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Multi-Seed *Zea* Pellets (MSZP) for increasing agroecosystem biodiversity

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Research Article

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Abstract

Intensive agricultural crop production is typically associated with low biodiversity. Low biodiversity is associated with a deficit of ecosystem services, which may limit crop yield (e.g., low pollination of insect-pollinated crops) at the individual field level or exacerbate the landscape-level impacts of intensive agriculture. To increase biodiversity and enhance ecosystem services with minimal loss of crop production area, farmers can plant desirable non-crop species near crop fields. Adoption of this practice is limited by inefficiencies in existing establishment methods. We have developed a novel seed-molding method allowing non-crop species to be planted with a conventional corn (*Zea mays* L.) planter, reducing labor and capital costs associated with native species establishment. Common milkweed (*Asclepias syriaca* L.) was selected as a model native species, because *Asclepias* plants are the sole food source for monarch butterfly (*Danaus plexippus* L.) larvae. Stratified *A. syriaca* seeds were added to a mixture of binder (maltodextrin) and filler (diatomaceous earth and wood flour) materials in a 3D-printed mold with the dimensions of a corn seed. The resulting Multi-Seed *Zea* Pellets (MSZP), shaped like corn seeds, were tested against non-pelleted *A. syriaca* seeds in several indoor and outdoor pot experiments. Molding into MSZP did not affect percent emergence or time to emergence from a 2-cm planting depth. Intraspecific competition among seedlings that emerged from an MSZP did not differ from competition among seedlings that emerged from a cluster of non-pelleted seeds. These findings demonstrate the potential of MSZP technology as a precise and efficient method for increasing agroecosystem biodiversity.

Introduction

Agricultural intensification tends to reduce the diversity of non-crop plants in agroecosystems (Storkey and Neve 2018; Tscharntke et al. 2005). Reduced non-crop diversity can reduce the benefits associated with a diverse community at both field and landscape levels. The range of benefits associated with plant diversity is broad. For example, diverse communities of non-crop plants in crop fields may cause less crop yield loss than non-diverse communities, which tend to be dominated by a few highly competitive weed species (Storkey and Neve 2018). Diverse plant communities are also more likely to support natural enemies of crop pests (Landis et al. 2005). In addition, non-crop plants on farms provide important resources for mobile organisms such as pollinators (Kovács-Hostyánszki et al. 2017; Nicholls and Altieri 2013). Agroecosystems that support rare arable non-crop species contribute to the conservation of these species (Albrecht et al. 2016). Thus, non-crop diversity often improves agricultural outcomes and minimizes the ecological consequences of habitat loss and fragmentation.

Some strategies for increasing non-crop diversity involve reversing trends toward intensification. For example, a greater diversity of plant species can typically survive in cropping systems with lower chemical inputs and reduced tillage relative to intensive "conventional" systems (Menalled et al. 2001; Murphy et al. 2006; Storkey and Neve 2018). This trend reflects the greater diversity of ecological niches available in less-intensive systems and the absence of strong selective pressures such as heavy herbicide use. However, not all farmers are able to adopt less-intensive management practices. Reducing agrochemical inputs and soil disturbance is a knowledge-intensive process that may require the purchase of new equipment or exploration of new markets.

Strategies involving direct manipulation of non-crop diversity may be more immediately feasible for more farmers. One key strategy is the establishment of desirable non-crop plants along field margins or on marginal land not profitable for crop production. Depending on

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the species sown and landscape context, these vegetated strips may provide multiple benefits. These benefits can include the attraction and facilitation of beneficial insects or other organisms, reduction of nutrient loss and pollution, and/or decreased erosion (Haddaway et al. 2018). In Europe and the United States, the practice of establishing wildflower strips is supported by an extensive scientific literature, and farmers who implement this practice are often eligible for financial compensation (Kleijn et al. 2019). However, farmers sometimes perceive wildflower strips negatively (Kleijn et al. 2019). Direct and opportunity costs associated with wildflower strip establishment may contribute to these negative perceptions. Additional challenges may include labor requirements, the difficulty of seeding large areas without specialized equipment, and the slow growth of many wildflower species (Xerces Society for Invertebrate Conservation and U.S. Department of Agriculture Natural Resources Conservation Service 2018).

The species composition and location of wildflower strips should generally be controlled so that wildflowers do not invade crop fields or compete with crops. It is true that non-crop plants within crop fields can have positive effects on the crops. For example, common milkweed (*Asclepias syriaca* L.) supports beneficial insects that attack crop pests (DiTommaso et al. 2016). However, competition against crops tends to dominate at high non-crop plant densities. Other negative effects on crops could involve resource-independent interference (e.g., early-season changes to light quality) or pests harbored by non-crop plants. To avoid risking crop yield losses, farmers can plant non-weedy wildflowers outside the field area. Seed technology may help increase the precision and efficiency of these planting operations.

Seed pelleting methods (i.e., seed coating methods in which the coated seed is much larger than the uncoated seed or shaped differently) are often developed for small-seeded, high-value horticultural crops that need to be planted with precision (Afzal et al. 2020). Seed agglomeration is a coating process in which multiple seeds are aggregated into propagules that may or may not be uniform in size and shape. Agglomerates may be produced with pan or rotary seed coaters, extrusion equipment, or molding technology (Afzal et al. 2020). For example, a molding technique was developed to produce cylindrical pellets containing multiple seeds of lettuce (*Lactuca sativa* L.) or tomato (*Solanum lycopersicum* L.) (Sikhao et al. 2015).

In addition to crop planting applications, seed pelleting and agglomeration technologies have several potential applications in restoration contexts (Gornish et al. 2019; Pedrini et al. 2020). For example, bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Á. Löve] seeds penetrated clay soil crusts more effectively when several seeds were agglomerated into one dispersal unit (Madsen et al. 2012). Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) seeds are very small, so seed pelleting technologies to produce conglomerates may improve delivery through a broadcast seeder (Hoose et al. 2019) or increase seedling emergence (Madsen et al. 2016). Pellets containing materials like activated carbon could also help protect seeds from chemical herbicides (Brown et al. 2019; Madsen et al. 2014).

This article reports a new application of seed agglomeration technology: a novel seed-molding method for planting wildflowers in agroecosystems. Our method differs from other agglomeration methods in that Multi-Seed *Zea* Pellet(s) (MSZP) containing wildflower seeds are produced in the size, shape, and density of corn (*Zea mays* L.) seeds. These MSZP are intended to be planted with a conventional corn planter. *Asclepias syriaca* was used as the model wildflower species, because the disappearance of *Asclepias* spp. (milkweed) plants from agroecosystems is largely responsible for the dramatic decline in North American monarch butterfly (*Danaus plexippus* L.) populations since the 1990s (Pleasants 2017; Stenoien et al. 2018). Although it is possible for *A. syriaca* to behave as a weed in crop fields, this species is not a major concern in systems that are frequently tilled or treated with herbicides such as glyphosate (DiTommaso et al. 2016). It is not likely that planting *A. syriaca* along field borders or in non-cropped areas of an agroecosystem would pose a threat to crops, especially if the *A. syriaca* stand is eventually terminated with tillage or herbicides. For example, allowing *A. syriaca* to grow for 3 yr before termination would likely support *D. plexippus* productivity with little risk to crops. In addition to detailing the novel MSZP method, we present the results of laboratory, greenhouse, and outdoor assessments of emergence, early growth, and competition as proof of concept. Our experiments addressed the following questions:

1. Does agglomerating *A. syriaca* seeds into MSZP inhibit or delay emergence?
2. How does planting depth affect emergence from MSZP?
3. How does planting density affect emergence and early growth?
4. Are *A. syriaca* plants from MSZP likely to compete substantially with nearby corn?

Materials and Methods

Seed Germination and Stratification

Seeds of an *A. syriaca* ecotype from Vermont, USA, were provided by Ernst Conservation Seeds (Meadville, PA, USA). Two preliminary experiments demonstrated that the Vermont ecotype would work well in New York, where the experiments took place (data not shown). First, a germination experiment was conducted on an ecotype from Pennsylvania, USA, also obtained from Ernst Conservation Seeds, alongside the Vermont ecotype. Second, an outdoor pot experiment was conducted to measure the emergence and early growth of the Vermont and Pennsylvania ecotypes as well as three populations collected near Ithaca, NY, USA. In both these preliminary experiments, the Vermont ecotype performed at least as well as other ecotypes or populations, so we used only the Vermont ecotype for further research.

Preliminary laboratory experiments were also conducted to measure percent germination of the Vermont ecotype in different germination environments and following different stratification durations. These experiments were conducted despite previous research on *A. syriaca* germination (see "Results and Discussion"), because germination rates in non-crop species frequently differ between ecotypes or seed lots. The preliminary experiment on germination environments primarily served to select a germination environment for the experiment on stratification duration and to ensure that greenhouse conditions for further experiments were reasonable. The preliminary experiment on stratification duration primarily served to select a stratification duration for seeds used in further experiments and to provide a sense of what percent germination might be expected from this particular ecotype and seed lot under optimal conditions.

For the preliminary experiment on germination environments, three environments were selected to represent plausible field conditions: 10/30 C with an 8-h photoperiod, 15/25 C with a 14-h photoperiod, and 20/30 C with an 8-h photoperiod. Each

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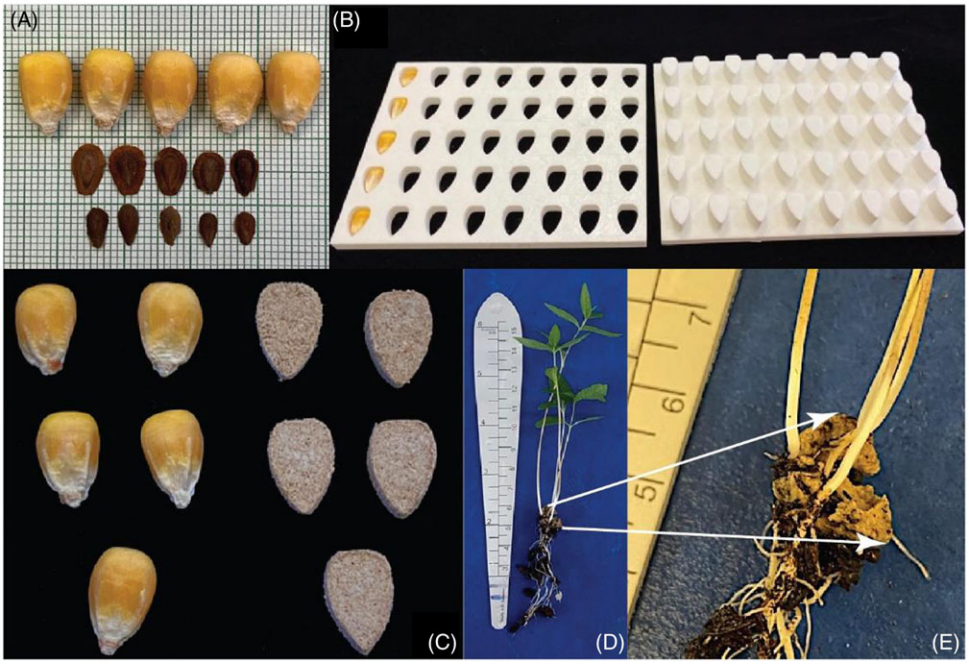


Figure 1. Development of Multi-Seed *Zea* Pellets (MSZP). (A) Corn seeds (top row), intact *Asclepias syriaca* seeds (middle row), and dewinged *A. syriaca* seeds (bottom row) on a 1-mm grid. (B) 3D-printed molds the size and shape of a corn seed. (C) Corn seeds (left) and MSZP containing *A. syriaca* seeds (right). (D, E) Emergence of multiple *A. syriaca* seedlings from a single MSZP.

treatment was maintained in a laboratory germination chamber. For each treatment, four replicate plastic boxes (10 by 10 cm) each containing 25 non-stratified seeds on moistened blue blotter paper (Anchor Paper, St Paul, MN, USA) were placed in the germination chamber and checked at 8, 14, and 21 d.

A separate preliminary experiment tested the effect of stratification duration (0, 1, 2, or 4 wk at 5 C in darkness). As in the experiment on germination environment, four replicates of 25 seeds were subjected to each stratification duration. Following stratification, seeds were germinated on moistened blotter paper at 15/25 C with a 14-h photoperiod and checked at 7 and 14 d.

Data from these preliminary experiments were not analyzed statistically, because a single germination chamber was used for each treatment.

MSZP Development

Asclepias syriaca seeds were pelleted into MSZP with the dimensions of corn seeds (Figure 1). To fit multiple *A. syriaca* seeds into a single MSZP, seeds were sorted by size, and the outer, winglike portion of the pericarp was removed. Seeds too large to pass through the #13.5 sieve (5.36 mm) were discarded, because large seeds would be harder to fit into MSZP. Seeds small enough to pass through the #10 sieve (3.97 mm) were discarded, because unusually small seeds might have lower germinability. Dry seeds of intermediate size were gently processed with a hand deawner/debearder (Hoffman Manufacturing, Inc, Corvallis, OR, USA) to remove the winglike portion of the pericarp (Figure 1A). This dewinging did not injure the seeds. The 100-seed weight of dewinged *A. syriaca* seeds was approximately 0.393 g, which was less than the 100-seed weight of intact *A. syriaca* seeds (0.422 g). Corn seeds are much larger (100-seed weight of 28.6 g). After dewinging, seeds were again sieved, and seeds too large to pass through the #10 sieve were discarded. Seeds that passed through the #10 sieve were stratified for 2 wk at 5 C.

MSZP were composed of maltodextrin (dextrose equivalent 16.5 to 19.5; Sigma-Aldrich, St Louis, MO, USA), diatomaceous earth (Perma-Guard, Albuquerque, NM, USA), and wood flour (Lignocel natural wood fibers C 120 GE; J. Rettenmaier & Söhne (JRS), Rosenberg, Germany). Maltodextrin served as a dry binder powder, whereas diatomaceous earth and wood flour were selected as low-density filler materials to produce a density similar to corn seed. Maltodextrin, diatomaceous earth, and wood flour were thoroughly mixed in a ratio of 20%, 40%, and 40% by weight, then combined with water to form a dough.

The dough was pressed into custom 3D-printed molds the size and shape of corn seeds (Figure 1B). Molds were printed at Cornell University from acrylonitrile butadiene styrene. While the dough was in molds, dewinged and stratified *A. syriaca* seeds were inserted into the center of each MSZP. Three *A. syriaca* seeds were inserted into most MSZP; the planting density experiment also tested MSZP containing five seeds. We expected that including three seeds per MSZP would ensure that at least one seedling emerged from most MSZP without excessive intraspecific competition. Five *A. syriaca* seeds represents the largest number that can easily fit into a pellet with the dimensions of a corn seed. After seeds were inserted, MSZP were removed from the molds and dried in a forced-air drying oven (Gallenkamp-Plus Oven, Loughborough, UK) at 30 C for 3 h.

The densities of corn seeds and MSZP were determined with a float/sink method in solutions of hexane and chloroform (Taylor et al. 1982). The densities of corn seeds, MSZP pellets without *A. syriaca* seeds, and MSZP containing three *A. syriaca* seeds were all in the range of 1.25 ± 0.05 g cc^{-1}. A sample size of 10 was used based on preliminary studies using the float/sink method with both corn and MSZP.

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Greenhouse and Outdoor Experiments

We performed three greenhouse and outdoor experiments. The first experiment tested the effects of planting depth and pelleting into MSZP on *A. syriaca* emergence. This experiment was conducted in outdoor pots in Ithaca, NY, USA (42.448°N, 76.460° W) and replicated in the greenhouse. The second experiment tested the effects of planting density and pelleting into MSZP on *A. syriaca* emergence and early growth. This experiment was conducted in outdoor pots in Ithaca, NY, USA. The third experiment tested the effects of *A. syriaca* planting time, relative to corn planting time, on early growth. This experiment was conducted in the greenhouse, maximizing potential growth rates. For the outdoor experiments, monthly mean average temperature was 19.6 C in June, 20.1 C in July, 21.5 C in August, and 16.8 C in September (Northeast Regional Climate Center 2022). Total precipitation was 11.2 cm in June, 16.1 cm in July, 15.8 cm in August, and 13.3 cm in September (Northeast Regional Climate Center 2022). The duration of daylight was 15:05 h on June 1, 15:14 h on July 1, 14:27 on August 1, and 13:08 h on September 1 (U.S. Naval Observatory 2022).

The first run of the planting depth experiment was conducted in outdoor pots. On August 4, 2021, forty 2.8-L pots were filled with a 3:1 by volume mixture of field soil from Mount Pleasant, NY (Mardin channery silt loam; coarse-loamy, mixed, active, mesic Typic Fragiudepts) and Cornell Soil Mix (peat, vermiculite, and perlite in a 2:2:1 ratio by volume amended with calcium, lime, and 10–5–10 N–P–K). After pots were filled with soil, they were watered daily to promote soil settling until seeds were planted on August 14. On August 14, one MSZP containing three *A. syriaca* seeds and three additional "free" seeds (i.e., seeds not molded into MSZP) were planted into each pot. Both the MSZP seeds and the free seeds had been dewinged and stratified (2 wk at 5 C). The free seeds were also coated with a red colorant (seed colorant TSC-180786, Standard Colors, High Point, NC, USA). Although the red colorant did not impede germination in a preliminary experiment, we acknowledge that it represents a difference between free seeds and seeds in MSZP. The colorant was not used elsewhere in this research. In each pot, the three free seeds were planted in a cluster (in contact with each other, to mimic the clustering of the three seeds within each MSZP). This cluster of free seeds was planted 8 cm from the MSZP planted in the same pot. Within each pot, a single planting depth was used for all *A. syriaca* seeds (i.e., the free seeds were planted at this depth and the MSZP was planted so that its center reached this depth). Pots were assigned to planting depths of 2, 3, 4, and 5 cm according to a completely randomized design (10 pots per treatment, for a total of 40 pots).

Pots were checked for emergence daily until September 26, 2021, at which point the emergence period had ended. Pots were rearranged on August 25 and September 8, and pot orientations were changed on August 26 and September 13. Due to sunny, dry conditions resulting in a hard, cracked soil surface, pots were watered to field capacity on August 23, 25, 26, 30, and 31; and September 20. Otherwise, pots were rainfed. No fertilizer was applied, because the starter fertilizer in the Cornell Soil Mix was considered sufficient for this short-term study.

The second run of the planting depth experiment was conducted under greenhouse conditions (20 to 26 C during the day, 18 to 23 C at night, 15-h photoperiod in addition to natural light). The materials and experimental design used were identical to the first run of the experiment with the following exceptions: (1) no red colorant was applied to the free seeds, (2) the MSZP in each pot was separated from the cluster of free seeds by a distance of 6 cm, and (3) circles of lightweight no-see-um mesh, cut to pot diameter, were buried in pots at the depth of planting. These mesh circles were placed because greenhouse pots, unlike the outdoor pots in the first run of this experiment, were watered three times per week. The watering rate was adjusted as needed to maintain moderate surface soil moisture. Mesh circles were intended to prevent this frequent watering from washing seeds farther down into the soil than would be expected under field conditions. The mesh circles were buried at the intended planting depth (2, 3, 4, or 5 cm) on the day that soil was potted (November 8, 2021). On the day of planting (November 13), we added soil if necessary to ensure that mesh circles remained at the intended planting depth, because soil in some pots had settled after potting. Pots were rearranged every two weeks and checked daily for emergence until the experiment was terminated on December 11, 2021. Emergence dates were not recorded for three seedlings, so these seedlings were excluded from the time-to-emergence analysis.

The planting density experiment was conducted outdoors. The pot size and soil were identical to the first run of the planting depth experiment. Soil was potted and seeds were planted on June 16, 2021. The experiment was set up as a completely randomized design with seven treatments and 10 replicates, for a total of 70 pots. In the five free-seed treatments, we planted seeds at densities of one, two, three, four, or five seeds per pot. The free seeds were planted in a cluster in the center of the pot at 2-cm depth. In the remaining two treatments, we planted MSZP containing three or five seeds. One MSZP was planted in the center of the pot at 2-cm depth (center of the MSZP). In addition to the 70 treatment pots, we included 10 control pots (soil only) to check for possible *A. syriaca* contamination in the soil. *Asclepias syriaca* is present in the surrounding area, so it is possible that some wild seeds were present in the field soil used in the experiment. No *A. syriaca* plants were observed in the control pots. However, three *A. syriaca* plants in the 70 treatment pots were identified as contaminants, because they emerged at the side of the pot rather than in the seedling cluster in the center of the pot. We did not include these contaminants in the data set presented here.

We checked each pot for emergence or mortality daily until the experiment was terminated (September 9 to 13, 2021). At emergence, we marked each seedling with a colored rubber band to distinguish it from other plants in the same cluster. Each week, we measured the height of each plant (soil surface to growing point). The only defoliating herbivorous insects observed on the plants were larvae of *D. plexippus*. These larvae (including those on the soil surface or apparently dead) were counted weekly from August 18, when they were first observed, until the end of the experiment. Larval counts represent conservative estimates. In addition to rainfall, pots were watered on June 16, 24, and 26; July 5 and 25; and August 4, 5, 13, 16, 23, 25, 30, and 31. Pots were fertilized once on August 5 (4.93 ml per pot, Miracle-Gro® Shake 'n Feed All Purpose Plant Food 12–4–8, Scotts, Marysville, Ohio, USA). Pots were rearranged on June 30; July 14 and 28; and August 11 and 25. At the end of the experiment, each pot with living plants was assessed twice for evidence of defoliation (presumably by *D. plexippus*). In each assessment, the degree of defoliation was described qualitatively. These descriptions were later used to sort pots into "no herbivory," "low herbivory," and "high herbivory" categories. If the two assessments for a pot disagreed, the pot was sorted into the category indicating more severe damage. The aboveground biomass of each plant was placed in an

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individual bag, dried (58 to 60 C, 4 d), and weighed. It was not possible to separate the root systems of plants occupying the same pot, so belowground biomass was washed, dried, and weighed as a total for each pot.

In our final experiment, we tested the effect of relative planting time on competition between *A. syriaca* and corn under greenhouse conditions (20 to 26 C during the day, 18 to 23 C at night, 15-h photoperiod in addition to natural light). This experiment was set up as a randomized complete block design with six blocks of 10 pots, for a total of 60 pots. Pots were 9.7 ± 0.1 L and filled with Cornell Soil Mix. Each block contained two pots each of the following five treatments: (1) one MSZP planted 2 wk before one corn seed ("early planting"), (2) one MSZP planted simultaneously with one corn seed ("simultaneous planting"), (3) one MSZP planted 2 wk later than one corn seed ("late planting"), (4) two MSZP ("*A. syriaca* monoculture"), or (5) two corn seeds ("corn monoculture"). Each MSZP contained three *A. syriaca* seeds. MSZP and corn seeds were planted at a depth of 2 cm (base of the MSZP or seed), 8 cm apart from the other MSZP or corn seed in the same pot. Soil was potted, and MSZP in the early planting treatment were planted on October 13, 2021. MSZP in the simultaneous planting treatment, MSZP in the *A. syriaca* monoculture treatment, and all corn seeds were planted on October 27. MSZP in the late planting treatment were planted on November 10.

Pots were checked for emergence or mortality daily. *Asclepias syriaca* seedlings were marked with colored bands to distinguish multiple plants emerged from the same MSZP. Water was added whenever the top few centimeters of soil appeared dry. Pots were rearranged within blocks every two weeks. On November 15, an insecticide (dinotefuran, Safari®, Valent USA, Walnut Creek, CA, USA) was sprayed at the recommended rate to control aphids in the greenhouse room. The experiment was terminated on December 11 to 12, 2021. Individual live plants were later dried and weighed (aboveground and belowground biomass separated, 58 to 60 C for 41 d). Two samples (one aboveground *A. syriaca* and one belowground corn) were lost during this process so are not included in the biomass data set. In the corn monoculture, the root systems of the two corn plants could not be separated so were washed, dried, and weighed together. The combined root biomass was divided between the two corn plants by assuming that both plants had the same root-to-shoot ratio; therefore, the plant with greater aboveground biomass would also have greater belowground biomass.

Statistical Analysis

Data analysis was performed in R (4.1.0; R Core Team 2021). Unless otherwise noted, measures of error are standard error (SE), seedling cluster was treated as the experimental unit to avoid pseudo-replication (i.e., response variables represent the mean of seedlings in the cluster rather than individual seedlings), and $\alpha = 0.05$. For parametric analyses, residual plots, the Shapiro-Wilk test of normality, and Levene's test for homogeneity of variance were used to check normality and homoskedasticity of residuals.

For the planting depth experiment, data were analyzed by linear models testing the effects of treatment (free seeds or seeds from MSZP), planting depth (2, 3, 4, or 5 cm), and their interaction on percent emergence or time to emergence. The outdoor and indoor runs of the experiment were analyzed separately. An inverse transformation was applied to time to emergence in the outdoor run. Untransformed responses were graphed as mean ± 1 SE, and transformed responses were graphed as back-transformed estimates ± 1 SE obtained by the delta method (package EMMEANS). Multiple comparison of means was performed with Tukey's HSD test (package EMMEANS). In addition, the nonparametric Wilcoxon signed-rank test (paired by pot) was used to test whether treatment affected within-cluster variation in time to emergence (i.e., SE of the three seeds per MSZP or free-seed cluster).

In the planting density experiment, the effects of treatment (cluster of one to five free seeds, MSZP containing three seeds, or MSZP containing five seeds) on percent emergence, time to emergence, within-cluster variation in time to emergence, and percent mortality were evaluated with the nonparametric Kruskal-Wallis rank-sum test. A linear model was used to test the effects of treatment and date (as factor: August 18, August 25, September 2, or September 9 to 13) on the total number of observed *D. plexippus* larvae across all replicates. A chi-square test was used to test for an association between treatment and *A. syriaca* defoliation level (none, low, or high; pots without living plants were excluded). For the height analysis, each plant was included in the per-pot mean for all dates on which the plant had passed the cotyledon stage and had not died. Linear models tested the effects of date, treatment, and their interaction on the square root of per-pot mean height or the cubic root of within-pot height SE. Tukey's HSD test was used to determine which slopes differed (*emtrends* in package EMMEANS). Treatment effects on mean height within dates were evaluated by ANOVA followed by Tukey's HSD test. Untransformed height data were graphed.

Biomass data from the planting density experiment were analyzed with linear models testing how treatment affected per-pot biomass, how the number of live plants at harvest (as a factor) affected per-pot biomass, how treatment affected per-plant biomass, and how the number of live plants at harvest affected per-plant biomass. Aboveground and belowground biomass data were analyzed separately. Square-root transformations were applied to the per-plant models for aboveground and belowground biomass by treatment and belowground biomass by number of live plants. A logarithmic transformation was applied to the per-plant model for aboveground biomass by number of live plants. Untransformed responses were graphed as mean ± 1 SE, and transformed responses were graphed as back-transformed estimates ± 1 SE obtained by the delta method. Multiple comparison of means was performed with Tukey's HSD test. Additional linear models were used to test whether treatment or the number of live plants at harvest affected the square root of the within-pot SE of aboveground biomass.

In the relative planting time experiment, data on percent emergence and time to emergence in *A. syriaca* and biomass in both species were analyzed with linear mixed models in which treatment was a fixed effect and block was a random effect. Aboveground biomass and belowground biomass were analyzed separately. An inverse transformation was applied to time to emergence, corn aboveground biomass was squared, a square-root transformation was applied to *A. syriaca* aboveground biomass, and a cubic-root transformation was applied to *A. syriaca* belowground biomass. Similar models for aboveground *A. syriaca* biomass (square-root transformation) and belowground *A. syriaca* biomass (logarithmic transformation) compared the simultaneous-planting and *A. syriaca*-monoculture treatments. *Asclepias syriaca* biomass was also analyzed on a per-plant basis with linear mixed models containing the fixed effects of age at harvest, corn presence ("no" in the *A. syriaca* monoculture and one pot in which corn

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Table 1. Percent germination of *Asclepias syriaca* seeds by germination environment (mean ± 1 SE, $n = 4$).

Treatment	Germination		
	Day 8	Day 14	Day 21
	%		
10/30 C	8 ± 2	15 ± 3	16 ± 3
15/25 C	26 ± 7	44 ± 7	46 ± 8
20/30 C	10 ± 1	26 ± 3	27 ± 4

Table 2. Percent germination of *Asclepias syriaca* seeds by stratification duration (mean ± 1 SE, $n = 4$).

Treatment	Germination	
	Day 7	Day 14
	%	
Control	25 ± 7	32 ± 7
1 wk at 5 C	74 ± 6	84 ± 3
2 wk at 5 C	87 ± 3	92 ± 2
4 wk at 5 C	89 ± 5	92 ± 3

did not emerge, "yes" otherwise), and their interaction in addition to the random effect of pot nested within block. For these models, a square-root transformation was applied to aboveground biomass and a cubic-root transformation was applied to belowground biomass. Data were graphed on the transformed scales with 95% confidence intervals and the *y* axes were relabeled to the original scales.

Results and Discussion

Germination and Stratification

The preliminary laboratory experiments on germination conditions and stratification duration established the baseline germinability of our seed lot and ensured that greenhouse and outdoor experiments reflected the true germination potential of these seeds. Percent germination of *Asclepias syriaca* at 15/25 C was 26% after 8 d, 44% after 14 d, and 46% after 21 d (Table 1). Stratification appeared to increase percent germination (Table 2). After 2 wk of stratification at 5 C, percent germination at 15/25 C was 87% after 7 d and 92% after 14 d. Preliminary studies suggested that drying stratified seeds and germination in darkness did not affect percent germination, compared with no drying and germination at 15/25 C with 14-h light (AG Taylor and MT Loos, unpublished data).

These results are consistent with the literature on *A. syriaca*, a species in which stratification releases physiological dormancy. For example, Bandara et al. (2019) found that an increasing stratification duration (1, 5, or 9 C for 4, 8, or 12 wk) increased percent germination. After 12 wk of stratification, percent germination was generally high (up to 100%) under alternating temperature regimes of 10/20, 15/25, or 15/30 C but lower at 6/15 C. Similarly, Finch et al. (2019) found that percent germination increased with increasing stratification duration and increasing germination temperature. Percent germination at 15/25 C was greater than 90% after 4 wk of stratification. Percent germination at 6/15 C was much lower and continued to increase with stratification durations up to 20 wk. The lack of a light requirement for germination of stratified *A. syriaca* seeds is also consistent with previous work (Baskin and Baskin 1977). Seed pelleting or agglomeration may reduce germination and emergence in species that require light for germination.

Planting Depth Experiment

We define percent emergence as a percentage of the three seeds per MSZP or free-seed cluster; for example, 33% emergence would indicate that one of the three seeds emerged. In the outdoor run of the planting depth experiment, percent emergence was influenced by treatment (free seeds or seeds from MSZP; $P = 0.002$), planting depth (2, 3, 4, or 5 cm; $P < 0.001$), and the interaction between treatment and planting depth ($P = 0.03$). In the MSZP treatment, percent emergence was 70% at 2 cm and decreased with increasing planting depth to 13% at 5 cm (Figure 2A). At least one seedling emerged from each MSZP planted at 2 cm; however, no seedlings emerged from 6 of the 10 MSZP planted at 5 cm. Percent emergence of free seeds was 80% at 2 or 4 cm but 50% at 3 or 5 cm (Figure 2A). Time to emergence was influenced by treatment ($P < 0.001$), planting depth ($P < 0.001$), and their interaction ($P = 0.03$). Time to emergence increased with increasing planting depth in the MSZP treatment but did not vary with planting depth in the free-seed treatment (Figure 2B). There was more variation in time to emergence among the three seeds per MSZP than among the three seeds per free-seed cluster ($P = 0.02$).

In the indoor run of the planting depth experiment, percent emergence was influenced by planting depth ($P < 0.001$) but not by treatment ($P = 0.2$) or the interaction of planting depth and treatment ($P = 0.6$). The free-seed and MSZP treatments did not differ at any planting depth (Figure 2C). In the free-seed treatment, percent emergence from 5 cm was lower than percent emergence from 3 cm. No seedlings emerged from 5 cm in the MSZP treatment. Neither planting depth nor treatment affected time to emergence (Figure 2D). There was equal variation in time to emergence among the three seeds per MSZP and the three seeds per free-seed cluster ($P = 0.8$).

It is notable that molding into MSZP did not affect percent emergence or time to emergence from a planting depth of 2 cm in either run of the experiment. In the outdoor trial, increasing planting depth reduced percent emergence and increased time to emergence among seeds from MSZP. This trend could indicate that seedlings expended some energy in emerging from the MSZP, which was then unavailable for preemergent growth. In the indoor trial, molding into MSZP did not affect percent emergence or time to emergence from any planting depth. Unlike pots in the outdoor trial, pots in the indoor trial were watered on a regular schedule and not exposed to heavy rain or high temperatures. If these differences reduced soil surface hardness in the indoor trial relative to the outdoor trial, they might help explain the lack of an MSZP effect in the indoor trial.

More generally, the planting depth experiment demonstrated *A. syriaca* emergence from all tested planting depths, although the 5-cm depth appeared to be suboptimal. Emergence from 5 cm was 0% to 63% of emergence from 2 cm, depending on treatment (free seeds or MSZP) and experimental context (outdoor or indoor). Similarly, a previous growth chamber experiment revealed that percent emergence was highest for seeds buried between 0.5- and 4-cm depths and very low for seeds buried at 7 cm (Yenish et al. 1996). Another experiment found that percent emergence was highest for seeds buried at 0.5 or 1 cm and decreased with planting depths up to 5 cm (Bhowmik 1978). A third experiment demonstrated that increasing planting depth (up to 6 cm) decreased percent emergence; this decreasing trend was steeper in a sandy loam relative to a silty clay loam (Evetts and Burnside 1972). Taken together with the results of our planting depth experiment, these findings suggest that *A. syriaca* emergence

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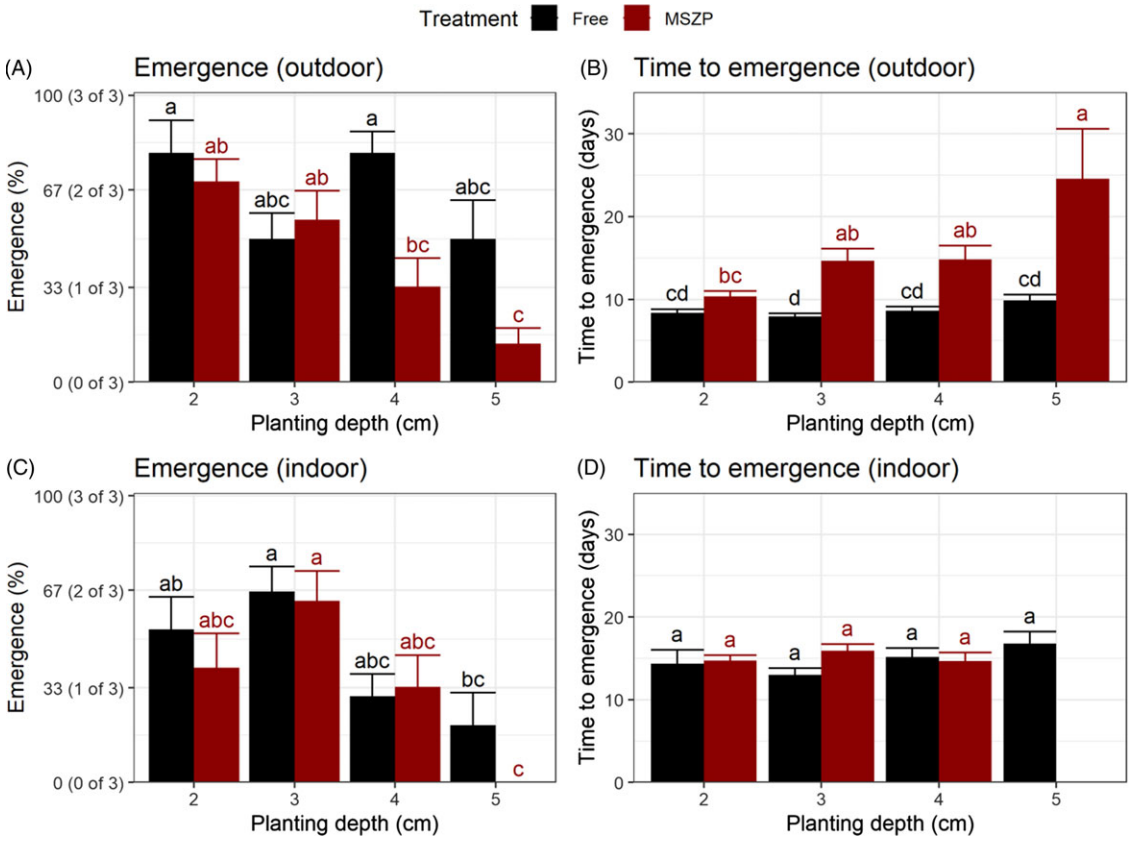


Figure 2. *Asclepias syriaca* emergence in planting depth experiment. (A, C) Percent emergence and (B, D) time between planting and emergence were evaluated in (A, B) outdoor and (C, D) indoor runs of the same experiment. Free (non-molded) *A. syriaca* seeds and Multi-Seed *Zea* Pellets (MSZP) were planted at depths of 2, 3, 4, or 5 cm. Percent emergence is defined as a percentage of the three seeds per MSZP or free-seed cluster; e.g., 33% emergence would indicate that one of the three seeds emerged. All data are presented as mean ± 1 SE ($n = 10$). Within panels, columns labeled with the same letter are not significantly different according to Tukey's HSD test.

from a typical corn planting depth (4 to 6 cm; Cornell University n.d.) would be lower than emergence from shallower depths. This conclusion is relevant to the design of a system in which MSZP containing *A. syriaca* seeds would be planted using a corn planter.

Planting Density Experiment

The planting density experiment compared seven treatments: free seeds planted in clusters of one, two, three, four, or five seeds; MSZP containing three seeds; and MSZP containing five seeds (Figure 3). Median percent emergence varied from 60% to 100% across treatments (mean 60% to 84%; Table 3). Median and mean times to emergence were 13 or 14 d for all treatments (Table 3). Over the course of the 3-mo experiment, disease and *D. plexippus* herbivory resulted in high mortality (Table 3). Treatment did not significantly affect percent emergence ($P = 0.3$), time to emergence ($P = 0.2$), within-cluster variation in time to emergence ($P = 0.7$), or percent mortality ($P = 0.3$).

The lack of a treatment effect on emergence from 2 cm was consistent with our planting depth experiment, but not necessarily predictable from the literature. Madsen et al. (2012) suggested that agglomerating several *P. spicata* seeds could improve emergence if the seedlings emerging from an agglomerated pellet together generated enough force to penetrate a clay soil crust. If this facilitation effect had occurred in our experiment, we likely would have observed higher percent emergence from MSZP or free-seed clusters containing more seeds. Differences between our experiment and the research on *P. spicata* include the larger size of *A. syriaca* seeds and different soil media.

Danaus plexippus larvae were observed in all seven treatments (Figure 3E; Table 4). The number of observed larvae varied by date ($P = 0.02$) but not by treatment ($P = 0.2$). By the end of the experiment, most pots containing live plants showed some evidence of herbivory. Herbivory level varied across treatments ($P = 0.02$), but there was no evidence that planting seeds as MSZP either increased or decreased herbivory (Table 5). These results cannot be interpreted as a formal test of *D. plexippus* preference, because the *D. plexippus* data were limited and collected from a single array of pots. Similarly, our results do not represent a test of *D. plexippus* performance, because data on larval development were not collected and no larvae reached maturity. It is likely that most larvae were lost to predation, although other outcomes such as disease, parasitism, or departure from the natal plant may also have occurred. A few larvae were still present on the plants when the experiment ended. Laboratory and field experiments are required to test whether *A. syriaca* planting method affects *D. plexippus* productivity.

Mean plant height increased with date (Figure 4). In addition to date ($P < 0.001$), plant height was affected by treatment ($P < 0.001$) and the interaction between treatment and date ($P < 0.001$). Height increased more quickly in pots with one free seed or MSZP containing three seeds, relative to pots with four or five free

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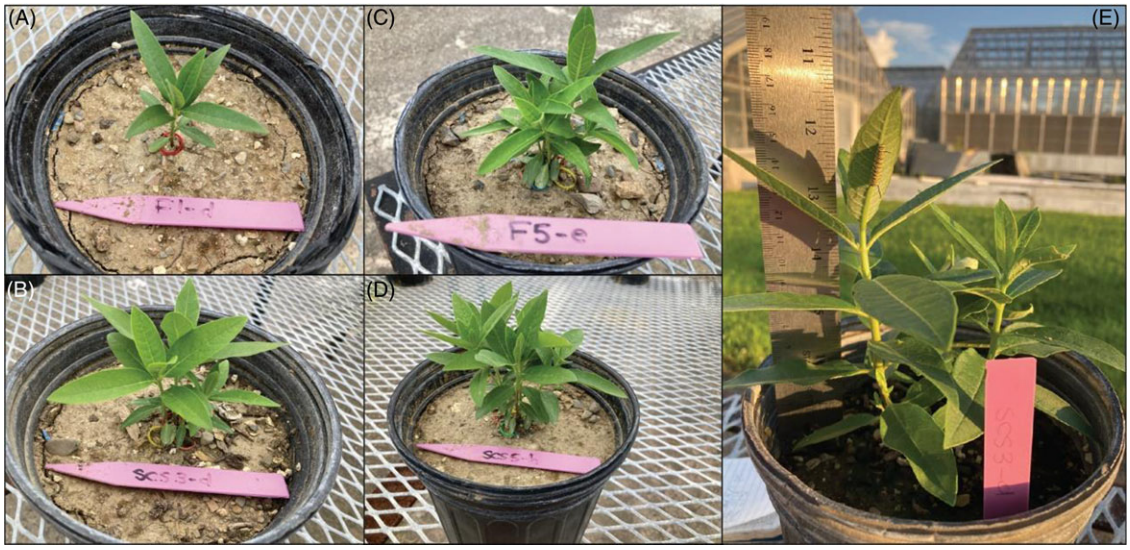


Figure 3. *Asclepias syriaca* seedlings in planting density experiment. (A) Representative pot in the F1 (one free seed) treatment on July 24, 2021. (B) Representative pot in the MSZP3 (one Multi-Seed *Zea* Pellet containing three seeds) treatment on July 24, 2021. (C) Representative pot in the F5 (five free seeds) treatment on July 24, 2021. (D) Representative pot in the MSZP5 (one Multi-Seed *Zea* Pellet containing five seeds) treatment on July 24, 2021. (E) Representative pot in the MSZP3 treatment on August 21, 2021.

seeds. Within-pot variation in height increased at the same rate in treatments with three or five free seeds compared with treatments with MSZP. Differences in mean height between treatments were not significant within dates, except that plants were taller in the treatment with one free seed, relative to the treatment with five free seeds, on August 25 ($P = 0.04$).

Biomass measurements revealed no effect of treatment on aboveground or belowground biomass per pot (Figure 5A). The number of live plants per pot at harvest did not affect aboveground biomass per pot ($P = 0.08$) but did affect belowground biomass per pot ($P = 0.003$; Figure 5B). Belowground biomass per pot was lower in pots that contained one live plant at harvest, relative to pots that contained two, three, or five live plants. Aboveground biomass per plant was not affected by treatment ($P = 0.07$; Figure 5C) but was affected by the number of live plants at harvest ($P < 0.001$; Figure 5D). Aboveground biomass per plant was higher in pots that contained one or two live plants at harvest relative to pots that contained three or four live plants. Neither treatment nor the number of live plants at harvest affected within-pot variation in aboveground biomass. Belowground biomass per plant varied with treatment ($P = 0.01$) and the number of live plants per pot at harvest ($P < 0.001$).

Although *A. syriaca* height and biomass were affected by treatment in the planting density experiment, there was no indication that molding into MSZP affected seedling growth. Variation in growth was best explained by the number of plants growing together (i.e., as an effect of intraspecific competition). These results are consistent with previous research showing that intraspecific competition can reduce plant size in *A. syriaca* (Kula et al. 2020). From a practical standpoint, our results suggest that there is little advantage to creating MSZP containing five or more *A. syriaca* seeds. Under favorable growing conditions, MSZP containing three seeds are likely to produce at least one emerged seedling and achieve good biomass production.

Relative Planting Time Experiment

In the relative planting time experiment, percent emergence of corn was 98%, and percent emergence of *A. syriaca* was 62%. Percent emergence of *A. syriaca* did not vary with planting time treatment (2 wk before corn, simultaneous with corn, 2 wk after corn, or *A. syriaca* monoculture). Mean time to emergence was 5 d in corn (median: 5 d) and 15 d in *A. syriaca* (median: 14 d). In *A. syriaca*, there was a large range in time to emergence (the final seedling emerged at 45 d after planting), but time to emergence did not vary with treatment. Only one corn plant and two *A. syriaca* plants died over the course of the experiment.

Neither aboveground corn biomass nor belowground corn biomass was affected by treatment. Aboveground corn biomass was 17.1 ± 2.1 g when *A. syriaca* was planted before corn, 16.2 ± 2.2 g when *A. syriaca* was planted simultaneously with corn, 18.5 ± 2.1 g when *A. syriaca* was planted after corn, and 14.1 ± 0.9 g in the corn monoculture ($P = 0.08$). Belowground corn biomass was 4.4 ± 0.6 g when *A. syriaca* was planted before corn, 3.8 ± 0.8 g when *A. syriaca* was planted simultaneously with corn, 5.5 ± 0.9 g when *A. syriaca* was planted after corn, and 3.8 ± 0.5 g in the corn monoculture ($P = 0.14$).

Both aboveground *A. syriaca* biomass and belowground *A. syriaca* biomass were affected by treatment. Aboveground *A. syriaca* biomass was 0.39 ± 0.09 g per seedling when planted before corn, 0.17 ± 0.03 g when planted simultaneously with corn, 0.01 ± 0.00 g when planted after corn, and 0.17 ± 0.03 g in the *A. syriaca* monoculture ($P < 0.001$). Belowground *A. syriaca* biomass was 0.16 ± 0.05 g when planted before corn, 0.07 ± 0.03 g when planted simultaneously with corn, 0.002 ± 0.001 g when planted after corn, and 0.05 ± 0.01 g in the *A. syriaca* monoculture ($P < 0.001$). However, neither aboveground *A. syriaca* biomass nor belowground *A. syriaca* biomass was affected by the difference between simultaneous-planting and *A. syriaca*-monoculture treatments. Similarly, *A. syriaca* aboveground and belowground biomass increased with age at harvest ($P < 0.001$) but was not affected by the presence of corn or the interaction of age of harvest and corn (Figure 6).

The finding that *A. syriaca* did not reduce corn growth, regardless of planting time, is unsurprising, given that corn emerged more quickly than *A. syriaca* and subsequently grew much more quickly. Consequently, even the *A. syriaca* clusters planted before corn planting did not have a measurable impact on corn growth. To

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Table 3. Percent emergence, time to emergence (days after planting), and mortality (percentage of emerged plants) in the planting density experiment (mean ± 1 SE, median).[a]

Propagule	Seeds per pot	Emergence	Time to emergence	Mortality
		— % —	— d —	— % —
Free	One	70 ± 15, 100	13 ± 0, 13	14 ± 14, 0
Free	Two	70 ± 13, 100	13 ± 0, 13	56 ± 18, 75
Free	Three	60 ± 8, 67	13 ± 1, 13	30 ± 15, 0
Free	Four	75 ± 11, 88	13 ± 0, 13	37 ± 16, 0
Free	Five	84 ± 8, 90	13 ± 1, 13	43 ± 14, 33
MSZP	Three	77 ± 7, 67	14 ± 1, 14	13 ± 10, 0
MSZP	Five	62 ± 7, 60	14 ± 1, 13	19 ± 11, 0

[a]Each pot contained a cluster of one, two, three, four, or five non-molded ("free") seeds or a Multi-Seed *Zea* Pellet (MSZP) containing three or five seeds. Percent emergence is defined as a percentage of the seeds planted in a treatment; e.g., 50% emergence from a cluster of four free seeds would indicate that two seedlings emerged. $n = 10$.

Table 4. *Danaus plexippus* larvae observed in the planting density experiment.[a]

Date	Number of larvae							
	F1	F2	F3	F4	F5	MSZP3	MSZP5	Total
August 18	0	0	1	0	0	1	2	4
August 25	1	0	0	4	0	2	4	11
September 2	0	1	5	4	3	5	3	21
September 9 to 13	1	2	1	0	1	2	1	8
Total	2	3	7	8	4	10	10	

[a]Each pot contained a cluster of one, two, three, four, or five non-molded "free" (F) seeds or a Multi-Seed *Zea* Pellet (MSZP) containing three or five seeds. Data represent the total number of larvae observed across all 10 pots in each treatment. At the end of the experiment, 48 of the 70 pots contained living plants.

Table 5. Evidence of *Danaus plexippus* herbivory in the planting density experiment.[a]

Herbivory assessment	Number of pots							
	F1	F2	F3	F4	F5	MSZP3	MSZP5	Total
No living plants	4	6	3	4	3	1	1	22
No evidence of herbivory	3	1	0	3	6	2	3	18
Low herbivory damage	1	3	7	1	0	4	3	19
High herbivory damage	2	0	0	2	1	3	3	11
	%							
Percent herbivory	0.5	0.75	1	0.5	0.14	0.78	0.67	

[a]Each pot contained a cluster of one, two, three, four, or five non-molded "free" (F) seeds or a Multi-Seed *Zea* Pellet (MSZP) containing three or five seeds. Data represent the number of pots in each category on September 9 to 13. Percent herbivory represents the percentage of pots with living plants that showed either low or high levels of defoliation. $n = 10$.

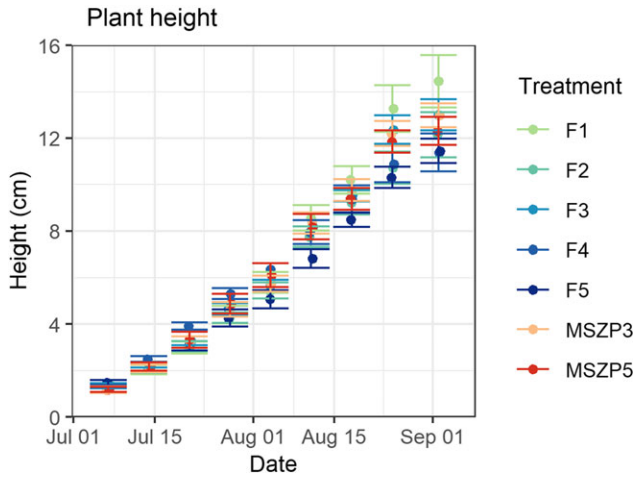


Figure 4. *Asclepias syriaca* height in planting density experiment. Each pot contained a cluster of one, two, three, four, or five non-molded seeds (free seeds, F1–F5) or a Multi-Seed *Zea* Pellet containing three or five seeds (MSZP3, MSZP5). All data are presented as mean ± 1 SE ($n = 8.1 \pm 0.2$).

exert a strong inhibitory effect on corn, *A. syriaca* would likely have needed to emerge much earlier than corn. Studies conducted before the introduction of glyphosate-tolerant crops (and concomitant decline of agricultural *A. syriaca* populations) demonstrate that it is possible for *A. syriaca* to reduce crop yield. Cramer and Burnside (1982) found that *A. syriaca* populations of 11,100 to 36,600 plants ha^{-1} caused corn stand losses up to 15% and yield losses up to 10%. Shortly before the introduction of glyphosate-tolerant crops, a review article concluded that *A. syriaca* was an increasing problem in crops including corn, soybean [*Glycine max* (L.) Merr.], and grain sorghum [*Sorghum bicolor* (L.) Moench], causing yield losses as high as 10% to 30% (Bhowmik 1994). Although less competitive than other broadleaf weeds, this perennial species was considered difficult to eradicate without nonselective herbicides. Therefore, we do not discount the possibility that *A. syriaca* could behave as a weed if reintroduced into agroecosystems and not controlled effectively. However, the decline of *Asclepias* spp. since the introduction of glyphosate-tolerant crops indicates that any competition arising from local *A. syriaca* restoration can be effectively controlled. Herbivory by *D.*

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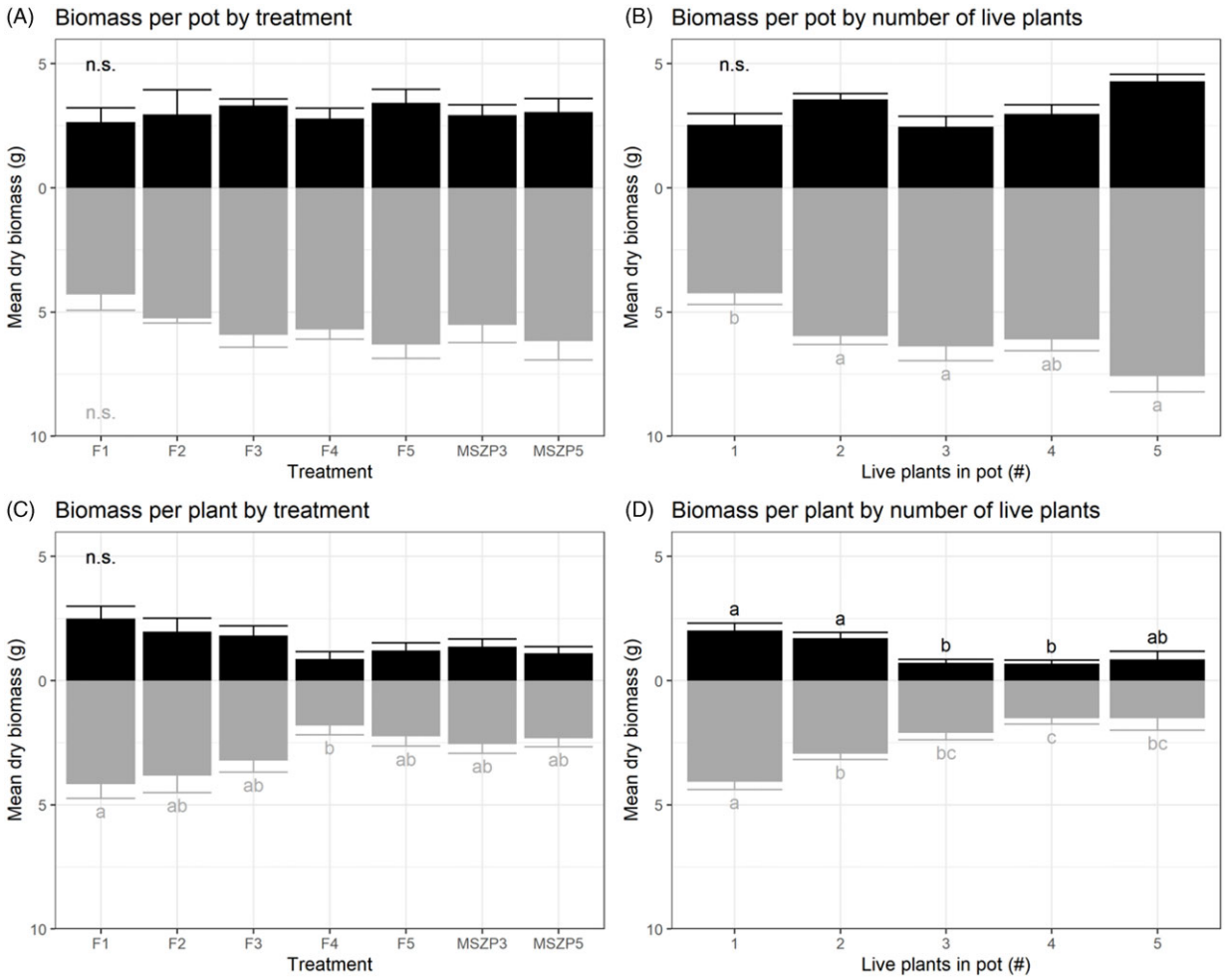


Figure 5. *Asclepias syriaca* biomass in planting density experiment. Each pot contained a cluster of one, two, three, four, or five non-molded seeds (free seeds, F1–F5) or a Multi-Seed *Zea* Pellet containing three or five seeds (MSZP3, MSZP5). Biomass data are presented as (A, B) total biomass per pot or (C, D) mean biomass per plant. Columns represent mean biomass by (A, C) treatment or (B, D) the number of live plants at the end of the experiment. Black columns represent aboveground biomass and gray columns represent belowground biomass. Within panel and response variable (aboveground or belowground biomass), columns labeled with the same letter are not significantly different according to Tukey's HSD test (n.s., not significant). All data are presented as mean ± 1 SE; (A, C) $n = 6.9 \pm 0.7$; (B, D) $n = 9.6 \pm 2.5$.

plexippus might also mitigate competition from a low density of *A. syriaca* plants.

The relative planting time experiment did not demonstrate a competitive effect of corn on *A. syriaca*. High nutrient, water, and light availability were likely responsible for the absence of a competitive effect. In more resource-limited environments, early competition from sorghum or weed species can reduce *A. syriaca* growth (Evetts and Burnside 1975). Competition from corn can suppress *A. syriaca* in field contexts (Yenish et al. 1997). For these reasons, our results should not be interpreted as evidence that competition between *A. syriaca* and corn is unlikely. The relative planting time experiment successfully demonstrated a major difference between *A. syriaca* and corn emergence and early growth rates but did not provide insight into possible competitive dynamics under resource-limited field conditions.

Future Directions

The three greenhouse and outdoor experiments demonstrated good *A. syriaca* emergence and early growth under a variety of conditions, including different planting depths, planting densities, and planting times relative to corn. In all experiments, seedlings successfully emerged from MSZP and reached the soil surface. Thus, the overarching goal of this research (to demonstrate the potential of MSZP as a seed-delivery technology for desirable native species) was achieved. Planting MSZP with a corn planter would likely reduce labor and capital costs associated with the establishment of wildflower strips in agroecosystems.

Our experiments provide proof of concept for MSZP technology. Future research should test whether MSZP can be effectively planted with a corn planter. If the MSZP crumble within the planter, it is possible to increase their three-dimensional strength by changing the pellet composition or applying more compressive force during molding. However, such modifications may reduce seedling emergence (Sikhao et al. 2015). Field testing and adoption of MSZP technology will require scaling up MSZP production. It is possible that commercial molding machines, such as those used for candy production, might be adapted for this purpose. Future research should also explore the potential of MSZP for simultaneous planting of multiple species. Agglomeration facilitates the even distribution of morphologically diverse seeds (Gornish et al. 2019), which could include wildflower seeds, cover crop seeds, or any other ecologically desirable seeds. This technology could even enable a low density of non-crop seeds to be planted among crop

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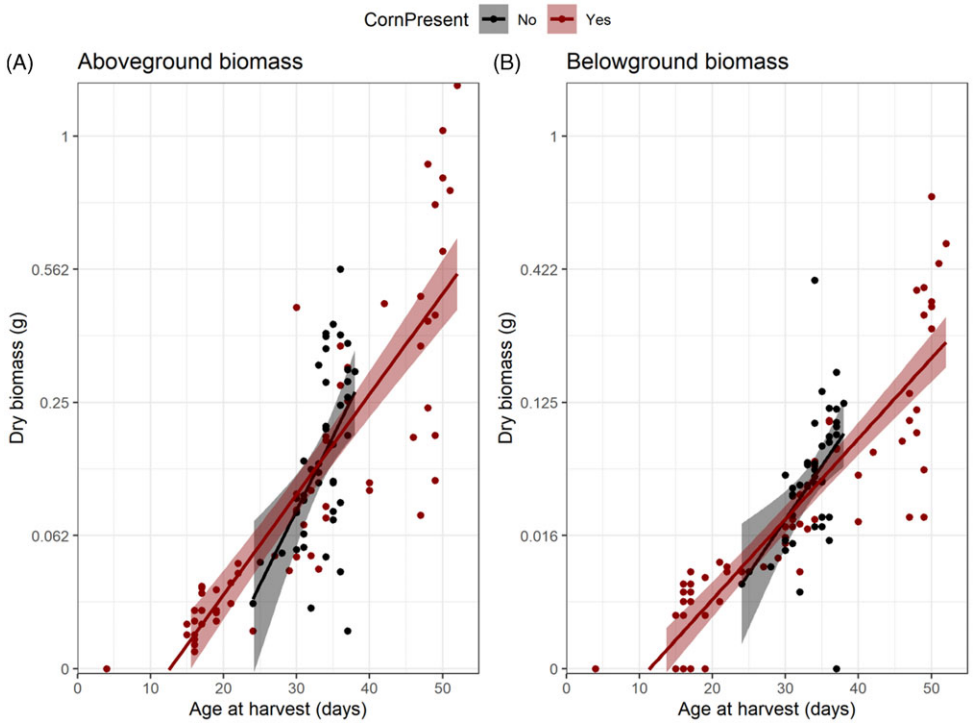


Figure 6. *Asclepias syriaca* biomass in relative planting time experiment. (A) Aboveground and (B) belowground biomass at harvest increased with plant age at harvest (days since emergence) but were not affected by the presence of corn in the same pot. Lines represent linear regressions with 95% confidence intervals.

seeds. We also note that our seed-molding method could be adapted to create pellets with the size, shape, and density of relatively large crop seeds other than corn seeds, such as soybean or cotton (*Gossypium hirsutum* L.) seeds. Small non-crop seeds might also be molded into pellets with the dimensions of small crop seeds, such as wheat (*Triticum aestivum* L.) seeds, and planted with a grain drill. Given appropriate non-crop species and seeding rates, this precise method of increasing agroecosystem biodiversity could provide ecosystem services with little risk to crop yield.

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