



Original Article

Milkweed Establishment in Restored Central Missouri Prairie

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ABSTRACT The decline of the Eastern North American monarch butterfly (*Danaus plexippus*) population continues to be a major concern among conservationists. Numerous publications and organizations suggest that establishing milkweed (*Asclepias* spp.), at a landscape scale, is necessary to increase the monarch population. The translocation of commercially grown containerized plugs is commonly recommended to establish milkweed. During 2017, we used a split-plot design to examine the factors of age of prairie restoration, plant type, and land preparation method on survival of translocated milkweed in restored prairie in central Missouri, USA. Milkweeds were translocated to 16 0.81-ha sites, half classified as young (<8 yr) and half as old (>9 yr) prairie restorations. We randomly selected 8 sites to establish bare-root milkweed seedlings and 8 sites were planted with milkweed seedling plugs. Each site contained paired 0.4-ha plots. At the plot level we tested 2 land preparation techniques, disking with the application of pre-emergent herbicide and application of a grass herbicide. Milkweeds were planted at the density of 200 milkweeds/0.40 ha as suggested by the Missouri Monarch and Pollinator Conservation Plan. We conducted a census to determine milkweed survival 2 growing seasons after establishment. Two-year survival of translocated bare-root milkweed ($\bar{x} = 0.26$, 95% CI = 0.12–0.49) was nearly 5 times greater than that of milkweed seedling plugs ($\bar{x} = 0.05$, 95% CI = 0.02–0.13). Milkweed in disked plots had greater survival ($\bar{x} = 0.18$, 95% CI = 0.10–0.31) than in plots treated with grass herbicide ($\bar{x} = 0.08$, 95% CI = 0.04–0.16). Milkweed survival in older restored prairies ($\bar{x} = 0.07$, 95% CI = 0.03–0.18) was lower than that in newer prairie plantings ($\bar{x} = 0.21$, 95% CI = 0.09–0.41). Our results suggest translocation of bare-root milkweed seedlings, combined with disking and application of pre-emergent herbicide, is the preferred alternative. We suggest translocation of milkweed may be a feasible method of enhancing monarch habitat. However, meeting the challenge of restoring milkweed at recommended densities in established grasslands may be limited if milkweed seedling plugs are used. © 2020 The Wildlife Society.

KEY WORDS *Asclepias*, *Danaus plexippus*, milkweed establishment, Missouri, monarch butterfly, restored prairie.

Eastern North American populations of monarchs have declined dramatically based on surveys of wintering monarch butterflies (*Danaus plexippus*) in the oyamel (*Abies religiosa*) fir forests in Mexico (Monarchwatch.org 2018). The decline has been attributed to both deforestation of wintering grounds and loss of breeding habitat along migration routes through the midwestern United States

(Brower et al. 2012). Breeding habitat for monarchs must contain milkweed (*Asclepias* spp.) for which monarch larvae are an obligate species (Pitman et al. 2018). Pleasants (2017) describes 2 contributing factors that have led to the loss of monarch breeding habitat across the Midwest: 1) widespread use of glyphosate herbicides in agriculture, and 2) conversion of grassland habitats into agricultural production or development. Prior to the widespread use of glyphosate herbicides, milkweed patches within and on agricultural field margins supported greater densities of monarch eggs than patches in nonagricultural areas. Once those more robust and highly productive milkweed patches were removed from agricultural areas, nonagricultural areas such as pasture, acres enrolled in the Conservation Reserve Program, old fields, roadsides, and natural areas became the primary source of milkweeds for breeding monarchs (Pleasants and Oberhauser 2013).

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Missouri, USA, lies in the primary breeding range of the monarch butterfly and is seen as a priority for the restoration of milkweed at a landscape scale (U.S. Fish and Wildlife Service 2016). A concerted effort was made to bring greater focus to the plight of the monarch butterfly and pollinators in Missouri by holding a state summit in July 2015. Similar to the all hands on deck strategy outlined by Thogmartin et al. (2017), the Missouri summit engaged representatives from >40 agencies, nongovernmental organizations, businesses, and private landowners. From the many ideas generated during the summit emerged a vision of a monarch and pollinator habitat-conservation collaborative—Missourians for Monarchs—that combined the efforts of multiple entities and individuals to address awareness, education, and on-the-ground habitat improvements. Work of the collaborative resulted in a Missouri Monarch and Pollinator Conservation Plan (Missourians for Monarchs 2016). The goal of this plan is conserving, enhancing, and restoring monarch butterfly and pollinator habitat on public and private lands in Missouri. This goal supports the U.S. Fish and Wildlife Service 20-year national plan to develop 2.83 million ha of habitat resulting in >1.5 billion additional milkweed plants (Monarch Joint Venture 2019). It is estimated Missouri will need 7,700 ha of habitat a year, with a density of 200 milkweeds/0.40 ha, to meet habitat conservation needs (Missourians for Monarchs 2016). Public lands receive high priority in the Missouri Monarch and Pollinator Conservation Plan because of the possibility for sustained management on these lands (Missourians for Monarchs 2016). It is anticipated that milkweed will be established as part of the existing vegetation community on public lands.

We examined methods of establishing milkweed in restored prairie in central Missouri. We selected the 2 most common types of milkweed seedlings used in translocations, bare-root seedlings and container-grown seedlings often called plugs (Luna and Dumroese 2013, Monarch Joint Venture 2019). In consultation with land management biologists, we applied 2 treatments that reflect differing intensities of reducing existing plant competition. We translocated milkweed across a range of restored prairie ages. Our objectives were to estimate 2-year survival of translocated milkweed by seedling and treatment type and to test whether age of restored prairie affected establishment. We hypothesized that there would be no difference in survival by plant type and that greater survival of translocated milkweed would occur on younger prairie restorations and in disked treatments.

STUDY AREA

We conducted our project on Prairie Fork Conservation Area (PFCA) in Callaway County, Missouri (Fig. 1). The Missouri Department of Conservation (MDC) owns 290 ha and manages an adjacent 81 ha owned by the Missouri Prairie Foundation—these 2 parcels are collectively referred to as PFCA. We identified the distribution of historic prairie on PFCA based on the work of Schroeder (1981). We used an ecological classification system, developed for Missouri by Nigh and Schroeder (2002), to further define prairie soils. All

prairie soils had been in row-crop agriculture for >30 years. Reconstructions began in 2004 in the north portion of the area and progressed southward. To date, prairie reconstruction has taken place on 142 ha of the PFCA, making it one of the largest tracts of reconstructed prairie in Missouri.

To aid in large-scale restorations like those on PFCA, MDC collected locally grown native seed from remnant and restored prairies. Local ecotype seed was assumed to be adapted to local climate and biotic factors such as herbivory and pollinators (Mijnsbrugge et al. 2010). Most years, MDC was able to collect and plant a mixture of >200 species of native grasses and forbs, including up to 10 species of milkweeds (C. Newbold, Missouri Department of Conservation, personal communication). On PFCA new prairies are established by broadcasting the entire seed mixture in winter when snow is present.

Greater than 180 species of grasses and forbs were established in the reconstructed prairies on PFCA, including many species with high coefficients of conservatism (Ladd and Thomas 2015, Newbold et al. 2019). However, as a result of grass competition, milkweeds and other forbs were not as abundant as desired. Milkweed were estimated to have a density of <25 plants/ha on the reconstructed prairie plantings (C. Newbold, unpublished data). Older plantings on the northern half of PFCA were considered by managers to be heavily grass-dominated, whereas the newer plantings on the south end were not. This distinction is important because the age of the planting may determine the quantity and type of competition that may be present for milkweeds (Heslinga and Grese 2010). Reconstructed prairie communities were mowed in the establishment year and maintained by fire on a 2–3-year interval. Invasive species such as sericea lespedeza (*Lespedeza cuneata*) were spot-treated with herbicide as required.

METHODS

We identified 3 factors as potentially having the greatest effect on the establishment and survival of milkweeds within the reconstructed prairies on PFCA. The first was the type of propagule being planted. Both bare-root seedlings and seedling plugs are available for milkweed translocation. Bare-root seedlings are planted in the dormant season. Seedling plugs are typically grown from seed in greenhouses during late winter and early spring and planted in late spring or early summer.

The second factor pertained to site preparation methods to reduce competition from surrounding vegetation. Two site preparation methods were applied. The first was to mow sites with a rotary mower prior to planting, followed by the application of a grass-selective herbicide (Dakota, 26.4% clethodim, EPA Registration #83100-38-83979). This method killed or inhibited grasses actively growing at the time of application. The second site preparation method was to mow the sites, then disk the ground to bare-mineral soil, followed by application of a pre-emergent herbicide (Prowl, 38.7% pendimethalin, EPA Registration #241-418) prior to planting milkweeds. Pre-emergent herbicides inhibit meristem growth, preventing new shoots from sprouting.

The third factor was age of prairie reconstruction. Older plantings are considered grass-dominated, and primary

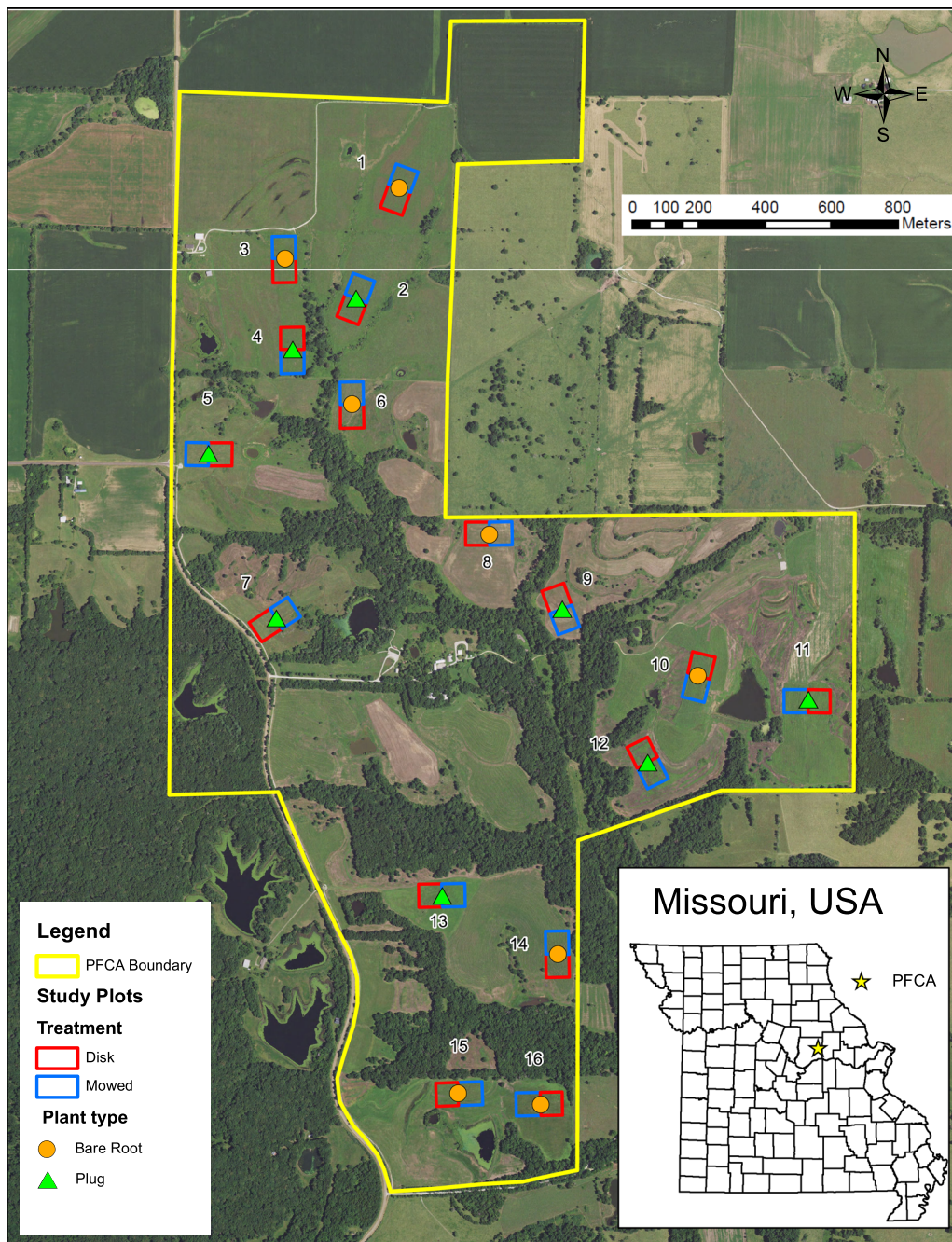


Figure 1. Location of study sites and plots within reconstructed prairies on Prairie Fork Conservation Area, Callaway County, Missouri, USA, where we examined the factors of age of prairie restoration, plant type, and land preparation method on survival of translocated milkweed in 2017.

competition is well-established perennial warm-season grasses such as big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*; Schroeder et al. 2012). In more recent prairie reconstructions, annuals such as species of foxtail (*Setaria* spp.) and ragweed (*Ambrosia* spp.) are the primary competitors. All plantings were on similar soil types identified as historically supporting prairie; therefore, we assumed age of prairie and geographic location on PFCA were not confounded.

Locating Study Sites and Milkweed Plantings

We selected 8 old reconstructed prairies, identified as those planted from 2004 to 2008 and 8 new reconstructed

prairies planted from 2010 to 2017. In each of these 16 reconstructed prairies, we systematically located a 0.81-ha study site (Fig. 1). We marked corners of the 64-m × 127-m study sites with T-posts. To minimize effects of untreated vegetation surrounding the study sites, we established a 4-m-wide buffer. Following the guidance of the Missouri Monarch and Pollinator Conservation Plan, our goal was to establish 200 milkweed stems/0.40 ha (Missourians for Monarchs 2016). We randomly assigned 8 of the 16 study sites to have bare-root milkweeds planted while the remaining half of the sites received milkweed seedling plugs. We then further divided each 0.81-ha

plots with an 8-m buffer between them. We mowed all 16 study sites to a height of 15 cm between 13 and 24 February 2017. We randomly assigned site preparation treatments of disking or grass herbicide to each plot. Following site preparation, we overlaid a 14-m \times 14-m grid on each plot, marking each node with a 10-cm \times 10-cm flag on a 40-cm-tall wire. This resulted in 196 individually marked locations on each plot with a 4-m spacing between them. We planted milkweeds at each marked location using dibble bars and plug planters. In the winter of 2018, we revisited all plots to replace deteriorated flags.

We planted common milkweed (*Asclepias syriaca*) bare-root seedlings, obtained from the MDC George O. White State Nursery, on 1 April 2017 on sites 1, 3, 6, 8, 10, 14, 15, and 16 (Fig. 1). Seedlings were 9 months of age and propagated using local ecotype seed in raised beds. The majority of bare-root seedlings were >25 cm tall and had substantial root mass. We conducted disking and application of pre-emergent herbicide at the plot level, within 1 week prior to planting. We applied a grass-selective herbicide to the other half of the plot 2–4 weeks after planting.

We planted common, tall green (*A. hirtella*), and swamp (*A. incarnata*) milkweed seedling plugs, on sites 2, 4, 5, 7, 9, 11, 12, and 13 on 3 June 2017 (Fig. 1). Plants were germinated in February 2017 by Grace Natives, a north Missouri wildflower nursery, from seed we collected the previous autumn at PFCA. Seedlings were grown in trays with 5-cm \times 5-cm cells containing a potting medium. Eighty-nine percent of the seedlings were common milkweed, 7% tall green milkweed, and 4% swamp milkweed. Seedlings averaged 18 cm tall and were typical of those propagated in containers. We mowed all sites planted with seedling plugs a second time 4 weeks prior to planting. At the plot level, we applied grass herbicide 2 weeks prior to planting. We completed disking of plots and application of pre-emergent herbicide 1 week prior to planting.

We mowed sites planted to bare-root seedlings to height of 40 cm (above the height of milkweed) in May–June 2017. We mowed sites with seedling plugs in July 2017. High mowing increases availability of light and reduces competition for resources among plants (Hillhouse and Zedler 2011, Natural Resources Conservation Service 2018). Once planted milkweed exceeded 40 cm in height, we only mowed between the rows to minimize the risk of damaging the milkweed plants. Mowing between rows was conducted twice, once in August–September 2017 and again in June–July 2018. We measured milkweed plant survival by conducting a complete census on each site between 7 July and 17 September 2018.

Statistical Analyses

We tested for differences in plant survival using PROC GLIMMIX (SAS Institute 2017). PROC GLIMMIX allows the analysis of nonnormally distributed data, in this case survival data with a binomial distribution. We entered survival data in the “events/trials” format, which was the ratio of the number of successful (alive) plants divided by the number originally planted ($N = 196/\text{plot}$). The logit link

function was used in the analysis. We present the mean survival rates from the LSMEANS statement, which were the result of applying the inverse link transformation to the least-square means reported in the estimate column. We also calculated odds ratios for each effect. We analyzed data by a $2 \times 2 \times 2$ factorial arrangement of treatments under a split-plot design. The main plot included the effects of age (old vs. new prairie plantings), the effects of plant (bare-root seedling vs. seedling plugs), and their interaction. We used the effect of site within age and plant as the denominator of F for the main plot effects. The subplot contained treatment effect (disked vs. grass herbicide) and all possible interactions with the main plot effects. The subplot effects used the residual mean square error as the denominator of F . Differences between means used Fischer’s protected least significant difference.

RESULTS

Survival of Milkweed Plants

Our analysis showed significant age ($F_{1,12} = 3.51$, $P = 0.086$), plant ($F_{1,12} = 8.09$, $P = 0.015$), and treatment ($F_{1,12} = 100.01$, $P < 0.001$) effects. Interactions were not influential in the analysis ($P > 0.100$). Mean survival of bare-root seedlings ($\bar{x} = 0.26$, 95% CI = 0.12–0.49) was greater than survival of seedling plugs ($\bar{x} = 0.05$, 95% CI = 0.02–0.13). Odds of survival for bare-root seedlings were 6.42 times greater (95% CI = 1.54–26.66) than odds of survival for seedling plugs. Untransformed survival of bare-root seedlings at the site level ranged from 0.09 to 0.85. Survival of seedling plugs was also variable at the site level, ranging from 0.00 to 0.29.

Mean survival of milkweed plants in old prairie plantings ($\bar{x} = 0.07$, 95% CI = 0.03–0.18) was lower than survival of milkweed plants in new prairie plantings ($\bar{x} = 0.21$, 95% CI = 0.09–0.41). Odds of survival of milkweed plants in old prairie plantings were 0.29 times lower (95% CI = 0.07–1.22) than odds of survival of milkweed plants in new prairie plantings.

Mean survival of milkweed plants in disked plots ($\bar{x} = 0.18$, 95% CI = 0.10–0.31) was greater than survival of milkweed plants in plots treated with grass herbicide ($\bar{x} = 0.08$, 95% CI = 0.04–0.16). Odds of survival for milkweed plants in disked plots were 2.41 times greater (95% CI = 1.99–2.92) than odds of survival for milkweed plants in plots treated with grass herbicide.

DISCUSSION

We found age of prairie restoration, preplanting treatment method, and seedling type all affected milkweed establishment. Our findings suggest that desired milkweed densities may not easily be attained when milkweed is translocated into an existing grassland. We suggest that public land managers may need to translocate milkweed plants at much greater densities than previously indicated if the goal is to establish the 200 consistency from earlier, replace stems with milkweeds/0.40 ha guideline outlined in the Missouri Monarch and Pollinator Conservation Plan (Missourians for Monarchs 2016). Furthermore, there is

both a focus and reliance on seedling plugs, grown by the commercial native plant industry, as the main source of plants used in milkweed translocations (Luna and Dumroese 2013, Monarch Joint Venture 2019). We found seedling plug survival to be significantly lower than bare-root stock. The <10% seedling plug survival we measured suggests commercially grown plugs may be a less viable option for any translocations of scale. Bare-root stock milkweed plants are of limited availability in comparison with seedling plugs (Monarchwatch.org 2019). The Monarch Conservation Implementation Plan lists “Increase understanding of milkweed propagation and cost-effective habitat establishment” as a high priority (Monarch Joint Venture 2019:35). We suggest conservation efforts directed at increasing the production of bare-root milkweed seedlings may greatly enhance milkweed restoration.

A review of the success of 249 plant species reintroductions and translocations (Godefroid et al. 2011) concluded that using bare-root seedlings resulted in a significantly greater survival rate, after 3 years, compared with seedlings rooted in potting soil. This study also found that reintroduction trials accompanied by ≥ 1 site preparation or management effort resulted in significantly greater survival rates starting in the first year. Our results showed a difference in 2-year survival of bare-root seedlings and seedling plugs between the 2 treatments. Plants in plots that received disking followed by application of pre-emergent herbicide had significantly greater survival than plots treated with a grass-selective herbicide. Therefore, we recommend site treatments that produce bare soil and limit competing plant growth when planting milkweed into reconstructed prairie. This is supported by our observation of greater plant survival in the new prairie reconstructions, which were assumed to have less grass competition.

The Monarch Butterfly Habitat Development Project identifies that the most immediate potential for milkweed habitat enhancement is on lands currently enrolled in the Wetlands Reserve Program (Natural Resources Conservation Service 2015). The protocol suggests prescribed burning or disking, followed by planting of milkweed plants as an appropriate conservation strategy. Our findings show that disking and pre-emergent herbicide application doubled the survival rate of bare-root seedlings and seedling plugs in comparison with plots treated with grass herbicide.

We did not design this project to test whether mowing affected plant survival. All of our sites were mowed in entirety before planting and periodically during the growing season. Therefore, mowing may be important to plant survival as it reduces plant competition. Mottl et al. (2006) suggested that survival of perennial herbaceous species may be successful using transplants when no weed control occurred beyond 2 years.

We recommend use of bare-root seedlings for milkweed translocations, but we raise concern over 2 factors. First, our seedling plug plantings contained 89% common milkweed with the remainder consisting of 2 other milkweed species. These were the plants available from the seeds collected the previous winter on PFCA. Each plot had a mix of species; however, the milkweeds were not distributed equally among

the plots nor did we record the species mix at the plot-level. Therefore, differences between bare-root and seedling plug survival could be due to a species effect that confounds our results. We suggest the dominance of common milkweed among the plugs allows for a relevant comparison of plugs versus bare-root, but caution that differential survival of milkweed species may be of concern when conducting translocations. Our second caution relates to the extent of variation we observed at the site level. We planted each milkweed type on the same date, using the same planting crew, and on similar soil-types. We assumed that because of their proximity, rainfall across sites did not vary. We considered sites to be homogenous. We must assume that unidentified site differences were responsible for the wide range, from 0.00 to 0.85, in site-level survival. We did not collect microsite information among the plots but it could be that small differences within sites affected plant survival. Kasten et al. (2016) found milkweed abundance to be highly variable along transects within sites, across the upper Midwest, where environmental conditions appeared homogenous. We caution those who may attempt large-scale translocations to expect variability in site-specific plant survival.

We contend, as suggested by Thogmartin et al. (2017), that public lands must play a major role in efforts to meet milkweed conservation goals. Thogmartin et al. (2017:6) stated “outside of converting corn and soy agriculture to land cover amenable to milkweed restoration, the greatest potential for gain in mean milkweed stem density occurred in the protected grassland and roadside sectors.” Their work suggests that success in the restoration of milkweed, presumed to be needed for monarch conservation in the Midwest, is critically linked to protected public lands (Thogmartin et al. 2017). Public land managers must consider broad-scale milkweed translocations as vital to achieving conservation goals. The Missouri Monarch and Pollinator Conservation Plan suggests >7,689 ha of land must be managed to achieve a density of 200 consistency with earlier, change milkweed stems to milkweeds/0.40 ha annually to meet conservation objectives (Missourians for Monarchs 2016). Private land incentives are available through federal, state, and nongovernmental organizations to those in Missouri who wish to enhance pollinator habitats. In Missouri, 121,408 ha were enrolled in conservation programs that may have potential to afford some degree of milkweed establishment from 2014 to 2018. Of these, 45,325 ha were in private ownership. Although monitoring efforts are in place, it is doubtful these lands and the associated management practices will provide milkweed densities of 200 consistency from earlier, change stems to milkweeds/0.40 ha suggested as necessary for monarch conservation (D. Duffin, Missourians for Monarchs, unpublished data).

MANAGEMENT IMPLICATIONS

Our results suggest that bare-root milkweed seedlings will establish with greater success than commercially grown seedling plugs. More aggressive site preparation, such as disking, will result in greater survival of translocated

milkweeds. Milkweed translocation into younger-aged restored prairie will foster greater plant survival than older restored sites. Conversely, efforts to establish milkweed at large scales may be hampered by multiple factors: a lack of availability of appropriate planting stock, low plant survival, variation in site-specific plant survival, and limitations in our knowledge of effective translocation techniques. Managers will also have to weigh the effects of intense site preparation in existing grasslands against competing management objectives. Disking and application of herbicide will have impacts on plant species composition and growth.

We raise concern over the apparent reliance on commercially grown plugs to meet the demand for milkweed restoration. We suggest that state and federal agencies, with support from organizations engaged in monarch conservation, begin efforts to cultivate bare-root milkweed plants to support translocation efforts.

It is critical in advancing our knowledge that conservation entities assess and report the success and failure of milkweed plantings, on both public and private lands. We suggest that future research be directed toward establishing guidelines for successful milkweed establishment. Agencies and organizations focused on monarch butterfly conservation should take the lead in support of a structured approach to monitoring milkweed translocations across varying landscapes.

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