2000; Kasten et al., 2016). Unmanaged pastures are estimated to support 200 stems/ha (Manzanares et al., 2022). Kaul and Wilsey (2019) found 3260 stems/ha in IA roadsides planted with native vegetation. Lukens and others (2020) found that conservation grasslands in the Upper Midwest generally hosted 1864 stems/ha. In similar grasslands, (Iowa Department of Natural Resources conservation plantings), Kaul (2019) found 921 stems/ha. In eastern Nebraska, Manzanares and others (2022) found ~3100 stems/ha in high-diversity prairie plantings and wildlife management areas. Though incredibly rare in the Cornbelt, remnant tallgrass prairies harbor by far the most milkweed stems (9522 stems/ ha) (Kaul and Wilsey, 2019). Our estimate of 2497 stems/ ha for CP-25 fields suggests the value of these fields for monarch habitat is better than many conservation grasslands in the Midwest, but not as high as native roadside vegetation in Iowa.

Expiring CP-25 CRP fields in eastern Iowa harbor considerably more milkweed stems than previous estimates of CRP fields. To our knowledge, the only attempt to characterize milkweed stem density in CRP lands specifically were in a modeling paper by Pleasants (2017) and a field survey by Hartzler and Buhler (2000). Pleasants used data from Hartzler and Buhler (2000) collected in 1999 to estimate milkweed stem density in CRP fields at 430 stems/ha. While neither Hartzler nor Pleasants attempted to differentiate the CRP practices being assessed, it is likely based on the overall makeup of practice acres around the time of the surveys that the practices observed would have been CP1 or CP2 grass dominated stands. CP-25 had only been authorized in Iowa two years before Hartzler conducted his surveys (Lange, pers comm). Our estimates suggest that recent CP-25 plantings provide nearly five times more monarch habitat than the types of CRP assessed in 1999.

Though CP-25 fields provide many milkweed stems, they provide considerably fewer than Pollinator Habitat (CP-42) plantings. Based on 46 recently established (age 3) CP-42 fields, Jackson and Meissen (in prep.) found that milkweed density averaged 4003 stems/ ha in pollinator plantings, 60% more than in CP-25 fields in the present study. While overall milkweed stems were higher in CP-42 plantings, common milkweed density was very similar between studies with 1957 stems/ ha in CP-42 and 1979 stems/ha in CP-25. The large difference between studies was due to an abundance of butterfly milkweed in CP-42 fields. Jackson and Meissen found 1791 stems/ ha of butterfly milkweed in CP-42 plantings, yet we found no butterfly milkweed in any of the CP-25 sites.

Seed mixes for monarch enhancement may not need to specify much common milkweed or whorled milkweed. While we do not have seed mix data for the sites we surveyed, milkweed was typically not specified in Iowa CRP seed mixes when our sites were seeded (Lange pers. comm). Thus, *A. syriaca* and *A. verticillata* appear to be "at-large" in eastern Iowa, and able to colonize many CRP fields at high density, even without seeding (Lukens et al., 2020). However, not all sites harbored "at-large" milkweeds, so relying purely on existing landscape seed sources for monarch habitat enhancement may not always result in success. Rather, conservation planners should confirm there are several sources of milkweed adjacent to enhancement areas before leaving common milkweed or whorled milkweed out of a seed mix. Alternatively, specifying very small amounts of common milkweed in seed mixes may be a reasonable trade off to both reallocate resources for other seed, and ensure some chance of establishing at least small milkweed populations during enhancement seeding.

When considering monarch habitat enhancement using seed addition, seed mixes should ensure focus on fall and spring nectar plants and milkweed species that do not typically colonize sites on their own. We found that most CP-25 sites had significant fall nectar plant abundance, but very little likely came from the seed mix. Spring forbs were practically absent. Seed mixes used to enhance monarch habitat should use higher rates of spring and fall nectar plant seeds to ensure they establish, especially in sites without much likelihood of passive nectar plant colonization. Butterfly milkweed and swamp milkweed were absent from CP-25 fields but are known to establish readily when seeded (Lukens et al., 2020, Jackson and Meissen in prep). While not necessarily as readily preferred by monarchs as host plants, butterfly milkweed is still used by monarchs if available (Pocius et al., 2022). Given their wide native range throughout much of the Midwest, we recommend butterfly milkweed and swamp milkweed form a core to all monarch enhancement seed mixes in most areas of the Corn Belt, with additions of common milkweed and whorled milkweed where sites are unlikely to receive milkweed from adjacent populations.

Further enhancement of CP-25 fields may prove useful given the current dominance of grass in these sites. Typical fields in our study were mostly composed (60% relative cover) of non-nectar plants. However, enhancement methods should be properly targeted to take existing site quality into account. Tillage, non-selective herbicide, or other destructive untargeted methods are not warranted in fields with well-developed milkweed and nectar plant populations. Interseeding and well-timed applications of grass-selective herbicides may provide an opportunity to increase floral resources without damaging existing milkweeds or nectar plants (see Task 2b, Results and Discussion). Ultimately, field checks done at the right time by well-trained staff are needed to help USDA conservation planners find and keep existing high quality monarch habitat while identifying opportunities where wholesale revegetation may be needed. While we did not directly measure the value of CP-1 (Establishment of Introduced Grasses and Legumes) or CP-2

(Permanent Native Grasses) practices, our survey of the literature suggests these fields have limited monarch value and probably represent a significant target for monarch habitat enhancement. However, these CP1/CP2 fields may be more challenging to enhance (see Task 3b, Results and Discussion), and enhancement plans may need to factor in significant time and resources for site preparation.

## *Task 2a: Assess approved methods for enhancing CRP on existing farms*

### *Enhancing existing CRP fields: Re-enrollment on-farm study*

*The following is an adaptation of a master's thesis submitted by Tristan Murphy in May 2023.*

*Background*

One particularly promising strategy for improving existing conservation lands in the Corn Belt is to enhance the many expiring low-quality CRP acres using interventions (usually reseeding) intended to increase monarch habitat quality through the re-enrollment process. Depending on NRCS staff assessment of field vegetation quality and other administrative considerations, landowners are often required to carry out some kind of enhancement process on their existing fields to qualify for re-enrollment. There is a need to understand the contribution of enhancing existing CRP acres, both to estimate the actual and potential contribution of the strategy to increase the amount and quality of monarch habitat. Our objective was to examine and compare non-native grass, warm-season grass, forb coverage, and milkweed density before and after the application of enhancement practices in 10-15 year old expiring and renewed CP-25 fields.

*Methods*

*Site Selection*

Site location, spatial data, and landowner contact information for 1,268 expiring CRP CP-25 contracts in Iowa were provided by the United States Department of Agriculture Farm Service Agency. we selected sites based on existing CRP contracts that were 1-3 years from expiration with plans to renew and contacted potential landowners to see if they would agree to participate in our study. Contracts were ranked based on being within driving distance of the University of Northern Iowa (120 km), time of expiration, and on willingness to participate. From this list of sites, 17 sites were selected (Fig. 2.1.1). The decision for enhancement is made at the local county NRCS office in collaboration with the landowner. We had no access to information regarding how sites were inspected or evaluated, how new contracts were created, how or why enhancements were chosen, the size of the enhanced area, or how seed mixes were chosen for overseeding.

Of the original 17 sites, only four sites were chosen to be enhanced by the NRCS and landowners at renewal (Table 2.1.1). The four 2021 sites were split into separate contracts in 2022 that ranged from 1.54% to 89.2% of the original site area sampled in 2021. The reasoning for the site size reduction is unclear. All stand enhancements involved pairing a stand disturbance with an addition of seed. Other than this general similarity, sites were highly variable in aspects of seed mixes added, disturbances applied, enhancement area, and administrative context of management. Half the sites re-enrolled small portions of the initial stand (1.5 - 3 ac) into CP-42 pollinator habitat while maintaining the majority of the original stands as-is (we assume they re-enrolled these portions back into CP-25). One site (36.3 ac) re-enrolled into CP-1 but still conducted enhancement equivalent to other sites. One site (5.8 ac) re-enrolled back into CP-25. The reasoning for the site size reduction or choice of site disturbance was unclear.

*Table 2.1.1.* Sites surveyed in 2021 and 2022 showing Iowa county, planting date, site size before and after enhancement, and *enhancement strategies of re-enrolling CP-25 plantings. ND= No Data*

| *Site* | *County* | *2021 Size (ha)* | *Year planted* | *Site Enhancement* | *Proportion enhanced* |
|---|---|---|---|---|---|
| 1 | Iowa | 14.7 | 2016 | Spring Burn | 100% |
| 2a/2b | Winneshiek | 38.9 | 2007 | Spring Burn/ Fall and Spring Tillage | 3.18% |
| 3 | Fayette | 2.69 | 2006 | Fall and Spring Herbicide | 89.2% |
| 4a/4b | Floyd | 12.5 | 2007 | Fall Herbicide, Fall Tillage/ Fall and Spring Herbicide, Fall Tillage | 4.88% |
| 5 | Winneshiek | 4.26 | ND | | |
| 6 | Hardin | 2.27 | 2011 | | |
| 7 | Winneshiek | 0.94 | 2011 | | |
| 8 | Fayette | 0.85 | ND | | |
| 9 | Fayette | 5.76 | 2011 | | |
| 10 | Butler | 8.86 | ND | | |
| 11 | Poweshiek | 12.8 | 2001 | | |
| 12 | Iowa | 35.1 | 2007 | | |
| 13 | Iowa | 17.5 | 2007 | | |
| 14 | Iowa | 27.2 | 2007 | | |
| 15 | Iowa | 19.7 | 2007 | | |
| 16 | Iowa | 29.7 | 2006 | | |
| 17 | Floyd | 21.6 | ND | | |

Nearly all landowners planted seed mixes in late-spring and used a Pheasants Forever seed mix. One landowner designed their own seed mix, procuring seed from a variety of sources. All seed mixes included at least two species of milkweed, and all seed mixes included common milkweed. All areas were seeded mid-May to late-June using a native seed drill. In order to better link establishment outcomes to the enhancement strategy, we controlled seed mix design by providing “bump-up” seed mixes to participating landowners (Table 2.1.2). “Bump-up” seed mixes were tailored to each site to equalize seeding rates of several fast-establishing species that can be easily identified. In our case, we chose “sentinel” species that could be easily detected due to their early, reliable germination and distinctive seedling characteristics. Seed amounts provided varied as some seed mixes contained these species already and needed more or less than others to meet our established seeding rates.

Enhancement practices create disturbance to promote establishment of an overseeding mix chosen by the landowner and local NRCS office. Creating disturbance in the soil and removing vegetative competition allows seeds to have access to sunlight, water, and other nutrients. The landowner has several options for enhancement practices, and wide latitude in how they are accomplished in terms of timing and intensity. General practices are burning, tillage and herbicide application. Burning is a common practice employed in CRP contracts. Burning removes litter, suppresses shrubs and other woody vegetation, and promotes the growth of established plants. Burning also removes much of the plant litter from the existing vegetation and allows better light penetration for seedling establishment.

*Table 2.1.2 Bump-up seed mixes provided to participating landowners.*

| *Species* | *Seeding Rate (PLS seeds/ $m^2$)* |
|---|---|
| *Dalea purpurea* | 53.8 |
| *Desmodium canadense* | 2.1 |
| *Chamaecrista fasciculata* | 10.8 |
| *Heliopsis helianthoides* | 5.4 |
| *Monarda fistulosa* | 43.0 |

Disking is the process of disturbing soil using a disk. Disking disrupts root systems of plants, which can lead to more effective establishment of seed mixes. Unfortunately, this process can also easily lead to the establishment of non-native grasses. As most farmers have access to a disc implement, this is a common method for site enhancement.

Herbicide application is also a common method to change vegetation structure. Herbicide application "increases wildlife habitat value by suppressing grasses, inhibiting woody plant growth, reducing the accumulation of plant residue, and increasing sunlight penetration to the ground" (CRP Required Management Practices n.d.). Herbicide application, particularly using broad-spectrum systemic herbicides, is also commonly used as a method to prepare sites for contract renewal. This method has the potential to kill most plants at a site which gives a good opportunity for sown seeds to establish without intense competition.

We tried to work with landowners to standardize stand disturbances in order to improve our ability to make statistical inferences about common stand disturbance methods. However, unpredictable events (e.g. contractor mistakes, pre-existing management decisions) limited our ability to standardize any particular method at sites. Thus, nearly all sites had a unique combination of stand disturbances (though the two burned sites shared a comparable disturbance method).

*Data collection and analysis*

Random points were created within the site shapefiles utilizing ArcPro and served as starting points for transects. These randomly generated points were uploaded to Garmin GPS units for location in the field. Each point was applied a random bearing between 0 and 359 and every other point was assigned the opposite bearing. Vegetative composition was measured in 100 1-$m^2$ quadrats (0.5 m × 2 m) using parallel transects. Quadrats were placed every 5 m of the transect starting at the randomly generated point. The length of the transects varied based on how far I could measure at each site without hitting the edge, but none exceeded 100 m in length. The total length of transects for each site was 500 m. Quadrats and transects were not reused again the following sample season. For sites undergoing enhancement, this process was repeated to generate a new random sample in 2022.

Field assistants underwent a rigorous three-week training in plant identification, which allowed us to identify plants to species level using vegetative characteristics. All plants over 10 cm in height were identified to species level except in cases where the identification was not possible in the field. Every 10 m along the transect using the tape measure as a guide, the percent cover of all plants presents in the 0.5 $m^2$ quadrat was recorded using the Daubenmire cover class system (Daubenmire 1959). In 2021 bare ground and litter were not included in the cover classes but they were measured in 2022. In these cases where identification was not possible, we collected samples in a plant press and identified them later in the lab. Milkweed stems were identified to species and counted if present every 5 m in a 1 $m^2$ quadrat. These methods were repeated for sites surveyed in summer 2022, though quadrats were sampled every 10m. Sampling occurred in June and July of the respective year.

To evaluate the establishment of our bump-up seed mix, we counted the seedlings of the six species utilizing a 0.125 $m^2$ quadrat and the same transect method as 2021 and 2022. For each site, 50 quadrats were sampled. The sampling was completed in July 2022.

### *Data Analysis*

We compared the vegetation characteristics of sites that were required to enhance vegetation with those that were not using a Welch's t-test to accommodate unequal sample size and variances. We used the average absolute cover site value for each vegetation group after the Box-Cox transformation was utilized to determine the best data transformation to achieve normality.

To examine the effect that enhancements had on vegetation, the average absolute cover for each vegetation group in 2021 was subtracted from the 2022 value to find the change in absolute cover. All enhancement types and their changes were included together in this one-sample t-test. The mean of six change values for each vegetation group was used to test the null hypothesis of no change in absolute cover of the existing vegetation groups due to enhancement.

To determine the loss of total vegetation at each site, we subtracted the 2022 absolute cover value from the 2021 absolute cover value. Because bare ground was not measured in the 2021 sampling, no formal statistical analysis was possible. However, extensive observations during vegetation sampling suggest that bare ground was in the 0-5% cover class for the vast majority of quadrats at each site in 2021.

We also wanted to estimate the degree of enhancement success at each enhanced site, and we compared sentinel species seedling density in the first year (emergence) to established plant density of sentinel species in the third year (establishment). We used a Paired t-test to assess whether sentinel species plant density was different in year one compared to year three. We also summarized species specific changes in density from emergence to establishment for each sentinel species.

The reenrolled area at each site was a small proportion of the original area sampled in 2021 and we could not know in advance where enhancements would take place. Thus, direct comparison of 2021 and 2022 vegetation was problematic. Four sites had no quadrats sampled in the 2022 enhancement areas. Of 850 total quadrats in 2021, only sixty quadrats fell in the 2022 enhancement. However, since the placement of 2021 sampling transects was random, and standard deviations and coefficients of variation for absolute cover classes were low, we assumed site uniformity and proceeded to compare vegetation before and after enhancement.

*Results*

*Consistency of CRP enhancement recommendations*

Prior to carrying out the study, we assumed that the decision to require enhancement would be related to some major deficiency in vegetation quality, such as a lack of nectar-bearing forbs or very high cover of non-native grasses. However, all vegetation categories showed no significant difference. Average warm-season grass cover, forb cover, and milkweed stem density did not differ between enhanced and unenhanced sites (p= 0.77, 0.36, and 0.50, respectively) (Table 2.2.3). In addition, non-native grass cover did not differ between enhanced and unenhanced sites (p= 0.13). Of the four sites we assessed, two applied enhancement treatments and overseeding to less than 5% of their original area. Two landowners overseeded the majority (89-100%) of their site.

*Table 2.1.3 Monarch resources (nectar bearing forb cover, milkweed stem density) and grass composition (native warm-season grass cover, non-native grass cover) among sites that were required or not required to enhance at the time of contract re-enrollment. Measures reported are average cover ± SE, with the exception of milkweed density (stems per square meter ± SE).*

| *Site Type* | *Forb cover* | *Milkweed stems $m^{-2}$* | *Warm-season Grass Cover* | *Non-Native Grass Cover* |
|---|---|---|---|---|
| Enhanced | 28.10 ± 4.70 | 0.20 ± 0.06 | 25.03 ± 10.22 | 13.00 ± 6.42 |
| Not Enhanced | 30.01 ± 4.30 | 0.27 ± 0.08 | 13.74 ± 3.12 | 26.55 ± 4.44 |

*Vegetative survey of newly enhanced fields*

After the sites were enhanced by landowners in 2022, vegetative characteristics varied from site to site. All six sites averaged 18.12% forb cover (range 2.55% -38.80%); 9.85% warm-season grass cover (range 0.10% to 23.35%); and 3.43% non-native grass cover (range 0.40% to 7.05%) (Table 2.1.4). Average milkweed stem density for all six sites was 0.29 stems/$m^2$ (range 0 to 0.68 stems/$m^2$). The sites with the lowest absolute forb coverage (Site 3 & Site 4b) had herbicide applied twice, while the two highest sites (Site 1 & Site 2a) were enhanced with spring burning. The highest absolute cover for non-native grasses was observed at Site 1 which was enhanced with a spring burn, while the lowest site (0.40% cover observed) underwent fall and spring herbicide application.

In 2022 after enhancement treatments we observed large and consistent reductions in absolute cover of vegetation within the area that was enhanced ($p<0.01$) (Table 2.1.5). Bare ground (including thatch), which is normally less than 5% in eastern Iowa CRP fields on typical soils (personal observations) but was not measured in 2021, averaged 67% in 2022 at the newly enhanced sites. Changes in cover of forbs and non-native grasses groups were insignificant.

Milkweed stem density did not change after enhancement. We found cover of warm-season grasses to be significantly reduced (with marginal statistical evidence) by an average of 20%.

*Table 2.1.4. Monarch resources (nectar bearing forb cover, milkweed stem density) and grass composition (native warm-season grass cover, non-native grass cover) after enhancements applied. Measures reported are average cover ± SE, with the exception of milkweed density (stems per square meter ± SE).*

| *Site* | *Enhancement* | *Forb cover* | *Milkweed stems $m^{-2}$* | *Warm-season Grass Cover* | *Non-Native Grass Cover* |
|---|---|---|---|---|---|
| 1 | Spring burn | 38.80 ± 2.91 | 0.00 ± 0.00 | 9.20 ± 2.15 | 7.05 ± 1.62 |
| 2a | Spring burn | 15.35 ± 2.12 | 0.60 ± 0.22 | 23.35 ± 2.43 | 1.55 ± 0.55 |
| 2b | Fall and spring tillage | 18.75 ± 3.24 | 0.10 ± 0.07 | 0.10 ± 0.07 | 6.25 ± 2.78 |
| 3 | Fall and spring herbicide application | 2.55 ± 0.65 | 0.22 ± 0.17 | 4.40 ± 1.03 | 0.40 ± 0.13 |
| 4a | Fall herbicide application and tillage | 27.10 ± 2.83 | 0.10 ± 0.055 | 15.50 ± 2.33 | 2.85 ± 0.84 |
| 4b | Fall and spring herbicide application, fall tillage | 6.15 ± 1.03 | 0.28 ± 0.11 | 6.55 ± 1.47 | 5.15 ± 0.97 |
| *Mean* | | *18.12* | *0.22* | *9.85* | *3.88* |

*Seedling establishment after enhancement*

Seedling emergence and establishment were variable but sentinel species were consistently present across sites (Fig. 2.1.3). In mid-summer 2022, most of the sentinel wildflower species had emerged as seedlings on all sites. Seedling emergence rates (seedlings per seed planted x 100) varied from 0.01-30%. Overall, seedlings were present at an average density of 8.18 ± 2.35 seedlings/$m^2$. During follow-up assessment in 2024, we found that sentinel species abundance was again variable among sites. At least some sown species were present in all sites. Some sites increased in sentinel species abundance while some decreased- no obvious patterns were associated with these trends. Across all enhanced sites, established seedlings were present at an average density of 10.38 ± 4.41 seedlings/$m^2$. When comparing average seedlings across years, we did not find a difference in sentinel species abundance from emergence to establishment ($t$ = 0.44, $p > 0.5$). *Monarda fistulosa* and *Heliopsis helianthoides* were the best establishing species, and *M. fistulosa* increased substantially by year three (Fig. 2.1.3). *Desmodium canadense* increased over time but remained at somewhat low density. *Chamaecrista fasciculata* did not change over time. *Dalea purpurea* decreased substantially over time, and was the only sentinel species on average across all sites to decrease.

*Table 2.1.5. Changes in vegetation cover from 2021 to 2022 after enhancements applied.*

| | *Absolute Cover* | | | *Absolute Cover Change* | | | |
|---|---|---|---|---|---|---|---|
| *Site* | *2021 All Vegetation* | *2022 All Vegetation* | *Vegetation Change* | *Warm Season Grasses* | *Non-native Grass* | *Forbs* | *Milkweed Stem Density* |
| 1 | 76.25 ± 2.62 | 63.60 ± 3.57 | -12.65 | 0.95 | -21.70 | -0.45 | -0.06 |
| 2a | 73.40 ± 3.27 | 41.25 ± 1.86 | -32.15 | -30.30 | -1.15 | -1.00 | 0.27 |
| 2b | | 38.80 ± 4.69 | -34.60 | -53.55 | 3.55 | 2.40 | -0.23 |
| 3 | 60.95 ± 2.92 | 7.60 ± 1.18 | -53.35 | -8.25 | -17.80 | -27.05 | 0.04 |
| 4a | 55.20 ± 2.87 | 49.80 ± 2.86 | -5.40 | -10.05 | 0.50 | -0.10 | -0.13 |
| 4b | | 22.05 ± 1.85 | -33.15 | -19.00 | 2.80 | -21.05 | 0.05 |
| *Mean* | *66.45* | *37.18* | *-28.55* | *-20.03* | *-5.63* | *-7.88* | *-0.01* |

*Discussion*

The impact of enhancing existing CRP fields for greater monarch habitat value will depend on several processes. First, only fields that are truly of low habitat quality at the end of contract should be selected for enhancement. If only a portion of the field is going to be enhanced, it should be an area of particularly low habitat quality. Second, the enhancement practice chosen by the landowner must successfully shift existing vegetation to more bare ground and to vegetation that is not very competitive which may allow for better establishment of overseeded species while also minimizing forb and milkweed loss.

In order to utilize the CRP to its maximum potential to increase monarch habitat, the lowest quality of the initial 17 sites would have been chosen for enhancement. We found no evidence that quality was related to enhancement decisions. This suggests that there is either no standard vegetative characteristic examined by NRCS offices, that standards vary from office to office, or that enhancement decisions are based on administrative, rather than ecological criteria. A lack of enhancement consistency for a program as large as the CRP is detrimental to conservation efforts not only for the monarch butterfly, but also to other endangered species. If low monarch habitat quality sites are not being enhanced, as the present study observed, monarch habitat and resources will not be increased to the extent of conservation goals.

Assuming that the correct sites are chosen, the enhancement methods must remove competing vegetation and leave desirable vegetation like milkweeds and forbs as well as provide an opportunity for seed mixes to establish. The enhancements we observed resulted in significant decreases of total vegetative cover and warm season-grasses. These changes may promote successful establishment of overseeded species by removing competition. While an increase in milkweed density and forb cover would be ideal, observing no significant change in their absolute abundance is still a positive outcome as this means these beneficial habitat elements were not diminished relative to grass cover.

All species included in the bump-up mix that we provided landowners established on at least one site. Seedling establishment ranged from 0.01-30%, demonstrating that these enhancement methods created sufficient disturbance to facilitate new recruitment of nectar-bearing forbs at least initially. With one exception, the species found as seedings in the initial enhancement year remained at similar abundances or increased in abundance by the third year after enhancement. Common species used in forb enhancements (e.g. *Monarda fistulosa*) can likely be expected to establish to some extent when seeded after enhancement.

While we did not find any statistically significant trends among different enhancement types, we noted that the sites that employed intense enhancement activities over multiple seasons (especially those employing herbicide) more consistently retained or improved emerging seedlings. Other enhancements led to losses of seeded species over time, which may suggest these sites may provide less effective monarch habitat over time. Further research is needed to more rigorously test the effects of enhancement intensity.

Ultimately our results show that enhancement activities do seem to work in concept, though high variation in success among sites may lead to challenges in meeting recovery goals. To make CRP enhancements more consistently successful, site-preparation activities that most effectively remove existing plant cover should be prioritized. While our results are somewhat promising, it is important to note that they applied to only a small (<6%) proportion of their entire field at two of the four farms sampled. The areas chosen for enhancement were all close (<100 m) to a road or dwelling; there is no record of whether the area was of particularly low vegetation quality.

## *Task 2b: Assess approved methods for enhancing CRP experimentally*

### *Mid-contract management plot experiment*

*Background*

While most of the required gains in monarch habitat require land use change from cropland to conservation grassland or low diversity CRP practices (e.g. CP1) to high diversity practices (eg. CP-42), opportunities exist to improve existing stands of nearly all CRP practices that use mixed native vegetation through required mid-contract management. There is a general tendency for many prairie reconstructions to become grass dominated and drive forb abundance down over time, especially when seed mixes include high rates of large warm-season grasses (Grman et al., 2021). There is potential for mid-contract management to prevent or delay the process of warm-season grass dominance while improving monarch nectar and host plant abundance.

Existing mid-contract management options generally include 1) prescribed fire, 2) disk tillage, or 3) herbicide application. Evidence for the impact of fire on milkweed abundance is mixed- some studies show negative (Towne and Kemp, 2008), positive (Ricono et al., 2018), and no impact (Leone et al., 2019) on milkweed abundance after burning. Fire has a generally positive effect on native forb abundance (Howe, 2011) and flower production (Richards and Landers, 1973), but typical spring timing of burns reduces spring forb abundance (Howe, 1994) and increases warm-season grass dominance (Howe, 2011). No studies exist that evaluate the impact of disking on any type of native vegetation, though one study in pastures showed a temporary decrease in smooth brome with spring disking (Renz et al., 2009), but otherwise was an ineffective option. Herbicide application in many recent popular CRP practices (e.g. CP-42) is limited to grass-specific herbicide, which can be effective at reducing the abundance of some less aggressive cool-season non-native grasses and increasing forb abundance (Barnes, 2007; Ruffner and Barnes, 2010). However, no studies exist that examine whether grass-selective herbicide can reduce warm-season grass abundance and increase monarch habitat quality in different CRP practices.

Our objective is to evaluate the effectiveness of grass-selective herbicide as a mid-contract management option to increase monarch habitat quality in a variety of different types of CRP practices. We conducted a field experiment to assess whether milkweed stem density, forb abundance, and forb flowering change after application of mid-contract management using herbicide.

*Methods*

The experiment, located at the Northeast Research and Demonstration Farm, was applied to existing prairie research plots (established 2015) with varying seed mixes based on CRP program specifications. The original experiment consisted of 36 research plots using a split-plot design with two spatial blocks. Eighteen plots (20 × 28 ft each) were established in each block. Within each block, we randomly established three replicate plots of seed mixes in 40 × 28 ft strips and a mowing treatment was applied to one randomly selected half of each strip. Seed mix treatments in the experiment varied in grass to forb ratio, and mimic NRCS approved CRP mixes commonly planted in Iowa. Three seed mixes were established in 2015: the economy mix modeled the CP-25 Rare and Declining Habitat Practice (3:1 grass/forb by seeding density), the diversity mix modeled the CP-43 Prairie Strips Practice (1:1), and the pollinator mix modeled the CP-42 Pollinator Habitat Practice (1:3). Mowing treatments consisted of 1) mowing four times throughout the first growing season, and 2) unmowed.

We applied one herbicide treatment to half of the experimental plots in 2021. We used clethodim, a grass selective herbicide commonly used to treat perennial grasses. We randomly selected subplots to receive herbicides using a restricted randomization procedure that ensured we did not apply herbicide to more of the mowed vs. unmowed plots. Three weeks prior to herbicide treatment, we mowed treatment plots at 5 in. height to create a uniform, actively growing stand of vegetation. The farm superintendent applied clethodim (Clethodim 2E) at 0.5 lb/ac along with adjuvant on Aug 30. The untreated plots served as a control group.

We collected baseline monarch habitat measures (milkweed density, forb density, inflorescence density, and grass density) before (July 2021) and after (July 2022,2023) herbicide treatment. See Meissen et al. (2020) for detailed methods for plant sampling. We also made observations and took photographs of each plot approximately 40 days after treatments to assess initial injury. We did not make formal measurements during this visit. To evaluate the effect of grass selective herbicide on monarch habitat metrics among different seed mix types, we used linear mixed effects (LME) models. We analyzed LME models in R using ANOVA (R Development Core Team 2024) to test for main effects and interactions. To assess the importance of herbicide treatments among each seed mix in the first and second years post-treatment, we used contrasts using the package emmeans in R (Lenth 2024). We found no significant main effects or interactions with mowing in our model, so we do not report or discuss this treatment further.

*Results*

We observed that clethodim application resulted in visible injury to grass species but not other plants. We found meristematic tissue death in the herbicide treated grasses, but not in untreated grasses when we visited the site two months later (Fig. 2.2.1). Most treated grass plants had little to no living aboveground tissue present. We also found no impacts to forbs (including milkweed) or sedges; we observed strong growth in non-grass (Poaceae) species during our October visit (Fig 2.2.2). Some species, such as the monarch nectar plant *Symphyotrichum leave*, were even flowering.

Inflorescence density increased in the year after herbicide treatment. Overall inflorescence density increased in herbicide treated plots compared to controls (F = 6.27, $df = {}_{1,33}$, $p < 0.05$). One year after treatment, there were 156% more inflorescences in diversity mix plots treated with herbicide compared to control ($t = 2.36$, $df = {}_{1,33}$, $p < 0.05$) and 145% more inflorescences in economy mix herbicide plots compared to control ($t = 1.83$, $df = {}_{1,33}$, $p = 0.08$) (Fig. 2.2.3, 2.2.4). There was no treatment difference in the pollinator mix, and no difference in any mix two years after treatment. Species that flowered the most after herbicide treatments included *Ratibida pinnata*, *Pycnanthemum virginianum*, *Silphium laciniatum*, and *Monarda fistulosa*.

Grass selective herbicide temporarily increased forb density. Overall, forb density was higher in herbicide treated plots compared to controls (F= 16.48, $df = {}_{1,33}$, $p < 0.01$). One year after treatment, there were 54% more forb stems in diversity mix plots treated with herbicide compared to control ($t = 2.25$, $df = _{1,33}$, $p < 0.05$) and 175% more forb stems in economy mix herbicide plots compared to control ($t = 4.47$, $df = _{1,33}$, $p < 0.001$) (Fig. 2.2.5). There was no treatment difference in the pollinator mix, and no difference in any mix two years after treatment. Species that increased in abundance the most after herbicide treatments included *Ratibida pinnata*, *Monarda fistulosa,* and *Carex* spp. We could not detect an effect of herbicide on grass density in any mix or any year (Fig. 2.2.5).

Milkweed stem density was unaffected by herbicide treatment (Fig. 2.2.6). We found stem density to be highly variable in our study, but generally we found the most milkweed stems in the pollinator mix, followed by the diversity mix. We did not find any milkweeds in the economy mix throughout the experiment. Pre-herbicide milkweed density ranged from 0.27 ± 0.27 SE stems/$m^2$ in the diversity mix to 1.07 ± 0.59 SE stems/$m^2$ in the pollinator mix. Post-treatment, we found densities of 0.27 ± 0.27 SE stems/$m^2$ in both one-year and two-years post treatment in the diversity mix. In pollinator mixes after herbicide treatment, we found 0.93 ± 0.69 SE stems/$m^2$ one year after treatment and 0.67 ± 0.44 SE stems/$m^2$ two years after treatment.

*Discussion*

Our study demonstrates that grass selective herbicides can be effective at promoting some aspects of monarch habitat in a variety of seed mixes. Though the effects only lasted one growing season, clethodim treatment increased inflorescence and forb stem density by over 150% in grassy and grass-forb balanced seed mixes (resembling CP-25 and CP-43 plantings). Milkweed stem density remained unaffected with herbicide treatment, but it is encouraging that stem density was not negatively affected. We did not observe an increase in seedlings (data not shown) between treatments, so the increase in floral resources was most likely derived from existing plants. Release from competition with grasses allowed existing forbs to produce more stems and more flowers. That this management's effectiveness relies on existing forbs also means that grassy sites without a significant forb component would not see much benefit from grass selective herbicide. This reinforces the importance of seeding high quality seed mixes that can provide quality monarch habitat and optimizing seedling establishment.

It was surprising that we found herbicide effects on floral resources and forb density but not grass density. After all, clethodim necessarily only effects grasses, and any positive effects on forbs would be the result of competitive release from grasses. While we did not formally measure herbicide injury in this study, we observed widespread tissue death in grass species coincident with widespread healthy growth in sedges and forbs. It is unlikely the effects we found were due to the pre-treatment mowing needed for herbicide application and not the actual herbicide, because mowing typically benefits grass species as much or more than forbs (Meissen et al., 2020; Glidden et al., 2023). The more likely explanation for the increase in forb flowering and abundance without decreases in measured grass abundance is that the competitive release from grasses occurred in the weeks between herbicide application and the dormant season (typically November). Benefits to forbs accrued during this timeframe as forbs increased energy stores that were used in the following year's growing season. Clethodim is not lethal to large perennial plants, and its suppressive effect likely did not persist through dormancy.

The finding that a single disturbance event did not lead to lasting plant community change is not surprising in perennial grassland systems. Other common management methods result in temporary changes if applied only once, such as prescribed burns and mowing. While there are very few published studies that assess the effects of a single fire in a prairie reconstruction, Randa and Yunger (2001) assessed biomass change after common management treatments. The impact of a single fire and a single herbicide application were similar. Both prescribed fire and grass selective herbicide primarily increased forb abundance and did not have large effects on graminoids in relatively young restorations. Both studies showed temporary results. Tallgrass prairies only tend to change with disturbance when such methods are carried out with frequency; otherwise, stands managed with low frequency tend to closely resemble unmanaged stands.

While it improved monarch habitat, clethodim application poses risks to non-target organisms. Non-grass plants seem to be at especially low-risk from clethodim. In sensitive forb species, clethodim effects appear very minor to slightly positive. Lincoln and others (2018) found lightly reduced leaf length but increased flowering and seed production in a *Camassia* species when exposed to clethodim in Oregon grasslands. Clethodim poses a more substantial risk to invertebrates. There is limited evidence that clethodim causes negative effects in invertebrates, though related Group 1 herbicides have been shown to reduce growth of some butterfly species (Schultz et al., 2016). Clethodim may have an effect between fluazifop (limited negative effects) and sethoxydim (evident negative effects) on sensitive invertebrates. Unlike prescribed fire which is applied in the dormant season, herbicide application must be applied while plants are actively growing and when pollinators are using floral resources. This coincidence of herbicide application with pollinator activity means exposure to potentially harmful effects is high. Additional research is advised to understand the effects of clethodim on monarchs and other imperiled pollinators in the Corn Belt before clethodim application is promoted widely as a mid-contract management method.

For reconstructions with low grass abundance, clethodim application is not a useful management practice. Clethodim in these plantings did not decrease grasses further nor did it promote forbs. A prescribed burn would be more likely to promote forbs in these types of mixes, since litter is removed and may increase light to small plants (Glenn-Lewin et al., 1990). Because the use of grass selective herbicide is an approved option for CRP required management, it may be worth revisiting whether grass specific herbicide targeting warm season grasses should remain an approved mid contract management option for practices like CP-42. At minimum the practice should be considered a valid management practice only for pollinator plantings that have become demonstrably grass dominated.

Further research in the realm of using grass selective herbicides to promote forbs is needed. Timing of application is especially important aspect to investigate. The timing of application for our study was after nesting season, and when warm season grasses were around peak growth. Thus, pre-treatment mowing required to produce a uniform, treatable stand also mowed off milkweeds in the month of August, the most important time to provide high quality habitat. If herbicide applications in late spring/early summer can produce similar results as those applied in fall, net positive outcomes for monarch habitat (rather than the more ambiguous situation we found) would follow from grass selective herbicide application. Investigating application frequency is also important, and finding a minimum effective frequency is key to avoiding excessive non-target effects from this practice.

***Task 3a: Improving the long-term performance of new CRP enrollments for monarch habitat on existing farms***

***Assessing the grass component in seed mixes for monarch habitat: On-farm study***

*Background*

High quality monarch habitat consists of grassland with high abundance of milkweed and nectar plants, but attempts to restore diverse stands of mixed grasses and forbs often result in poor establishment or long-term persistence of forb species. Several authors have shown that the overabundance of grasses in the establishing stands of native grassland vegetation can lead to poor forb establishment or loss of forb abundance and diversity over time (Dickson and Busby, 2009; Grman et al., 2021). These and similar studies such as Meissen et al. (Meissen et al., 2020) and Peters and Schottler (2010) show that seed mix design, especially using a balanced grass to forb or forb dominated seeding rate (by seed density), plays a leading role in increasing forb establishment and limiting grass dominance.

Balanced grass-forb seed mix designs may not necessarily ensure successful forb establishment or long-term persistence. There is a tendency for many commercial seed mixes used in CRP practices (including those that encourage balanced grass forb seeding such as CP43 Prairie Strips) to prioritize simple, low cost seed mix designs that meet minimum specifications. This type of seed mix design typically leads to mixes with high seeding rates of a few readily available, low-cost warm-season grass species but few other species of grass-like plants (graminoids). Indeed, despite some degree of commercial availability and feasibility of use, most non-C4 graminoids, especially sedges, are underrepresented in prairie reconstructions (Kindscher and Tieszen, 1998; Sivicek and Taft, 2011). Seed mixes with low graminoid diversity and high abundance of common warm-season grasses may result in poor forb establishment and persistence, even when seed mixes have a well-developed forb component. Because monarch habitat requires an abundance of forbs to be high quality, seed mix designs used to improve monarch habitat may need to further consider the diversity of graminoids in the seed mix.

Timing of seeding may also play an important role in determining how well monarch habitat can be established in the context of differing seed mix designs. Even in seed mixes with low graminoid diversity and high abundance of common warm-season grasses, seeding in the dormant season may prevent overabundance of warm-season grasses by lowering initial establishment rates of dominant warm-season grasses while increasing forb establishment (Larson et al., 2011). In seed mixes that include diverse graminoids such as sedges and C3 grasses, seeding in the dormant season may increase establishment of non-C4 graminoids as well as forbs (Glidden et al., 2023), resulting in denser stands more resistant to invasive C3 grasses. While there is a generally well observed benefit to monarch nectar plant establishment with dormant seeding (Lukens et al., 2020), the importance of timing of seeding on milkweeds is still unclear.

Our objective was to investigate whether 1) graminoid diversity in seed mixes or 2) timing of seeding influences monarch habitat outcomes in prairie reconstruction by evaluating the effects of graminoid composition in seed mixes (diverse vs. simple) and timing of seeding (dormant vs. spring) on native plant establishment (including nectar plants and milkweeds) in prairie strips.

*Methods*

*Study site*

The study site is located at the Roadman Farm Demonstration Area near Dike, IA in Grundy County. The soils underlying the study site are primarily moderately well drained Kenyon loams, though significant areas are composed of somewhat poorly drained Clyde silty clay loams and Floyd loams (Natural Resources Conservation Service, 2021). Topographically, the study site is located on generally level ground where slopes do not exceed 5% grade. Land use prior to this study was agricultural, with corn and soybeans consistently grown in rotation at the site.

This study is part of a larger project developed and installed in partnership with Iowa State University's STRIPS program and Hertz Farm Management. The fields where this project was established were enrolled in the 2019 initial sign-up of the USDA's CP-43 Prairie Strips practice. The ISU STRIPS team determined strip placement in the field, while UNI researchers determined the makeup and location of study treatments on the placed strips.

We prepared the study site using tillage after crop production. In the summer of 2020, the farm operator grew corn throughout the site. The farm operator used a combine with a chopping corn head to harvest in October 2020. To break up the remaining residue, we used one pass of disc cultivation after harvest. The prepared seedbed was firm but generally covered by 50-75% corn residue.

*Study design*

To assess monarch habitat establishment in seed mix designs with varied graminoid composition and timing of seeding we installed an experiment with a split plot design in November 2020. We established a study area consisting of eight prairie strips, each approximately 1 ha. Individual strips were either 12.2 m wide and 750 m long or 24.4 m wide and 390 m long (Fig. 3.1.1). One strip was 12.2 m wide and 610 m long since a residential lot interrupted the strip. Another strip was slightly irregularly shaped to border a grain bin site. We randomly assigned a seed mix, diverse grass composition or simple grass composition, to each strip (whole plot). Within each strip, we randomly applied a seeding time treatment to each half of the strip at two levels: 1) dormant seeded and 2) spring seeded (split plot) (n=8).

In order to explore whether graminoid diversity influences monarch habitat outcomes we tested two seed mixes with contrasting composition of graminoids. We varied the rates and number of graminoid species included, but we held the forb composition constant (Appendix 2). We designed two seed mixes: 1) a 5 Grass Mix that included 4 common warm season grasses and 1 common cool-season grass (all planted at high rates), and 2) a 16 Grass Mix that included 8 warm-season grasses, 2 cool-season grasses, 5 sedges, and one rush (all planted at low to moderate rates). In 2020, the 16 Grass Mix was $1226/ ha, while the 5 Grass Mix was $1163/ ha. The diverse forb component matches those of seed mixes from similar studies (Meissen et al., 2020; Glidden et al., 2023). To ensure accuracy in seeding rates and seed purity, we calculated seeding rates for each species using pure live seed (PLS). We standardized the overall seeding rate among mixes at approximately 430 PLS seeds per square meter. We purchased seed from native seed nurseries in Iowa and adjacent states in January 2020 and stored the seed in a temperature and humidity controlled (4°C, 45% RH) cooler until planting. We weighed, bagged, and mixed the seed for each plot separately. To ensure soils were stabilized as prairie seedlings established, we included a nurse crop of oats in spring seeded treatments at a rate of 2.5 bu/ha and winter wheat in fall seeded treatments at a rate of 2.5 bu/ha.

We seeded the study site in the dormant and spring seasons of 2020-2021. We used a Truax FLX-86U no-till drill with a John Deere JD-5325 tractor to seed each treatment area. To minimize seed contamination between treatments, we cleaned out the drill after seeding different mixes. Dormant seeded treatments were planted November 20-21, 2020 while spring seeded treatments were planted May 24-26, 2021. We chose mid-November as a dormant seeding date since NRCS generally restricts planting earlier than November 15 in Iowa, and seeding later when ground temperatures were below freezing would have prevented the seed drill from operating properly. We chose a late-May spring seeding date primarily to reflect the timing preference of farmers establishing similar types of habitat in related CRP practices (CP-42) (Jackson and Meissen, 2019).

The farm operator conducted establishment mowing over the first growing season to control weed growth. We mowed vegetation throughout the 2021 growing season to ~ 10 cm when most vegetation reached approximately 1 m in height. Due to drought conditions throughout the 2021 growing season, weed regrowth was slower than normal and we reduced mowing frequency. The farm operator mowed once in mid-summer, and left the resulting thatch on site.

*Data collection and analysis*

We measured plant density and frequency in August 2021-2024, and used density estimates to calculate establishment metrics. We sampled later in the year to allow seedlings to grow to a size that allowed for confidence in seedling identification. We used QGIS to generate 10 random sampling points within polygons mapped to each treatment area. We applied a negative buffer of 2 m to the polygons to avoid sampling edges. In some areas, the prairie strips seeded did not exactly match the planting plan, resulting in sampling points outside the strip. In these cases, we re-positioned the sampling point 2 m inside and perpendicular to the true strip edge from the initially mapped sampling point. To sample plant composition at each random point, we used a modified nested quadrat sampling method described in the National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions (McColpin et al., 2019). In this method, observers record plant identity and presence in a series of nested quadrats (0.0625, 0.125, 0.25, 0.5, and 1 $m^2$). We additionally measured density of sown species in the 0.125 $m^2$ quadrats, where we counted and identified all individuals (ramets) of seeded species >10 cm tall. We also counted all milkweed stems in the 1$m^2$ quadrat area. We calculated frequency and species diversity metrics using the 1 $m^2$ quadrat measurements. We used a rarefied Shannon species diversity, i.e. effective species ($e^{H'}$), to quantify species diversity, as it has been shown to better represent the intuitive concept of biodiversity (Jost, 2006). We also assessed the frequency of high value monarch species (plant species documented in other published studies that were used for nectaring by monarchs or listed as high value by NRCS (Appendix 3).

To evaluate the effect of seed mix graminoid composition on monarch habitat establishment we used linear mixed effects (LME) models. We analyzed LME models in R using ANOVA (R Development Core Team 2021) to test for main effects and interactions. We modeled seed mix, timing of seeding, and interactions as fixed effects and whole-plot (strip) as a random effect. To meet the assumptions of normality and homoscedasticity of residual variance, we used a square root transformation for milkweed stem density and forb stem density. We present raw data in all figures, while we report and discuss results of analyses using transformed data.

### *Results*

Sown native species established relatively well across the experiment. On average across treatments, overall sown species density was 200.95 ± 20.14 SE stems/$m^2$ by the fourth growing season (Fig. 3.1.1). We detected no statistical differences on overall sown stem density between seed mix or planting season treatments. By the fourth growing season, grass density reached 184.7 ± 18.3 stems/$m^2$ while forb density was 16.3 ± SE 2.8 stems/$m^2$ averaged across all treatments (Fig. 3.1.2). Neither seed mix nor planting time had an effect on sown grass or forb density. Though we found no main effects or two-way interactions to be important, we did find a significant three-way interaction between seed mix, planting season and year when in the grass density model.

Diversity in established prairie strips increased with graminoid diversity of the seed mix. Prairie strips planted with the 16 graminoid mix were 24% more diverse than the 5 graminoid mix ($F= 12.81$, $df = {}_{1,6}$, $p < 0.05$) (Fig. 3.1.3). We found 23.49 ± 0.96 SE effective species in strips seeded with the diverse graminoid mix and 18.90 ± 0.56 SE effective species in the low diversity graminoid mix. Planting season did not affect diversity. However, we did observe that diversity was much higher in dormant season plantings during the first growing season, but that the effect disappeared by the second year.

Milkweed stem density increased with dormant seeding but was otherwise highly variable across our study (Fig. 3.1.4). Averaged across all treatments, milkweed stem density in the experimental prairie strips was 0.41 ± 0.11 SE stems/$m^2$. In the fourth growing season, milkweed abundance was 0.46 ± 0.19 SE stems/$m^2$ in dormant seeded strips, which was 32% greater than the milkweed abundance in the growing season seeded strips (0.35 ± 0.11 SE stems/$m^2$) ($F= 12.81$, $df = {}_{1,42}$, $p < 0.001$). The largest differences in milkweed abundance between planting season were observed in the first three years, where dormant seedings had four-fold more stems than growing season seedings. Graminoid diversity of the seed mix did not influence milkweed stem density.

The abundance of high value monarch species was generally high across all treatments. Important nectar and host plants across the study site occupied 94.4 % (SE 2.2%) of the quadrats we surveyed (Fig. 3.1.5). It is important to note that our estimates only measure species presence, not plant size or flowering. Thus, we estimate the most expansive measure for monarch habitat provision here. We detected no differences in the abundance of valuable monarch species between seed mix or planting season treatments. The most common high value monarch species were *Heliopsis helianthoides*, *Taraxacum officinale*, *Ratibida pinnata*, and *Asclepias syriaca*.

### *Discussion*

Diverse prairie strips established in former croplands provide good monarch habitat regardless of graminoid seeding diversity, particularly when seeded in the dormant season. Prairie strips in our study produced milkweed stem densities comparable to other conservation grasslands (Kaul and Wilsey, 2019; Lukens et al., 2020) Though the quality of individual strips was variable, the overall plant community metrics (native establishment, species diversity) of the prairie strips in this study are comparable to functional prairie reconstructions (Glidden et al., 2023). Further, there was approximately one or more high value monarch species per square meter, meaning that most of the measured area of these prairie strips provided some potential for hosting nectar or host plants. Other measures of monarch habitat like forb stem density were comparable to Glidden (2023), though considerably less than those in Meissen (2020). Dormant seeded prairie strips produced more milkweed stems but approximately similar stem density and diversity measures than growing season seeded strips. This finding is somewhat surprising, since other studies have not shown an effect of seeding time on milkweed establishment (Glidden et al., 2023). It is possible that the droughty conditions in the first growing season of this study led to poor outcomes in the growing season seeded strips, but milkweed stem density was still comparable in this study and Glidden (2023), particularly in the fourth growing season.

We were surprised to see a lack of impact of grass in the seed mix on plant communities. Graminoid density was approximately equal when comparing seed mixes, which is not what we expected. We anticipated highly grass dense stands to develop in the low graminoid diversity mix, while grass density would be moderate in the diverse graminoid mix, but we did not see this difference. We did observe differences in types of grass- the diverse mix had more cool-season species (data not shown). We also expected high grass density (especially dense stands of warm season grasses) to lead to decreases in forb density, though we did not find this either. It is possible that warm season grass density will continue to increase over time (indeed, in all strips grass density increased all 4 years of data collection) and lead to expected declines. However, based on our data from approximately the first half of a CRP contract, and from other experiments (Meissen et al., 2020; Glidden et al., 2023), it appears that continued provision of high forb density is achieved better by ensuring sufficient forb seeding density in seed mixes, and less by reducing highly competitive native grasses.

Our results show that seeding seed mixes with high forb and graminoid diversity in the dormant season may be one way to ensure establishment of effective monarch habitat in new CRP plantings. Cost associated with increasing species diversity in our study was only about 5 %., so seeding a mix with high graminoid diversity led to more diverse established stands without significantly increasing overall project costs. Dormant seeding in this study represented a no-cost way to enhance monarch outcomes, which is a similar finding to Glidden (2023), who found dormant seeding to improve pollinator outcomes for free.

### *Task 3b: Improving the long-term performance of new CRP enrollments for monarch habitat experimentally*

### *Enhancing cool-season grass stands for effective monarch habitat*

*Background*

Species-poor grasslands, particularly those dominated by introduced cool-season grasses, represent a significant portion of CRP acres across the U.S. CP-1 (Establishment of Introduced Grasses and Legumes), one of the most common CRP practices, covers over 2.7 million acres, with more than 1 million acres located in core monarch breeding areas of the Midwest. Currently, CP-1 lands are primarily managed for soil stabilization and basic wildlife habitat. However, by incorporating monarch host and nectar plants into these areas, monarch habitat could be expanded on existing conservation lands without compromising the original goals of the program. This approach offers a feasible way to address a pressing conservation need while working within the constraints of agricultural landscapes.

Enhancing species-poor grasslands is often achieved through the CRP re-enrollment process. Depending on NRCS assessments of vegetation quality and administrative considerations, landowners are frequently required to improve their fields to qualify for re-enrollment. This often involves transitioning from species-poor grasslands, such as CP-1 (Establishment of Introduced Grasses and Legumes) and CP-2 (Establishment of Native Grasses), to species-rich grasslands, such as CP-25 (Rare and Declining Habitat) and CP-42 (Pollinator Habitat), by adding seeds of native forbs and grasses. For sites targeting monarch recovery, milkweed and monarch nectar plants are also included in seed mixes.

Establishing additional species in existing grasslands requires creating disturbances to allow seedlings access to light, space, nutrients, and water. Without such gaps, added seeds rarely survive to establishment (Rabinowitz and Rapp, 1985; Williams et al., 2007). Mechanical methods, such as disking or tillage, can create temporary openings in vegetation, but they often fail to significantly reduce competition from sod-forming grasses and can increase soil erosion. For example, spring disking in pastures only temporarily decreased smooth brome cover, with little long-term impact on interseeding success (Renz et al., 2009). In contrast, herbicide application offers greater potential for creating suitable conditions for seedling establishment. Non-selective, systemic herbicides can induce widespread mortality of competing vegetation, resulting in larger areas of bare ground that persist for significant portions of the growing season. However, the duration and effectiveness of these gaps depend on the rate of recolonization from seed banks and the survival of plants that escape herbicide treatment.

While herbicides show promise, their success often hinges on the intensity of application. For instance, in Missouri, Newbold and others (2020) demonstrated that five herbicide applications over two full growing seasons were necessary to achieve reconstructed grasslands comparable to those established on recently cropped land. Such intensive preparation, however, conflicts with CRP regulations, which typically limit the timeframe for site preparation. The minimum level of herbicide intensity needed to establish native forbs and monarch habitat within shorter, more feasible timeframes remains unknown. Balancing the effectiveness of herbicide applications with regulatory and cost constraints remains a key challenge for scaling up monarch habitat enhancement efforts.

In this study, we evaluate the effectiveness of stand enhancement methods to increase monarch habitat quality in species-poor cool-season grasslands. We established research plots where we varied site-preparation intensity (herbicide application at three different frequencies) in existing cool-season grasslands, then interseeded with a diverse native monarch habitat seed mix. To assess monarch habitat provided by each site-prep treatment, we measured native species richness, stem density (of native grasses and native forbs), and cover (native plants, annual weeds, perennial weeds, and high value monarch plants). We also evaluated cost-effectiveness, and considered the cost of project materials with respect to the monarch habitat it provided.

*Methods*

The study site is located at Irvine Prairie near Dysart, IA in Benton County. The soils underlying the study site are poorly drained Colo-Judson silty clay loams (Natural Resources Conservation Service, 2022). Topographically, the study site is located on very gently sloping ground where slopes do not exceed 5% grade. Prior to this study, the site was used as a grass waterway and was annually hayed in early summer.

To assess stand enhancement methods (site-prep herbicide frequency) and seed mix designs with varied graminoid composition, we carried out a split-plot experiment where we varied seed mix design at the whole-plot level and herbicide frequency at the sub-plot level. We established the experiment throughout the north half of a waterway at Irvine Prairie, consisting of twelve 18.3 x 8.5 m whole plots. We randomly assigned a seed mix, diverse grass composition or simple grass composition, to each whole plot. Within each whole plot, we randomly applied an herbicide frequency treatment to three subplots (size 6.1 x 8.5m) at three levels: 1) no herbicide, 2) 1x application, 3) 2x application ($n$=36).

We evaluated three frequencies of herbicide application used to prepare sites for seeding a native seed mix. We compared 1) a control with no herbicide application, 2) 1x glyphosate application, and 3) 2x glyphosate application. Four weeks prior to herbicide treatment, we mowed treatment plots at 10 cm height to create a uniform, actively growing stand of vegetation. We applied herbicide for 1x and 2x sprayed plots on May 4, and again in the 2x plots on May 31. For all herbicide treatments, we applied a 41% glyphosate formulation with surfactant (EPA Reg. No. 86068-4-84009) at a rate of 1.68 kg/ha using a Solo piston pump backpack sprayer.

We used two seed mixes with contrasting composition of graminoids for this study. We varied the rates and number of graminoid species included, but we held the forb composition constant (Appendix 2). We designed two seed mixes: 1) a 5 Grass Mix that included 4 common warm season grasses and 1 common cool-season grass (all planted at high rates), and 2) a 16 Grass Mix that included 8 warm-season grasses, 2 cool-season grasses, 5 sedges, and one rush (all planted at low to moderate rates). The diverse forb component matches those of seed mixes from similar studies (Meissen et al., 2020; Glidden et al., 2023). To ensure accuracy in seeding rates and seed purity, we calculated seeding rates for each species using pure live seed (PLS). We standardized the overall seeding rate among mixes at approximately 430 PLS seeds per square meter. We purchased seed from native seed nurseries in Iowa and adjacent states in January 2022 and stored the seed in a temperature and humidity controlled (4°C, 45% RH) cooler until planting. We weighed, bagged, and mixed the seed for each plot separately. To ensure soils were stabilized as prairie seedlings established, we included a nurse crop of oats at a rate of 2.5 bu/ha.

We seeded the study site in the growing season of 2022. We used a Truax FLX-86U no-till drill with a John Deere JD-5325 tractor to seed each treatment area. To minimize seed contamination between treatments, we cleaned out the drill after seeding different mixes. All plots were planted June 2-3, 2022. We conducted establishment mowing once during the first growing season to manage annual weeds. We mowed treatment plots to 11.4 cm on October 4, when vegetation began senescing. We did not conduct additional establishment mowing because a previous study of prairie enhancement in cool-season grass stands showed limited benefits to mowing (Meissen, 2017).

*Data collection and analysis*

We measured plant density and canopy cover in September 2022. We sampled later in the year to allow seedlings to grow to a size that allowed for confidence in seedling identification. To sample plant density and canopy cover, we used five 0.25 $m^2$ quadrats spaced every 1 m along a 5 m transect placed randomly in each plot. To reduce edge effects, we did not lay quadrats within 1 m of plot borders. In each quadrat, we counted and identified all individuals (ramets) of seeded species. We recorded canopy cover values (Daubenmire classes) for each species and bare ground. To assess responses from functional groups, we summed ramets among species belonging to each group and calculated relative cover for each group. We also assessed responses of general vegetation types based on typical land management objectives of prairie strips (i.e. prioritizing native perennial plants of high conservation value). We defined the following classifications within this group: 1) sown species (sown forbs and graminoids), 2) ruderal weeds (annual or biennial species of any origin with a coefficient of conservatism (CoC) $\leq 1$), 3) perennial weeds (introduced perennial species). We also calculated cover of high value monarch species (plant species documented in other published studies that were used for nectaring by monarchs or listed as high value by NRCS) (Appendix 3) by summing cover midpoint estimates of high value species in each quadrat.

We estimated costs and cost-effectiveness of each treatment. We used cost estimates for custom rate seeding and mowing (Plastina, 2022), but used actual project costs for herbicide (Table 3.2.1). To estimate seed costs, we used the median seed price for 2022 for each species (derived from seed quotes and published lists), summed according to each amount in the seed mixes. To assess cost-effectiveness, we divided the number of observed ramets of sown species in each plot (2024 data) by the treatment input costs (seed, herbicide, seeding, and mowing) to estimate stems produced per dollar. We then used the inverse of stems per dollar multiplied by 1000 to estimate the cost to produce 1000 native stems. If total plot stem density was zero, we replaced the zero value with 1 to avoid errors associated with dividing by zero. We also estimated milkweed stems per dollar using the same method, though we use only costs for milkweed seed.

To evaluate the effects of herbicide frequency and seed mix we used generalized linear mixed effects (LME) models. We analyzed LME models in R using ANOVA (R Core Team 2024) to test for main effects and interactions. We modeled seed mix, herbicide frequency, and interactions as fixed effects and whole-plot as a random effect. To meet the assumptions of normality and homoscedasticity of residual variance, we used a log transformation for cost of producing 1,000 native stems. We used plot-level ramet counts for testing models with density-based response variables (milkweed stems, sown stems) and fit a Poisson distribution with the glmer package (Bates et al., 2015) for these measures. We conducted post-hoc comparisons of significant treatment effects using Tukey HSD tests with package emmeans (Lenth, 2024). We present raw data in all figures, while we report and discuss results of analyses using transformed data. We did not find an effect of seed mix on any of the metrics we investigated (with one exception: an interaction between seed mix and herbicide for total stem density), and do not expect to find such effects until stands have been established for several years (minimum year 4-5). Thus, we do not report or discuss seed mix effects in this report.

*Table 3.2.1. Estimated costs (USD per hectare) for each study treatment.*

| *Seed Mix* | *Site Prep Method* | *Planting Method* | *Establishment Management* | *Total Input Cost ($/ha)* |
|---|---|---|---|---|
| 5 Grass Mix ($1,136.10) | No Herbicide ($0.00) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,232.47 |
| 16 Grass Mix ($1,235.42) | No Herbicide ($0.00) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,331.79 |
| 5 Grass Mix ($1,136.10) | 1x Glyphosate ($135.88) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,368.35 |
| 16 Grass Mix ($1,235.42) | 1x Glyphosate ($135.88) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,467.67 |
| 5 Grass Mix ($1,136.10) | 2x Glyphosate ($271.77) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,504.24 |
| 16 Grass Mix ($1,235.42) | 2x Glyphosate ($271.77) | 1x Drill ($46.95) | 1x Mowing ($49.42) | $1,603.56 |

*Results*

We observed large differences in general plant composition resulting from herbicide frequency treatments (Fig. 3.2.1). The abundance of perennial weeds, compared to unsprayed control plots (97.7 ± 0.7 % relative cover), initially decreased dramatically in herbicide treatments, with decreases in relative cover of 63% in 1x sprayed plots and 88% in twice sprayed plots. By the third growing season, perennial weeds were again overwhelmingly dominant in 1x sprayed plots (88.2 ± 2.3 SE % relative cover). In twice sprayed plots, perennial weeds comprised the majority of the vegetation (63.5 ± 3.3 SE % relative cover). Tall fescue (*Schedonorus arundinaceus*), smooth brome (*Bromus inermis*), and alsike clover (*Trifolium hybridum*) were by far the most abundant perennial weeds. Annual/biennial weeds, mostly green foxtail (*Setaria viridis*) and witchgrass (*Panicum capillare*), increased with herbicide frequency, especially in the first growing season where relative cover of the group reached >80% in the twice sprayed treatment. Annual/biennial weeds were much less abundant by year three, with relative cover no greater than 6% in any treatment. Native grass and forbs were initially an insignificant component of the plant community in all treatments. By the third growing season, relative cover of native species was considerably higher in the twice sprayed plots (13.2 ± 2.6 SE % relative cover for forbs, 11.0 ± 2.0 SE % relative cover for grasses). Native species in no-herbicide and 1x sprayed plots remained relatively insignificant by the end of the study (with 1x spray we found only 3.3 ± 0.7 SE % relative cover for forbs and 3.0 ± 0.9 SE % relative cover for grasses).

Sown species richness increased with greater frequency of herbicide application during site prep ($p < 0.0001$) (Fig. 3.2.2). Plots sprayed twice had nearly twice as many species (8.2 ± 1.0 SE species) than those sprayed once (4.2 ± 0.9 SE species) ($p < 0.001$) and about ten times more than in no-herbicide plots (0.8 ± 0.2 SE species) ($p < 0.001$). Species richness did not change over time, and the species present in the first growing season was about the same as in the third growing season.

Sown species stem density increased in plots treated with herbicide during site prep. While we found at least some native species established in all treatments by the third growing season, stems were much more abundant with more frequent herbicide application ($p < 0.001$) (Fig. 3.2.3). Overall, we found 2.9 ± 0.7 SE ramets/$m^2$ in no-herbicide plots, while 1x sprayed plots produced 15.9 ± 5.0 SE ramets/$m^2$. We found 61.3 ± 10.3 SE ramets/$m^2$ in twice sprayed plots. Native forb stem establishment was higher with more frequent herbicide during site prep ($p < 0.001$). Forb density ranged from 1.6 ± 0.5 SE ramets/$m^2$ in no-herbicide plots to 13.5 ± 3.4 SE ramets/$m^2$ in twice sprayed plots. Native grass stem establishment was higher with more frequent herbicide during site prep ($p < 0.001$), with stem densities of 1.3 ± 0.4 SE ramets/$m^2$ in no-herbicide plots and 47.8 ± 8.6 SE ramets/$m^2$ in twice sprayed plots. Across all herbicide treatments, establishment was apparently complete in the second growing season, as native stems remained about the same density in the third growing season as in the second.

Metrics of monarch habitat increased with herbicide frequency during site prep, though milkweed stem density was highly variable. We found no milkweed in no-herbicide plots after three growing seasons; in fact, we did not find any throughout the entire study in this treatment. By the third growing season in herbicide plots, we found 0.27 ± 0.15 SE milkweed stems/$m^2$ in 1x and 0.67 ± 0.28 SE stems/$m^2$ in twice sprayed plots (Fig. 3.2.4). Given the high variability among milkweed observations, we could not detect a difference in milkweed stem density due to herbicide. Relative cover of high value monarch species increased with herbicide frequency ($p < 0.0001$). In established plantings (third growing season) we found that twice sprayed plots produced the highest relative cover of high value monarch species at 26.2 ± 2.1% relative cover, which was approximately two-fold greater than the 1x sprayed plots. High value monarch plants were more than twice as abundant in 1x sprayed (12.2 ± 2.2 % relative cover) compared with no-herbicide treatments (5.1 ± 1.5 % relative cover) (Fig. 3.2.5).

Frequent herbicide application during site prep increased cost-effectiveness. By the third growing season, plots sprayed twice were twenty-eight times more cost-effective (4.43 ± 1.15 SE $/1k stems) than those sprayed once (126.22 ± 81.64 SE $/1k stems) ($p < 0.01$) and nearly fifty times more cost effective than unsprayed plots (215.18 ± 106.49 SE $/1k stems) ($p < 0.001$). We found the cost to produce 1000 milkweeds in the most cost-effective scenario (twice glyphosate application) was $66.67.

*Discussion*

Our study demonstrates that species-poor grasslands dominated by cool-season grasses can be enhanced for monarch habitat, but the effectiveness of such enhancements depends heavily on site preparation methods. Simply seeding native species into existing cool-season grass stands, even with a no-till drill, resulted in negligible native species establishment. A single application of herbicide reduced the cover of dominant cool-season grasses by only half, leaving substantial competition for establishing seedlings. Under these conditions, only 5 sown ramets/$m^2$ established, a density typically associated with failed prairie reconstructions (Smith et al., 2010). These benchmarks, however, are based on seedings initiated on former croplands dominated by annual weeds, not highly competitive, fast-spreading perennial grasses. Given this, it is unlikely that the sparse native plants in stands prepared with a single herbicide application will persist long-term. Successful stand enhancement was only observed in plots sprayed twice during the early growing season. In these plots, sown species densities exceeded the 10 individuals/$m^2$ benchmark (Smith et al., 2010), milkweed stem density matched regional monarch habitat standards (Kaul and Wilsey, 2019), and high-value nectar forbs achieved significant cover.

While high-frequency herbicide applications prior to seeding facilitated the establishment of important nectar plants, they did not convert cool-season grasslands into native warm-season grasslands. Perennial weed cover was substantially reduced in plots sprayed twice, but recolonization occurred rapidly within two years. Invasive cool-season grasses, such as smooth brome, are known to reduce species diversity in temperate grasslands (Palit and DeKeyser, 2022), which raises concerns about the long-term persistence of monarch habitat plants established in our study.

Despite the high costs of frequent herbicide use, spraying twice before seeding was by far the most cost-effective method to produce monarch habitat in species-poor grasslands. We found that the cost to establish 1,000 native stems was nearly 30 times higher in plots sprayed once compared to those sprayed twice. Interestingly, a similar cost difference exists between the twice-sprayed plots and seedings planted into former cropland, with the latter being about 30 times more cost-effective (Meissen, 2020). When considering monarch habitat enhancement using seed alone, our results show that simple interseeding attempts in species-poor grasslands—even with a no-till drill that ensures ideal seed placement—are potentially wasteful of both seed and other resources. Monarch habitat was practically absent with this approach. Given the challenges of procuring sufficient native seed and their high expense, land managers should ensure enhancement efforts are efficient to avoid unnecessary resource use.

Compared to adding species after cropping, native plant establishment was lower in monarch-enhanced cool-season grass stands. Augmented cool-season grass stands were more dominated by perennial weeds and had lower native plant density than seedings planted into former cropland, though they still resembled diverse, functional prairie reconstructions (Glidden et al., 2023). Even in the twice-sprayed plots, third-year stem density was almost 500% lower, and species richness was 75% lower than reported by Meissen (2020) for a similar seed mix planted into recently cropped land. Newbold et al. (2020) found that grasslands sprayed five times achieved diversity measures comparable to those on former cropland, contrasting with our findings of lower diversity and establishment rates in twice-sprayed plots. This suggests that higher herbicide application rates (e.g., 3x or 4x) may improve native species establishment, though further research is needed to confirm this.

Further research is needed to refine methods for enhancing species-poor grasslands for monarch habitat. Given the challenges of improving cool-season grass stands, strategies to address failed plantings or upgrades should be explored. For example, grass-selective herbicide applications may improve the performance of monarch host and nectar plants in grass-dominated stands, offering a potential pathway to more successful habitat enhancement.

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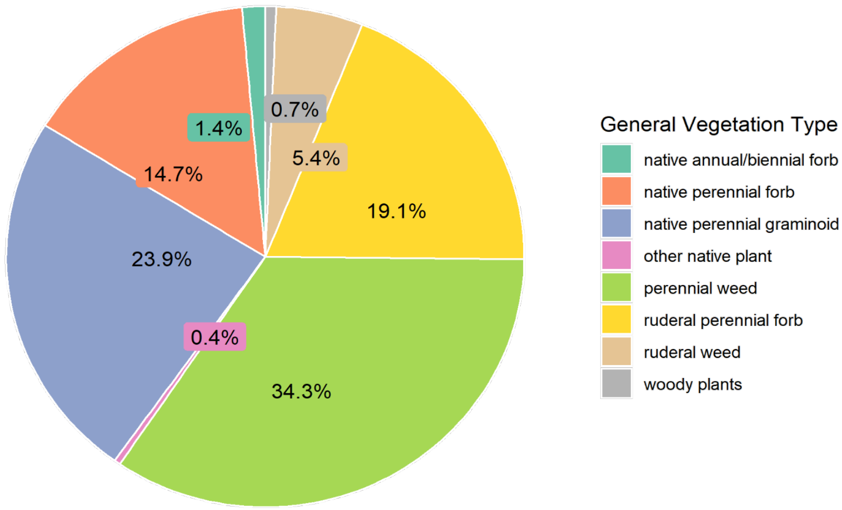


Figure 1.1.1 Relative cover (%) of general vegetation types in eastern IA expiring CP-25 fields.

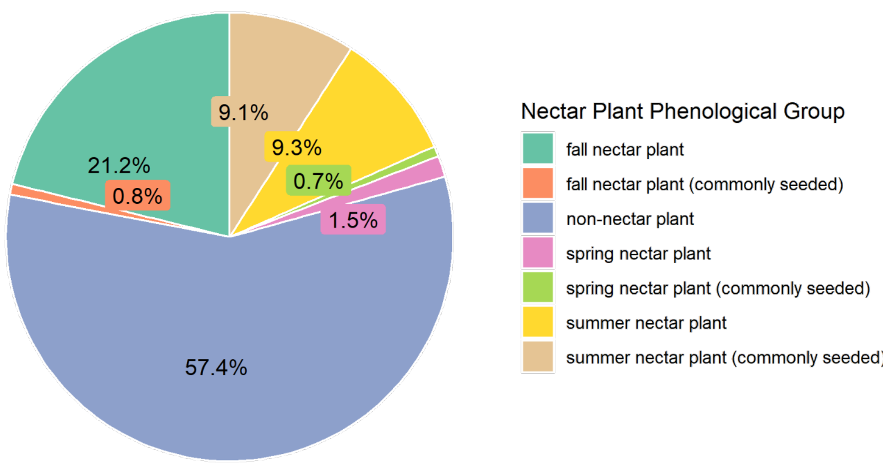


Figure 1.1.2. Relative cover (%) of nectar plant types in eastern IA expiring CP-25 fields.

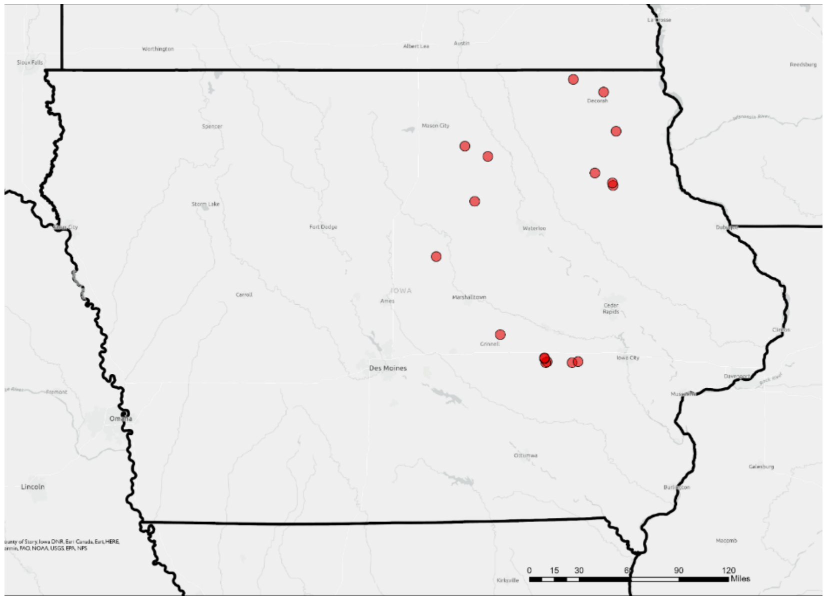


Figure 2.1.1. Map showing 17 sites sampled in 2021 and 2022.

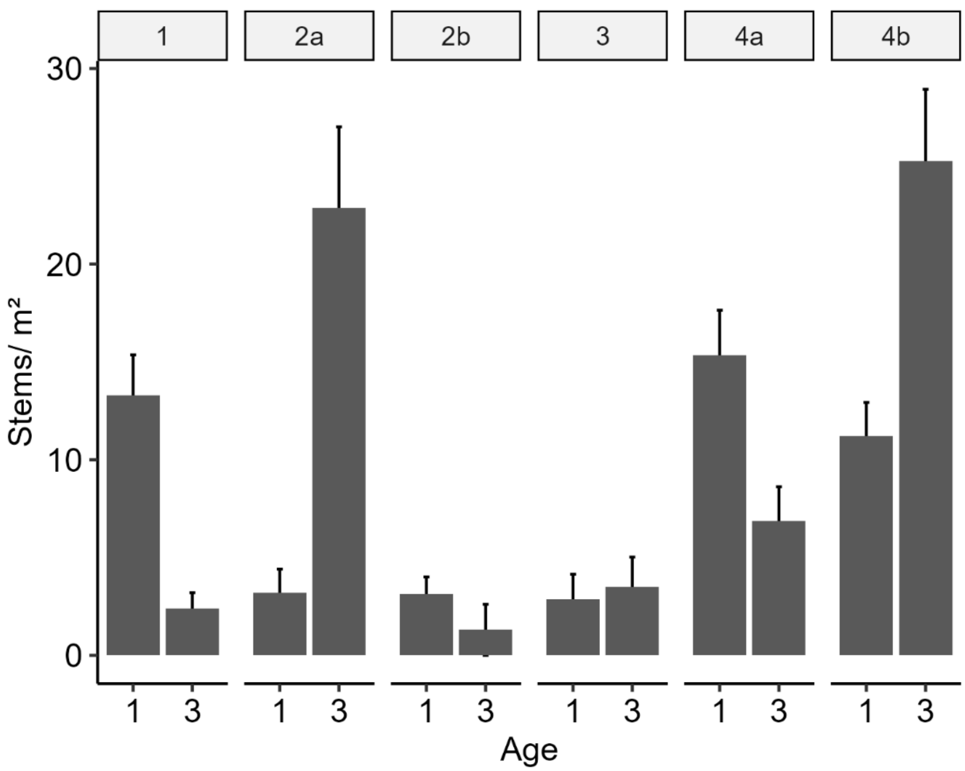


Figure 2.1.2. Establishment of sentinel species (stems per square meter ± SE) in year one (seedling emergence) and year three (plant establishment) after seeding at each enhancement site (top labels).

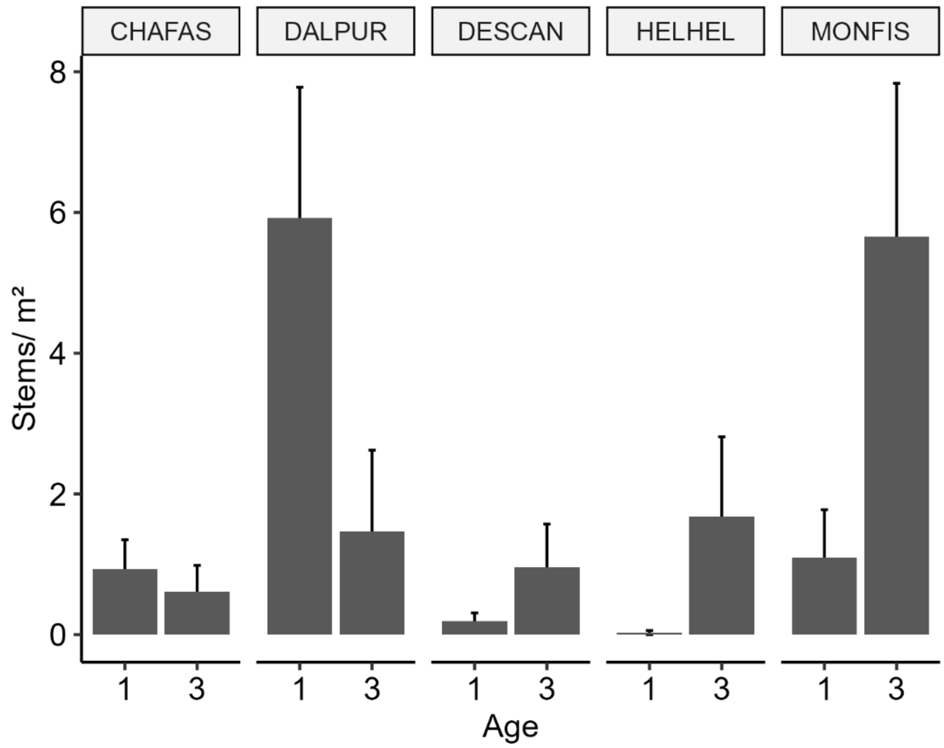


Figure 2.1.3. Establishment (stems per square meter ± SE) in year one (seedling emergence) and year three (plant establishment) for each sentinel species (top labels). CHAFAS= *Chamaecrista fasciculata*, DALPUR= *Dalea purpurea*, DESCAN=*Desmodium canadense*, HELHEL=*Heliopsis helianthoides*, MONFIS=*Monarda fistulosa*.

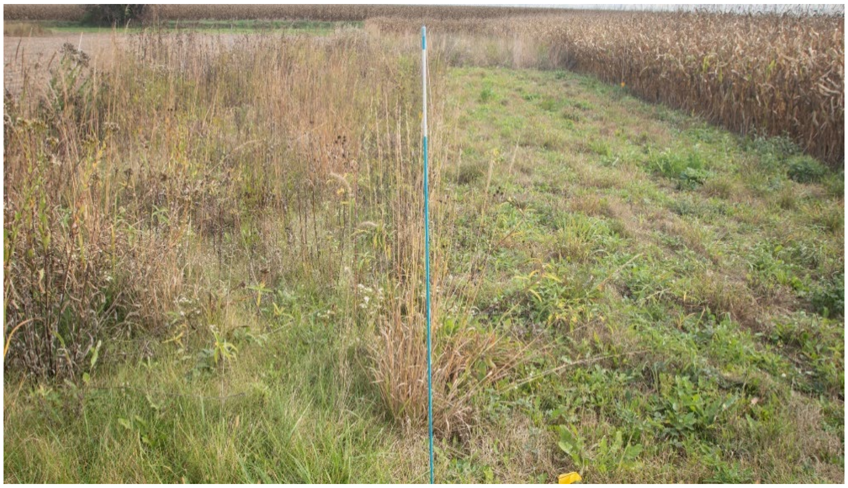


Figure 2.2.1 Experiment plots seen in October 2021. Left photo shows control (left half) and herbicide treated (right half) plots. Note the active growth of forbs and stunted, desiccated grasses in the herbicide treatment plots. Right photo shows death of meristematic tissues (brown, desiccated emergent leaf) in *Sorghastrum nutans*, suggesting effective herbicide treatment.


Figure 2.2.2. Vegetation observed in herbicide treated plots. Left photo shows regrowth of *Asclepias syriaca* after treatment among other native (Ratibida pinnata) and non-native (*Taraxacum officinale, Cirsium arvense*) forbs. Right photo shows vigorous growth of sedges (*Carex* cf. *brevior*) among stunted warm-season grasses.

Figure 2.2.3. Grass dominated Economy Mix one year after herbicide application. Clethodim plots can be seen to the left of the stakes in the center of the photo, with control plots to the right. Higher abundance of inflorescences are apparent in the clethodim treatment plots.

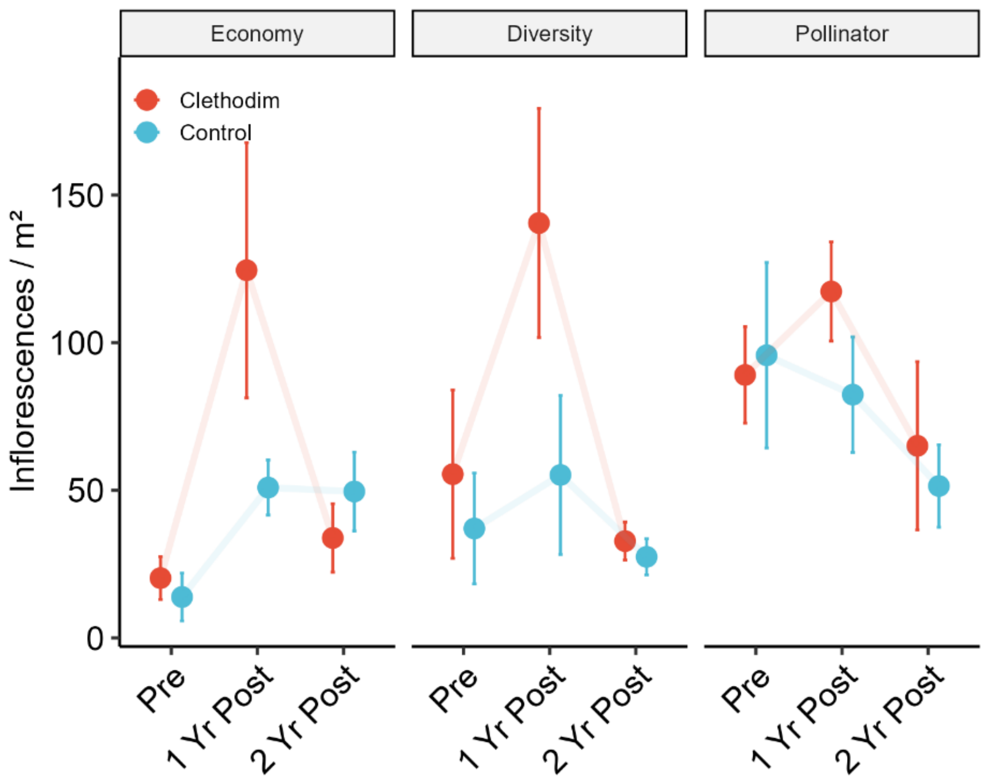


Figure 2.2.4. Floral resource abundance (inflorescence density ± SE) among seed mixes before and after grass-selective herbicide application.

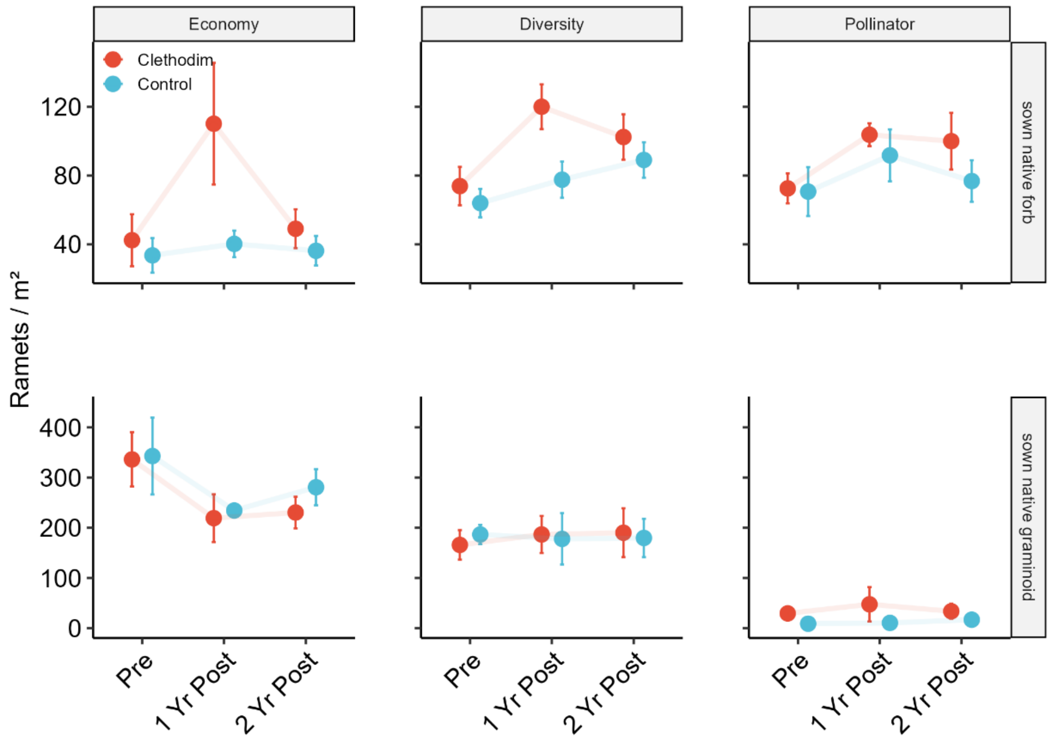


Figure 2.2.5. Sown grass and forb density ± SE among seed mixes before and after grass-selective herbicide application.

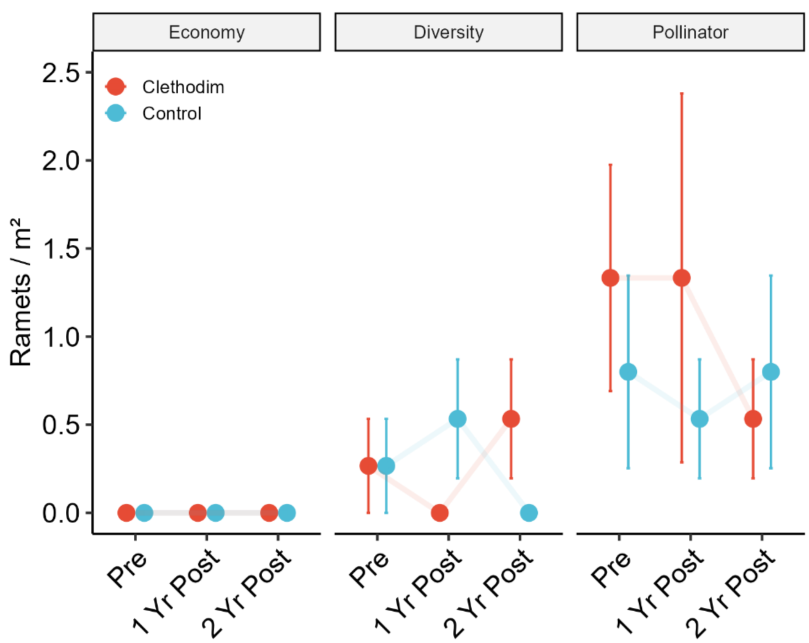


Figure 2.2.6. Milkweed stem density ± SE among seed mixes before and after grass-selective herbicide application.


Figure 3.1.1. Experiment map at the Roadman Farm in Grundy County, IA (left). Typical view of vegetation in well-established prairie strips in August 2024 (right).

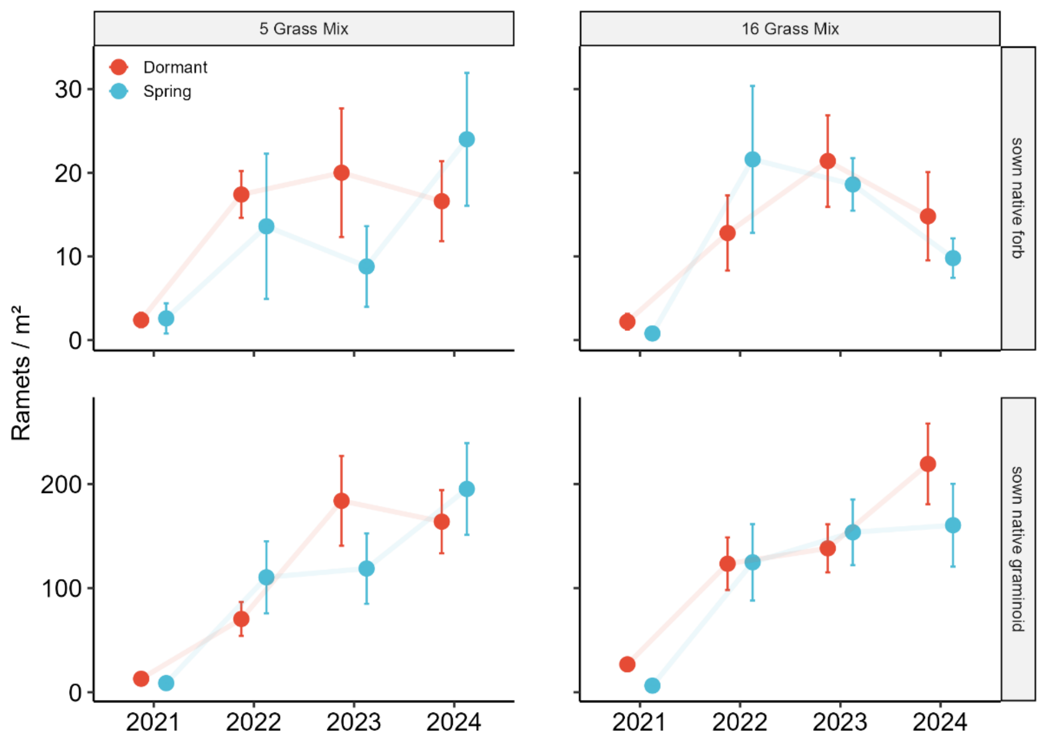


Figure 3.1.2. Stem density ± SE of sown graminoids and forbs in seed mixes with a 16 spp. or 5 spp. graminoid component seeded in the dormant or spring season.

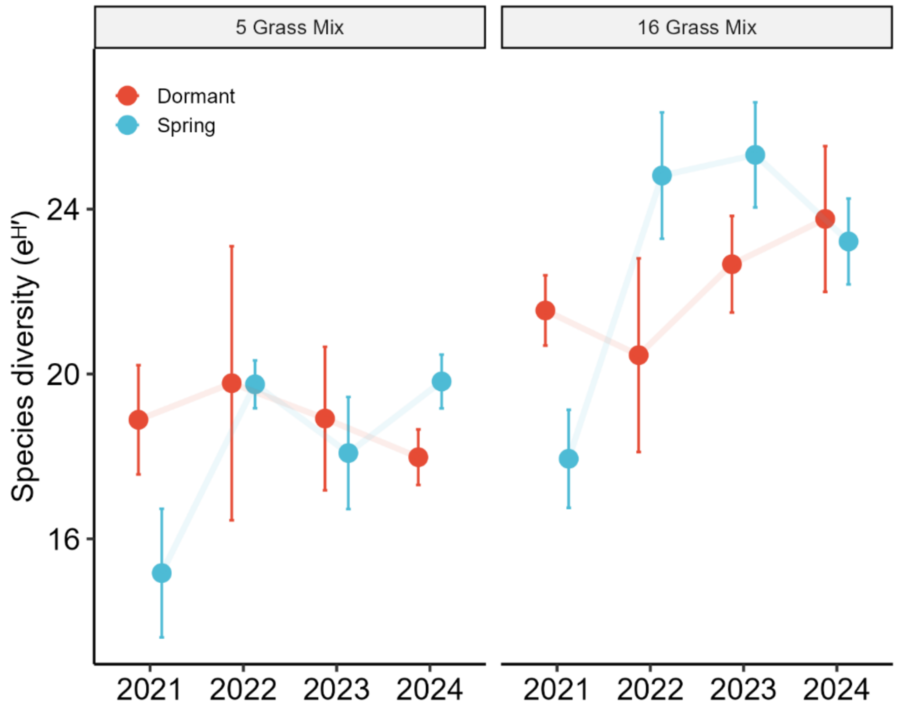


Figure 3.1.3. Species diversity (rarefied Shannon index) ± SE in seed mixes with a 16 spp. or 5 spp. graminoid component seeded in the dormant or spring season.

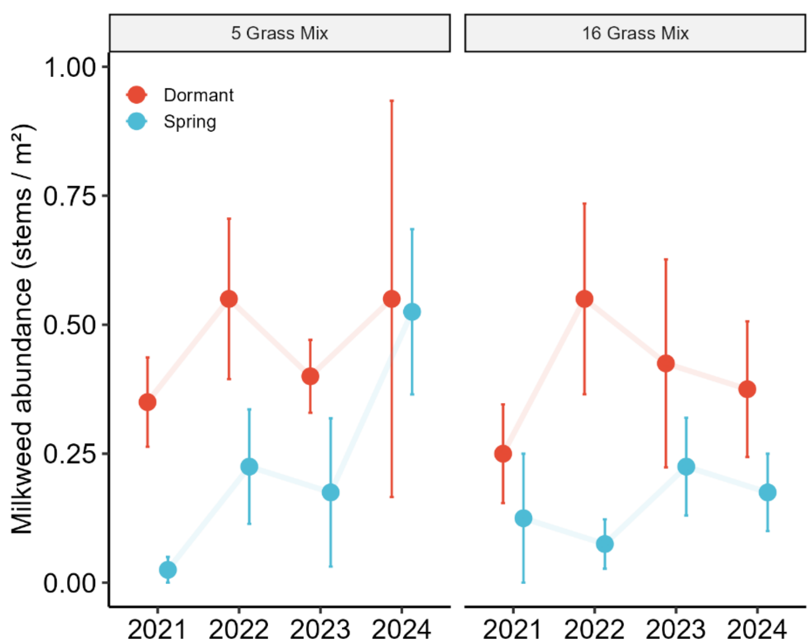


Figure 3.1.4. Milkweed (*Asclepias* spp.) stem density ± SE in seed mixes with a 16 spp. or 5 spp. graminoid component seeded in the dormant or spring season.

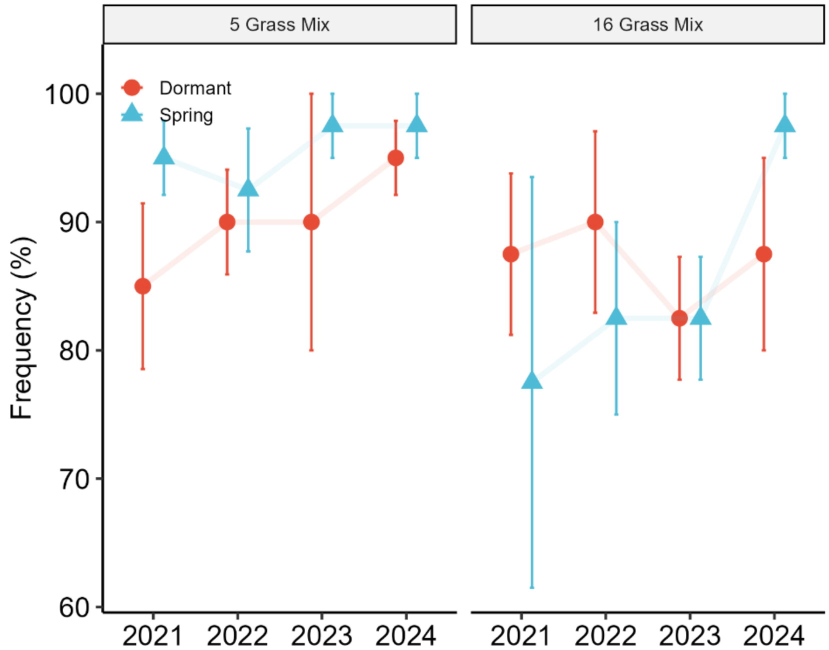


Figure 3.1.5. Proportion of quadrats occupied (%) ± SE of high value species for monarch habitat in seed mixes with a 16 spp. or 5 spp. graminoid component seeded in the dormant or spring season.

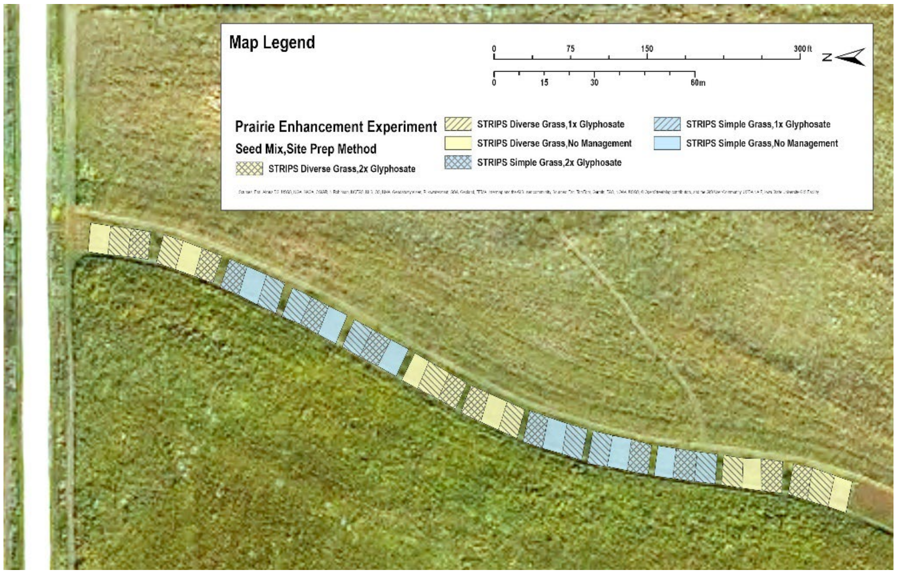


Figure 3.2.1 Experiment map. Site located in Eastern Iowa at Irvine Prairie (Benton County, IA).

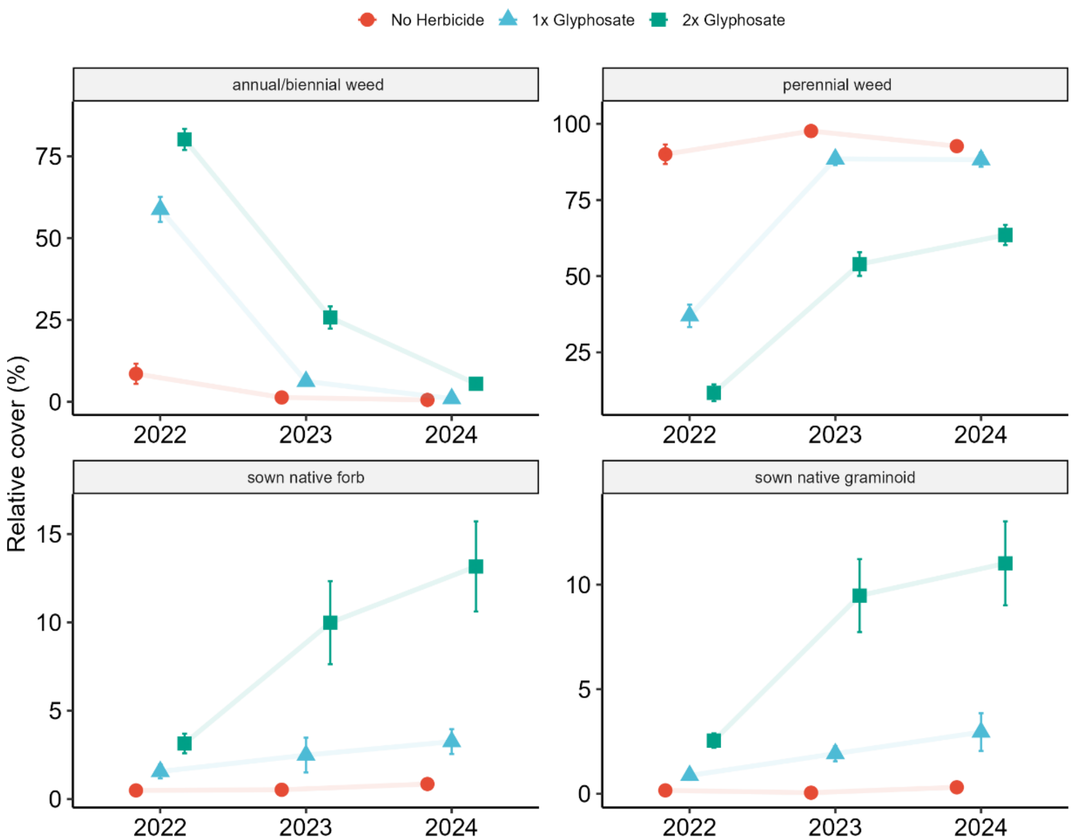


Figure 3.2.1 Relative canopy cover ± SE of sown grasses and forbs, perennial weeds, and ruderal (annual/biennial) weeds in plots treated with no herbicide, one application of glyphosate, or two applications of glyphosate.

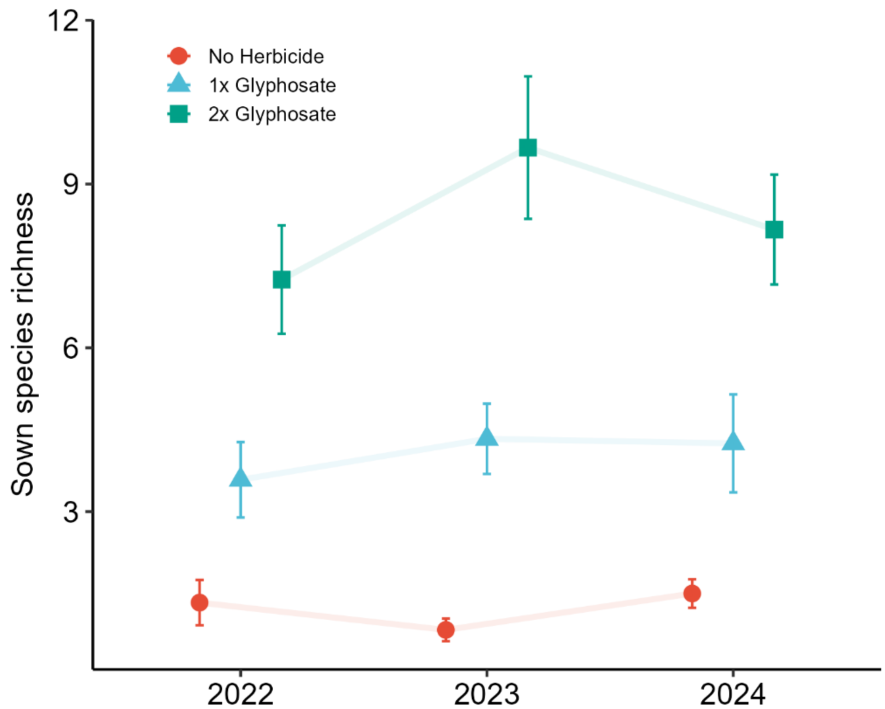


Figure 3.2.2 Sown species richness ± SE in plots treated with no herbicide, one application of glyphosate, or two applications of glyphosate.

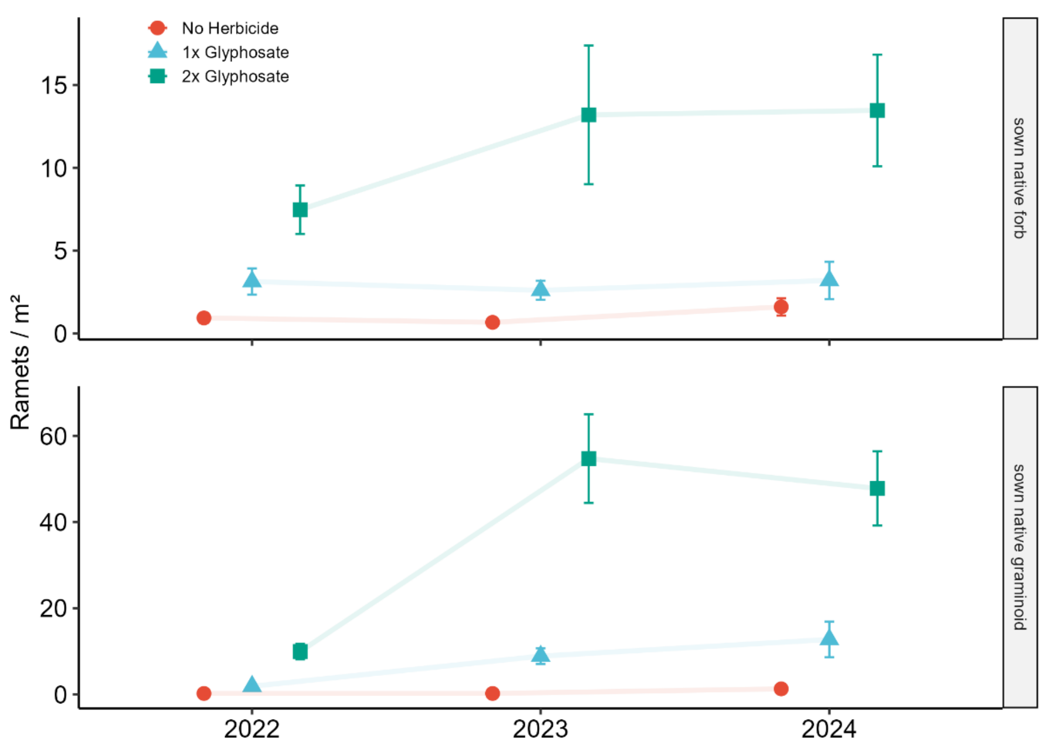


Figure 3.2.3 Establishment of sown graminoids and forbs (stem density ± SE ) in plots treated with no herbicide, one application of glyphosate, or two applications of glyphosate.

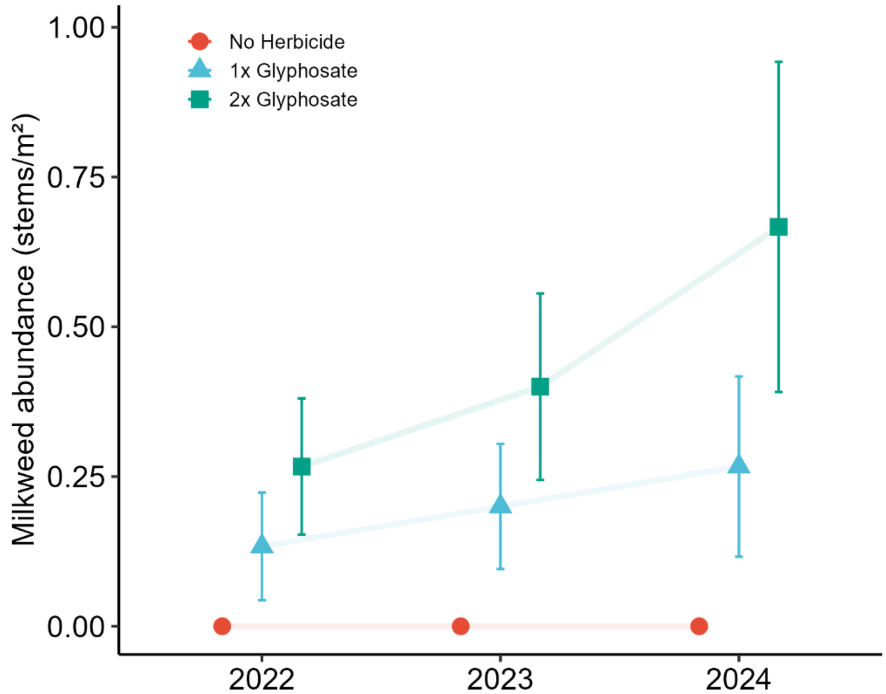


Figure 3.2.4 Milkweed abundance (all ramets of *Asclepias* spp.) ± SE in plots treated with no herbicide, one application of glyphosate, or two applications of glyphosate.

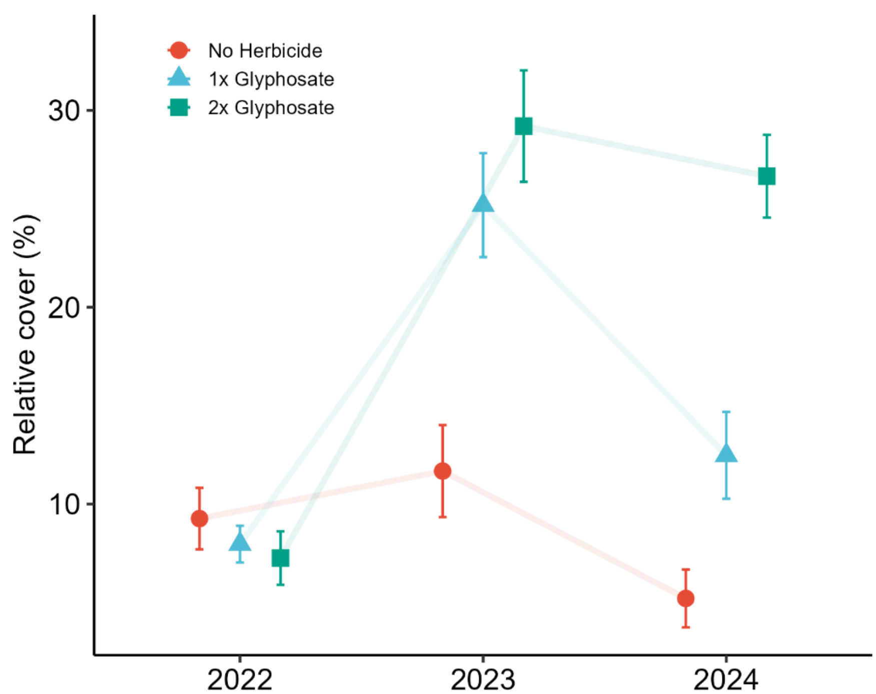


Figure 3.2.4 Relative canopy cover ± SE of plants with high value to monarchs in plots treated with no herbicide, one application of glyphosate, or two applications of glyphosate. High value species are those documented in other published studies to be used for nectaring by monarchs.

**Appendix 1. Common species found in expiring CP-25 fields in eastern Iowa.**

| *Scientific Name (ITIS)* | *Mean Freq.* | *SE¹* | *Mean Relative Cover* | *SE²* | *Vegetation Type* | *Monarch Value (NRCS 2019*)* | *Monarch Habitat Type* | *Nectar Plant* | *Typical CP-25 Seed Mix Frequency* |
|---|---|---|---|---|---|---|---|---|---|
| *Andropogon gerardii* | 0.63 | 0.07 | 0.20 | 0.04 | native perennial graminoid | none | non-nectar plant | non-nectar plant | |
| *Bromus inermis* | 0.66 | 0.08 | 0.20 | 0.04 | perennial weed | none | non-nectar plant | non-nectar plant | |
| *Solidago canadensis* | 0.64 | 0.07 | 0.17 | 0.03 | ruderal perennial forb | high | fall nectar plant | nectar plant | |
| *Poa pratensis* | 0.73 | 0.06 | 0.10 | 0.01 | perennial weed | none | non-nectar plant | non-nectar plant | |
| *Monarda fistulosa* | 0.48 | 0.06 | 0.04 | 0.01 | native perennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Ratibida pinnata* | 0.38 | 0.07 | 0.03 | 0.01 | native perennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Pastinaca sativa* | 0.49 | 0.07 | 0.03 | 0.01 | ruderal weed | low | summer nectar plant | nectar plant | |
| *Phalaris arundinacea* | 0.08 | 0.03 | 0.03 | 0.01 | perennial weed | none | non-nectar plant | non-nectar plant | |
| *Sorghastrum nutans* | 0.31 | 0.06 | 0.02 | 0.01 | native perennial graminoid | none | non-nectar plant | non-nectar plant | |
| *Solidago gigantea* | 0.09 | 0.03 | 0.02 | 0.01 | native perennial forb | high | fall nectar plant | nectar plant | |
| *Schizachyrium scoparium* | 0.10 | 0.03 | 0.01 | 0.01 | native perennial graminoid | none | non-nectar plant | non-nectar plant | |
| *Daucus carota* | 0.29 | 0.06 | 0.01 | 0.00 | ruderal weed | low | summer nectar plant | nectar plant | |
| *Taraxacum officinale* | 0.21 | 0.06 | 0.01 | 0.00 | perennial weed | low | spring nectar plant | nectar plant | |
| *Helianthus maximiliani* | 0.06 | 0.03 | 0.01 | 0.00 | native perennial forb | high | fall nectar plant | nectar plant | |
| *Solidago rigida* | 0.10 | 0.04 | 0.01 | 0.00 | native perennial forb | high | fall nectar plant | nectar plant | commonly seeded |
| *Zizia aurea* | 0.07 | 0.04 | 0.01 | 0.01 | native perennial forb | low | spring nectar plant | nectar plant | commonly seeded |
| *Erigeron annuus* | 0.13 | 0.03 | 0.01 | 0.00 | ruderal weed | high | summer nectar plant | nectar plant | |
| *Echinacea purpurea* | 0.09 | 0.04 | 0.01 | 0.00 | native perennial forb | very high | summer nectar plant | nectar plant | commonly seeded |
| *Cirsium discolor* | 0.20 | 0.03 | 0.01 | 0.00 | native annual/biennial forb | high | summer nectar plant | nectar plant | |
| *Asclepias syriaca* | 0.12 | 0.03 | 0.01 | 0.00 | ruderal perennial forb | very high | summer nectar plant | nectar plant | |
| *Desmodium canadense* | 0.07 | 0.03 | 0.01 | 0.00 | native perennial forb | low | summer nectar plant | nectar plant | |
| *Cirsium arvense* | 0.07 | 0.04 | 0.00 | 0.00 | perennial weed | high | summer nectar plant | nectar plant | |
| *Symphyotrichum pilosum* | 0.13 | 0.03 | 0.00 | 0.00 | ruderal perennial forb | high | fall nectar plant | nectar plant | |
| *Helianthus grosseserratus* | 0.04 | 0.03 | 0.00 | 0.00 | native perennial forb | very high | fall nectar plant | nectar plant | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Rudbeckia hirta* | 0.12 | 0.02 | 0.00 | 0.00 | native annual/biennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Calystegia sepium* | 0.12 | 0.05 | 0.00 | 0.00 | ruderal perennial forb | low | summer nectar plant | nectar plant | |
| *Spartina pectinata* | 0.03 | 0.03 | 0.00 | 0.00 | native perennial graminoid | none | non-nectar plant | non-nectar plant | |
| *Chamaecrista fasciculata* | 0.08 | 0.04 | 0.00 | 0.00 | native annual/biennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Bouteloua curtipendula* | 0.01 | 0.01 | 0.00 | 0.00 | native perennial graminoid | none | non-nectar plant | non-nectar plant | |
| *Symphyotrichum novae-angliae* | 0.02 | 0.01 | 0.00 | 0.00 | native perennial forb | very high | fall nectar plant | nectar plant | |
| *Galium aparine* | 0.03 | 0.01 | 0.00 | 0.00 | native annual/biennial forb | low | spring nectar plant | nectar plant | |
| *Vitis riparia* | 0.02 | 0.01 | 0.00 | 0.00 | other native plant | low | spring nectar plant | nectar plant | |
| *Cornus sericea* | 0.02 | 0.01 | 0.00 | 0.00 | woody plants | none | non-nectar plant | non-nectar plant | |
| *Ulmus pumila* | 0.01 | 0.01 | 0.00 | 0.00 | woody plants | none | non-nectar plant | non-nectar plant | |
| *Fraxinus pennsylvanica* | 0.01 | 0.01 | 0.00 | 0.00 | woody plants | none | non-nectar plant | non-nectar plant | |
| *Fraxinus pennsylvanica* | 0.01 | 0.01 | 0.00 | 0.00 | woody plants | none | non-nectar plant | non-nectar plant | |
| *Arctium minus* | 0.01 | 0.00 | 0.00 | 0.00 | ruderal weed | low | summer nectar plant | nectar plant | |
| *Lespedeza capitata* | 0.03 | 0.03 | 0.00 | 0.00 | native perennial forb | low | summer nectar plant | nectar plant | |
| *Pycnanthemum virginianum* | 0.03 | 0.03 | 0.00 | 0.00 | native perennial forb | high | summer nectar plant | nectar plant | |
| *Medicago lupulina* | 0.03 | 0.02 | 0.00 | 0.00 | perennial weed | low | summer nectar plant | nectar plant | |
| *Equisetum arvense* | 0.03 | 0.02 | 0.00 | 0.00 | other native plant | none | non-nectar plant | non-nectar plant | |
| *Morus alba* | 0.01 | 0.00 | 0.00 | 0.00 | woody plants | none | non-nectar plant | non-nectar plant | |
| *Parthenocissus quinquefolia* | 0.03 | 0.01 | 0.00 | 0.00 | other native plant | high | summer nectar plant | nectar plant | |
| *Apocynum cannabinum* | 0.04 | 0.01 | 0.00 | 0.00 | native perennial forb | high | summer nectar plant | nectar plant | |
| *Heliopsis helianthoides* | 0.02 | 0.01 | 0.00 | 0.00 | native perennial forb | very high | summer nectar plant | nectar plant | commonly seeded |
| *Achillea millefolium* | 0.03 | 0.02 | 0.00 | 0.00 | ruderal perennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Veronicastrum virginicum* | 0.02 | 0.01 | 0.00 | 0.00 | native perennial forb | high | summer nectar plant | nectar plant | commonly seeded |
| *Solanum carolinense* | 0.03 | 0.01 | 0.00 | 0.00 | ruderal perennial forb | low | summer nectar plant | nectar plant | |
| *Rudbeckia subtomentosa* | 0.01 | 0.01 | 0.00 | 0.00 | native perennial forb | high | fall nectar plant | nectar plant | |

**Appendix 2. Seed mixes planted as treatments at the Roadman Farm Demonstration Area and the Irvine Prairie Grass Stand Enhancement Experiment.**

***Diverse Graminoid Mix (16 graminoid species)***

| *Common Name* | *Scientific Name* | *Functional group* | *Seeds/m2* |
|---|---|---|---|
| big bluestem | *Andropogon gerardii* | warm-season graminoid | 10.76 |
| sideoats grama | *Bouteloua curtipendula* | warm-season graminoid | 37.67 |
| marsh muhly | *Muhlenbergia racemosa* | warm-season graminoid | 2.69 |
| switchgrass | *Panicum virgatum* | warm-season graminoid | 10.76 |
| little bluestem | *Schizachyrium scoparium* | warm-season graminoid | 37.67 |
| Indiangrass | *Sorghastrum nutans* | warm-season graminoid | 13.99 |
| composite dropseed | *Sporobolus compositus* | warm-season graminoid | 48.44 |
| prairie dropseed | *Sporobolus heterolepis* | warm-season graminoid | 0.54 |
| yellowfruit sedge | *Carex annectens* | cool-season graminoid | 10.76 |
| Bicknell's sedge | *Carex bicknellii* | cool-season graminoid | 2.15 |
| shortbeak sedge | *Carex brevior* | cool-season graminoid | 8.61 |
| heavy sedge | *Carex gravida* | cool-season graminoid | 0.22 |
| troublesome sedge | *Carex molesta* | cool-season graminoid | 4.31 |
| Canada wildrye | *Elymus canadensis* | cool-season graminoid | 8.61 |
| Virginia wildrye | *Elymus virginicus* | cool-season graminoid | 7.53 |
| poverty rush | *Juncus tenuis* | cool-season graminoid | 10.76 |
| Canadian anemone | *Anemone canadensis* | spring forb | 0.22 |
| candle anemone | *Anemone cylindrica* | spring forb | 0.54 |
| New Jersey tea | *Ceanothus americanus* | spring forb | 0.54 |
| foxglove beardtongue | *Penstemon digitalis* | spring forb | 10.76 |
| downy phlox | *Phlox pilosa* | spring forb | 0.22 |
| longbract spiderwort | *Tradescantia bracteata* | spring forb | 0.54 |
| bluejacket | *Tradescantia ohiensis* | spring forb | 1.08 |
| golden zizia | *Zizia aurea* | spring forb | 2.69 |
| swamp milkweed | *Asclepias incarnata* | summer forb | 1.08 |
| common milkweed | *Asclepias syriaca* | summer forb | 2.15 |
| butterfly milkweed | *Asclepias tuberosa* | summer forb | 0.32 |
| whorled milkweed | *Asclepias verticillata* | summer forb | 0.54 |
| Canadian milkvetch | *Astragalus canadensis* | summer forb | 10.76 |
| largeleaf wild indigo | *Baptisia lactea* | summer forb | 0.22 |
| partridge pea | *Chamaecrista fasciculata* | summer forb | 3.23 |
| stiff tickseed | *Coreopsis palmata* | summer forb | 0.43 |
| purple prairie clover | *Dalea purpurea* | summer forb | 10.76 |
| showy ticktrefoil | *Desmodium canadense* | summer forb | 1.61 |
| Illinois ticktrefoil | *Desmodium illinoense* | summer forb | 0.54 |
| tall cinquefoil | *Drymocallis arguta* | summer forb | 10.76 |
| pale purple coneflower | *Echinacea pallida* | summer forb | 2.15 |
| button eryngo | *Eryngium yuccifolium* | summer forb | 2.15 |
| flowering spurge | *Euphorbia corollata* | summer forb | 0.32 |
| northern bedstraw | *Galium boreale* | summer forb | 1.08 |
| smooth oxeye | *Heliopsis helianthoides* | summer forb | 5.38 |
| roundhead lespedeza | *Lespedeza capitata* | summer forb | 0.54 |

| | | | |
|---|---|---|---|
| wild bergamot | *Monarda fistulosa* | summer forb | 8.07 |
| wild quinine | *Parthenium integrifolium* | summer forb | 1.08 |
| whorled mountainmint | *Pycnanthemum pilosum* | summer forb | 8.07 |
| narrowleaf mountainmint | *Pycnanthemum tenuifolium* | summer forb | 10.76 |
| Virginia mountainmint | *Pycnanthemum virginianum* | summer forb | 10.76 |
| pinnate prairie coneflower | *Ratibida pinnata* | summer forb | 10.76 |
| blackeyed Susan | *Rudbeckia hirta* | summer forb | 8.07 |
| wholeleaf rosinweed | *Silphium integrifolium* | summer forb | 0.22 |
| compassplant | *Silphium laciniatum* | summer forb | 0.11 |
| purple meadow-rue | *Thalictrum dasycarpum* | summer forb | 0.54 |
| Culver's root | *Veronicastrum virginicum* | summer forb | 5.38 |
| white sagebrush | *Artemisia ludoviciana* | fall forb | 10.76 |
| tall thoroughwort | *Eupatorium altissimum* | fall forb | 2.69 |
| flat-top goldentop | *Euthamia graminifolia* | fall forb | 10.76 |
| closed bottle gentian | *Gentiana andrewsii* | fall forb | 5.38 |
| sawtooth sunflower | *Helianthus grosseserratus* | fall forb | 1.08 |
| stiff sunflower | *Helianthus pauciflorus ssp.* | fall forb | 0.22 |
| prairie blazing star | *Liatris pycnostachya* | fall forb | 1.08 |
| great blue lobelia | *Lobelia siphilitica* | fall forb | 10.76 |
| sweet coneflower | *Rudbeckia subtomentosa* | fall forb | 8.07 |
| stiff goldenrod | *Solidago rigida* | fall forb | 8.07 |
| showy goldenrod | *Solidago speciosa* | fall forb | 8.07 |
| smooth blue aster | *Symphyotrichum laeve* | fall forb | 5.38 |
| New England aster | *Symphyotrichum novae-angliae* | fall forb | 5.38 |
| skyblue aster | *Symphyotrichum oolentangiense* | fall forb | 2.69 |
| prairie ironweed | *Vernonia fasciculata* | fall forb | 2.69 |
| | *Overall total:* | | 433.04 |

***Simple Graminoid Mix (5 graminoid species)***

| *Common Name* | *Scientific Name* | *Functional group* | *Seeds/m²* |
|---|---|---|---|
| big bluestem | *Andropogon gerardii* | warm-season graminoid | 53.82 |
| switchgrass | *Panicum virgatum* | warm-season graminoid | 43.06 |
| little bluestem | *Schizachyrium scoparium* | warm-season graminoid | 32.29 |
| Indiangrass | *Sorghastrum nutans* | warm-season graminoid | 53.82 |
| Canada wildrye | *Elymus canadensis* | cool-season graminoid | 32.29 |
| Canadian anemone | *Anemone canadensis* | spring forb | 0.22 |
| candle anemone | *Anemone cylindrica* | spring forb | 0.54 |
| New Jersey tea | *Ceanothus americanus* | spring forb | 0.54 |
| foxglove beardtongue | *Penstemon digitalis* | spring forb | 10.76 |
| downy phlox | *Phlox pilosa* | spring forb | 0.22 |
| longbract spiderwort | *Tradescantia bracteata* | spring forb | 0.54 |
| bluejacket | *Tradescantia ohiensis* | spring forb | 1.08 |
| golden zizia | *Zizia aurea* | spring forb | 2.69 |
| swamp milkweed | *Asclepias incarnata* | summer forb | 1.08 |
| common milkweed | *Asclepias syriaca* | summer forb | 2.15 |
| butterfly milkweed | *Asclepias tuberosa* | summer forb | 0.32 |
| whorled milkweed | *Asclepias verticillata* | summer forb | 0.54 |
| Canadian milkvetch | *Astragalus canadensis* | summer forb | 10.76 |
| largeleaf wild indigo | *Baptisia lactea* | summer forb | 0.22 |
| partridge pea | *Chamaecrista fasciculata* | summer forb | 3.23 |
| stiff tickseed | *Coreopsis palmata* | summer forb | 0.43 |
| purple prairie clover | *Dalea purpurea* | summer forb | 10.76 |
| showy ticktrefoil | *Desmodium canadense* | summer forb | 1.61 |
| Illinois ticktrefoil | *Desmodium illinoense* | summer forb | 0.54 |
| tall cinquefoil | *Drymocallis arguta* | summer forb | 10.76 |
| pale purple coneflower | *Echinacea pallida* | summer forb | 2.15 |
| button eryngo | *Eryngium yuccifolium* | summer forb | 2.15 |
| flowering spurge | *Euphorbia corollata* | summer forb | 0.32 |
| northern bedstraw | *Galium boreale* | summer forb | 1.08 |
| smooth oxeye | *Heliopsis helianthoides* | summer forb | 5.38 |
| roundhead lespedeza | *Lespedeza capitata* | summer forb | 0.54 |
| wild bergamot | *Monarda fistulosa* | summer forb | 8.07 |
| wild quinine | *Parthenium integrifolium* | summer forb | 1.08 |
| whorled mountainmint | *Pycnanthemum pilosum* | summer forb | 8.07 |
| narrowleaf mountainmint | *Pycnanthemum tenuifolium* | summer forb | 10.76 |
| Virginia mountainmint | *Pycnanthemum virginianum* | summer forb | 10.76 |
| pinnate prairie coneflower | *Ratibida pinnata* | summer forb | 10.76 |
| blackeyed Susan | *Rudbeckia hirta* | summer forb | 8.07 |
| wholeleaf rosinweed | *Silphium integrifolium* | summer forb | 0.22 |
| compassplant | *Silphium laciniatum* | summer forb | 0.11 |
| purple meadow-rue | *Thalictrum dasycarpum* | summer forb | 0.54 |
| Culver's root | *Veronicastrum virginicum* | summer forb | 5.38 |
| white sagebrush | *Artemisia ludoviciana* | fall forb | 10.76 |
| tall thoroughwort | *Eupatorium altissimum* | fall forb | 2.69 |
| flat-top goldentop | *Euthamia graminifolia* | fall forb | 10.76 |

| | | | |
|---|---|---|---|
| closed bottle gentian | *Gentiana andrewsii* | fall forb | 5.38 |
| sawtooth sunflower | *Helianthus grosseserratus* | fall forb | 1.08 |
| stiff sunflower | *Helianthus pauciflorus ssp.* | fall forb | 0.22 |
| prairie blazing star | *Liatris pycnostachya* | fall forb | 1.08 |
| great blue lobelia | *Lobelia siphilitica* | fall forb | 10.76 |
| sweet coneflower | *Rudbeckia subtomentosa* | fall forb | 8.07 |
| stiff goldenrod | *Solidago rigida* | fall forb | 8.07 |
| showy goldenrod | *Solidago speciosa* | fall forb | 8.07 |
| smooth blue aster | *Symphyotrichum laeve* | fall forb | 5.38 |
| New England aster | *Symphyotrichum novae-angliae* | fall forb | 5.38 |
| skyblue aster | *Symphyotrichum oolentangiense* | fall forb | 2.69 |
| prairie ironweed | *Vernonia fasciculata* | fall forb | 2.69 |
| | *Overall total:* | | 433.90 |

**Appendix 3. Plant Species Designations for Monarch Habitat Quality**

| *Common Name* | *Scientific Name* | *NRCS Monarch Value* | *Source* |
|---|---|---|---|
| common yarrow | *Achillea millefolium* | NA | Adamson et al. 2018; Antonsen et al. 2021; Fisher and Bradbury 2022; Lukens et al. 2020 |
| blue giant hyssop | *Agastache foeniculum* | High | Lukens et al. 2020; NRCS 2019 |
| yellow giant hyssop | *Agastache nepetoides* | High | NRCS 2019 |
| purple giant hyssop | *Agastache scrophulariifolia* | High | NRCS 2019 |
| white snakeroot | *Ageratina altissima* | High | Adamson et al. 2018; NRCS 2019 |
| leadplant | *Amorpha canescens* | High | Adamson et al. 2018; NRCS 2019 |
| blue star | *Amsonia tabernaemontana* | High | NRCS 2019 |
| Indian hemp | *Apocynum cannabinum* | High | Antonsen et al. 2021; NRCS 2019 |
| clasping milkweed | *Asclepias amplexicaulis* | High | NRCS 2019 |
| poke milkweed | *Asclepias exaltata* | High | NRCS 2019 |
| tall green milkweed | *Asclepias hirtella* | High | NRCS 2019 |
| swamp milkweed | *Asclepias incarnata* | Very High | Adamson et al. 2018; Antonsen et al. 2021; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| oval leaf milkweed | *Asclepias ovalifolia* | High | Antonsen et al. 2021; NRCS 2019 |
| purple milkweed | *Asclepias purpurascens* | High | NRCS 2019 |
| showy milkweed | *Asclepias speciosa* | High | Antonsen et al. 2021; NRCS 2019 |
| prairie milkweed | *Asclepias sullivantii* | High | NRCS 2019 |
| common milkweed | *Asclepias syriaca* | Very High | Adamson et al. 2018; Antonsen et al. 2021; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| butterfly milkweed | *Asclepias tuberosa* | Very High | Adamson et al. 2018; Fisher and Bradbury 2022; NRCS 2019; Rudolph et al. 2006 |
| whorled milkweed | *Asclepias verticillata* | Very High | Adamson et al. 2018; Fisher and Bradbury 2022; NRCS 2019 |
| green milkweed | *Asclepias viridiflora* | High | NRCS 2019 |
| spider milkweed | *Asclepias viridis* | Very High | Adamson et al. 2018; NRCS 2019 |
| swamp marigold | *Bidens aristosa* | Very High | Adamson et al. 2018; NRCS 2019; Rudolph et al. 2006 |
| nodding bur marigold | *Bidens cernua* | High | NRCS 2019 |
| smooth beggarticks | *Bidens laevis* | High | NRCS 2019 |
| Ohio horse mint | *Blephilia ciliata* | High | NRCS 2019 |
| wood mint | *Blephilia hirsuta* | High | NRCS 2019 |
| false aster | *Boltonia asteroides* | High | NRCS 2019 |
| false boneset | *Brickellia eupatorioides* | High | Adamson et al. 2018; NRCS 2019 |
| musk thistle | *Carduus nutans* | NA | Antonsen et al. 2021; Lukens et al. 2020 |
| buttonbush | *Cephalanthus occidentalis* | Very High | Adamson et al. 2018; NRCS 2019 |
| leatherleaf | *Chamaedaphne calyculata* | High | NRCS 2019 |
| chicory | *Cichorium intybus* | NA | Fisher and Bradbury 2022 |
| tall thistle | *Cirsium altissimum* | Very High | Adamson et al. 2018; NRCS 2019 |
| Canada thistle | *Cirsium arvense* | NA | Antonsen et al. 2021; Lukens et al. 2020 |
| field thistle | *Cirsium discolor* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| Flodman's thistle | *Cirsium flodmanii* | High | Antonsen et al. 2021; NRCS 2019 |

| | | | |
|---|---|---|---|
| swamp thistle | *Cirsium muticum* | High | Keele et al. 2023; NRCS 2019 |
| wavy leaf thistle | *Cirsium undulatum* | NA | Antonsen et al. 2021 |
| bull thistle | *Cirsium vulgare* | NA | Antonsen et al. 2021; Fisher and Bradbury 2022; Keele et al. 2023; Lukens et al. 2020 |
| mistflower | *Conoclinium coelestinum* | High | Adamson et al. 2018; NRCS 2019 |
| horseweed | *Conyza canadensis* | NA | Lukens et al. 2020 |
| lance leaf coreopsis | *Coreopsis lanceolata* | High | NRCS 2019 |
| prairie coreopsis | *Coreopsis palmata* | High | Adamson et al. 2018; NRCS 2019 |
| tall coreopsis | *Coreopsis tripteris* | High | Adamson et al. 2018; NRCS 2019 |
| narrowleaf hawksbeard | *Crepis tectorum* | NA | Lukens et al. 2020 |
| common dittany | *Cunila origanoides* | NA | Rudolph et al. 2006 |
| white prairie clover | *Dalea candida* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| round head prairie clover | *Dalea multiflora* | High | NRCS 2019 |
| purple prairie clover | *Dalea purpurea* | High | Lukens et al. 2020; NRCS 2019 |
| dwarf larkspur | *Delphinium tricorne* | High | NRCS 2019 |
| Dutchman's breeches | *Dicentra cucullaria* | High | NRCS 2019 |
| flat topped aster | *Doellingeria umbellata* | High | Adamson et al. 2018; Keele et al. 2023; NRCS 2019 |
| narrow leaved coneflower | *Echinacea angustifolia* | High | Adamson et al. 2018; NRCS 2019 |
| pale purple coneflower | *Echinacea pallida* | High | Adamson et al. 2018; Fisher and Bradbury 2022; NRCS 2019 |
| purple coneflower | *Echinacea purpurea* | Very High | Adamson et al. 2018; Antonsen et al. 2021; Lukens et al. 2020; NRCS 2019 |
| annual fleabane | *Erigeron annuus* | NA | Lukens et al. 2020 |
| rattlesnake master | *Eryngium yuccifolium* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| tall boneset | *Eupatorium altissimum* | Very High | Adamson et al. 2018; NRCS 2019 |
| boneset | *Eupatorium perfoliatum* | High | Adamson et al. 2018; NRCS 2019 |
| late boneset | *Eupatorium serotinum* | Very High | Adamson et al. 2018; NRCS 2019; Rudolph et al. 2006 |
| grass leaved goldenrod | *Euthamia graminifolia* | Very High | Adamson et al. 2018; NRCS 2019 |
| trumpetweed | *Eutrochium fistulosum* | High | Adamson et al. 2018; NRCS 2019 |
| spotted Joe-pye weed | *Eutrochium maculatum* | Very High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| sweet Joe-pye weed | *Eutrochium purpureum* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| wild licorice | *Glycyrrhiza lepidota* | NA | Antonsen et al. 2021 |
| bitterweed | *Helenium amarum* | NA | Rudolph et al. 2006 |
| common sunflower | *Helianthus annuus* | Very High | Adamson et al. 2018; NRCS 2019 |
| woodland sunflower | *Helianthus divaricatus* | High | Adamson et al. 2018; NRCS 2019 |
| giant sunflower | *Helianthus giganteus* | High | Antonsen et al. 2021; NRCS 2019 |
| sawtooth sunflower | *Helianthus grosseserratus* | Very High | Adamson et al. 2018; NRCS 2019 |
| Maximilian sunflower | *Helianthus maximiliani* | High | Adamson et al. 2018; Antonsen et al. 2021; Lukens et al. 2020; NRCS 2019 |
| downy sunflower | *Helianthus mollis* | High | NRCS 2019 |
| western sunflower | *Helianthus occidentalis* | High | NRCS 2019 |
| stiff sunflower | *Helianthus pauciflorus* | High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| paleleaf sunflower | *Helianthus strumosus* | High | NRCS 2019 |
| Jerusalem artichoke | *Helianthus tuberosus* | High | NRCS 2019 |

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|---|---|---|---|
| showy sunflower | *Helianthus X laetiflorus* | High | NRCS 2019 |
| false sunflower | *Heliopsis helianthoides* | Very High | Adamson et al. 2018; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| hairy false goldenaster | *Heterotheca villosa* | NA | Antonsen et al. 2021 |
| mouse ear hawkweed | *Hieracium pilosella* | NA | Lukens et al. 2020 |
| twoflower dwarfdandelion | *Krigia biflora* | High | NRCS 2019 |
| blue lettuce | *Lactuca tatarica* | NA | Antonsen et al. 2021 |
| rough blazing star | *Liatris aspera* | Very High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| cylindrical blazing star | *Liatris cylindracea* | Very High | Adamson et al. 2018; NRCS 2019 |
| pinkscale blazing star | *Liatris elegans* | NA | Rudolph et al. 2006 |
| meadow blazing star | *Liatris ligulistylis* | Very High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| dotted blazing star | *Liatris punctata* | High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| prairie blazing star | *Liatris pycnostachya* | High | Adamson et al. 2018; NRCS 2019 |
| savanna blazing star | *Liatris scariosa* | High | Adamson et al. 2018; NRCS 2019 |
| marsh blazing star | *Liatris spicata* | High | Adamson et al. 2018; NRCS 2019 |
| Turk's cap lily | *Lilium superbum* | High | NRCS 2019 |
| hoary puccoon | *Lithospermum canescens* | High | NRCS 2019 |
| cardinal flower | *Lobelia cardinalis* | High | NRCS 2019 |
| great blue lobelia | *Lobelia siphilitica* | High | NRCS 2019 |
| alfalfa | *Medicago sativa* | NA | Antonsen et al. 2021 |
| yellow sweet clover | *Melilotus officinalis* | NA | Antonsen et al. 2021; Fisher and Bradbury 2022 |
| Virginia bluebells | *Mertensia virginica* | High | NRCS 2019 |
| wild bergamot | *Monarda fistulosa* | High | Adamson et al. 2018; Antonsen et al. 2021; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| spotted beebalm | *Monarda punctata* | High | Adamson et al. 2018; NRCS 2019 |
| common evening primrose | *Oenothera biennis* | NA | Lukens et al. 2020 |
| marbleseed | *Onosmodium bejariense* | High | Antonsen et al. 2021; NRCS 2019 |
| golden ragwort | *Packera aurea* | High | NRCS 2019 |
| roundleaf ragwort | *Packera obovata* | High | NRCS 2019; Rudolph et al. 2006 |
| rocky mountain bee plant | *Peritoma serrulata* | NA | Antonsen et al. 2021 |
| Pennsylvania smartweed | *Persicaria pensylvanica* | NA | NRCS 2019, Rudolph et al. 2006 |
| blue phlox | *Phlox divaricata* | High | NRCS 2019 |
| smooth phlox | *Phlox glaberrima* | High | NRCS 2019 |
| garden phlox | *Phlox paniculata* | High | NRCS 2019 |
| prairie phlox | *Phlox pilosa* | High | NRCS 2019 |
| obedient plant | *Physostegia virginiana* | High | Adamson et al. 2018; NRCS 2019 |
| wild plum | *Prunus americana* | High | Adamson et al. 2018 |
| hoary mountain mint | *Pycnanthemum incanum* | High | NRCS 2019 |
| hairy mountain mint | *Pycnanthemum pilosum* | High | NRCS 2019 |
| slender mountain mint | *Pycnanthemum tenuifolium* | High | Adamson et al. 2018; NRCS 2019 |
| Virginia mountain mint | *Pycnanthemum virginianum* | High | NRCS 2019 |
| yellow coneflower | *Ratibida pinnata* | High | Fisher and Bradbury 2022; Lukens et al. 2020 |
| smooth sumac | *Rhus glabra* | High | Adamson et al. 2018 |
| northern dewberry | *Rubus flagellaris* | High | NRCS 2019 |

| | | | |
|---|---|---|---|
| orange coneflower | *Rudbeckia fulgida* | High | NRCS 2019 |
| Black-eyed Susan | *Rudbeckia hirta* | High | Adamson et al. 2018; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| wild golden glow | *Rudbeckia laciniata* | High | NRCS 2019 |
| sweet black-eyed Susan | *Rudbeckia subtomentosa* | High | NRCS 2019 |
| brown-eyed Susan | *Rudbeckia triloba* | High | NRCS 2019 |
| blue sage | *Salvia azurea* | High | Adamson et al. 2018; NRCS 2019 |
| rosinweed | *Silphium integrifolium* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| compass plant | *Silphium laciniatum* | High | Adamson et al. 2018; NRCS 2019 |
| cup plant | *Silphium perfoliatum* | High | Lukens et al. 2020; NRCS 2019 |
| prairie dock | *Silphium terebinthinaceum* | High | NRCS 2019 |
| hemlock water parsnip | *Sium suave* | High | NRCS 2019 |
| Canada goldenrod | *Solidago canadensis* | High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| zigzag goldenrod | *Solidago flexicaulis* | High | NRCS 2019 |
| late goldenrod | *Solidago gigantea* | High | Antonsen et al. 2021; NRCS 2019 |
| early goldenrod | *Solidago juncea* | High | NRCS 2019 |
| Missouri goldenrod | *Solidago missouriensis* | NA | Antonsen et al. 2021 |
| gray goldenrod | *Solidago nemoralis* | High | Adamson et al. 2018; NRCS 2019 |
| downy goldenrod | *Solidago petiolaris* | NA | Rudolph et al. 2006 |
| upland white aster | *Solidago ptarmicoides* | High | NRCS 2019 |
| Riddell's goldenrod | *Solidago riddellii* | High | NRCS 2019 |
| stiff goldenrod | *Solidago rigida* | High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| wrinkleleaf goldenrod | *Solidago rugosa* | NA | Rudolph et al. 2006 |
| showy goldenrod | *Solidago speciosa* | Very High | Adamson et al. 2018; NRCS 2019 |
| elm leaved goldenrod | *Solidago ulmifolia* | High | NRCS 2019 |
| field sow thistle | *Sonchus arvensis* | NA | Antonsen et al. 2021 |
| meadowsweet | *Spiraea alba* | NA | Adamson et al. 2018 |
| wolfberry | *Symphoricarpos occidentalis* | NA | Antonsen et al. 2021 |
| manyray aster | *Symphyotrichum anomalum* | NA | Rudolph et al. 2006 |
| heart leaved aster | *Symphyotrichum cordifolium* | High | Adamson et al. 2018; NRCS 2019 |
| Drummond's aster | *Symphyotrichum drummondii* | High | NRCS 2019 |
| heath aster | *Symphyotrichum ericoides* | High | Adamson et al. 2018; Rudolph et al. 2006; NRCS 2019 |
| smooth blue aster | *Symphyotrichum laeve* | High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| panicled aster | *Symphyotrichum lanceolatum* | High | NRCS 2019 |
| calico aster | *Symphyotrichum lateriflorum* | High | NRCS 2019 |
| New England aster | *Symphyotrichum novae-angliae* | Very High | Adamson et al. 2018; Lukens et al. 2020; NRCS 2019 |
| aromatic aster | *Symphyotrichum oblongifolium* | Very High | Adamson et al. 2018; NRCS 2019 |
| sky blue aster | *Symphyotrichum oolentangiense* | High | NRCS 2019 |
| hairy aster | *Symphyotrichum pilosum* | High | NRCS 2019 |
| willow aster | *Symphyotrichum praealtum* | High | NRCS 2019 |
| crooked stem aster | *Symphyotrichum prenanthoides* | High | NRCS 2019 |
| swamp aster | *Symphyotrichum puniceum* | High | NRCS 2019 |
| silky aster | *Symphyotrichum sericeum* | High | NRCS 2019 |

| | | | |
|---|---|---|---|
| Short's aster | *Symphyotrichum shortii* | High | NRCS 2019 |
| prairie aster | *Symphyotrichum turbinellum* | High | NRCS 2019 |
| arrow leaved aster | *Symphyotrichum urophyllum* | High | NRCS 2019 |
| common dandelion | *Taraxacum officinale* | NA | Fisher and Bradbury 2022 |
| alsike clover | *Trifolium hybridum* | NA | Lukens et al. 2020 |
| red clover | *Trifolium pratense* | NA | Fisher and Bradbury 2022; Lukens et al. 2020 |
| white clover | *Trifolium repens* | NA | Fisher and Bradbury 2022 |
| blue vervain | *Verbena hastata* | High | NRCS 2019 |
| hoary vervain | *Verbena stricta* | High | Adamson et al. 2018; Antonsen et al. 2021; Fisher and Bradbury 2022; Lukens et al. 2020; NRCS 2019 |
| wingstem | *Verbesina alternifolia* | High | Adamson et al. 2018; NRCS 2019 |
| yellow crownbeard | *Verbesina helianthoides* | High | NRCS 2019 |
| white crownbeard | *Verbesina virginica* | High | NRCS 2019 |
| western ironweed | *Vernonia baldwinii* | High | NRCS 2019; Rudolph et al. 2006 |
| ironweed | *Vernonia fasciculata* | High | Adamson et al. 2018; Antonsen et al. 2021; NRCS 2019 |
| tall ironweed | *Vernonia gigantea* | High | Adamson et al. 2018; NRCS 2019 |
| Missouri ironweed | *Vernonia missurica* | High | NRCS 2019 |
| Culver's root | *Veronicastrum virginicum* | High | Adamson et al. 2018; NRCS 2019 |

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