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The Impact of Planting Season and Crop Residue on Germination, Reproductive Success, and Mass of Native Forbs

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THE IMPACT OF PLANTING SEASON AND CROP RESIDUE ON GERMINATION, REPRODUCTIVE SUCCESS, AND MASS OF NATIVE FORBS

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ABSTRACT

The lack of biodiversity in prairie restorations compared to native prairies is alarming, and restoring this diversity has been a key focus of research and restoration projects for years. This study aims to assess two variables: planting season and plant residue, for achieving success in forb establishment. This research was conducted in a greenhouse using mesocosms that were seeded in spring, summer, and fall with nine forb species. Half of each seasonal treatment received ground cover, while the other half did not. Two hypotheses were formed. The first was that the fall planting will be the most successful seasonal treatment having the best germination rates, reproductive successes, and above-ground biomass. The second was that the ground cover treatments will perform significantly better than those without in germination, reproductive success, and biomass. The spring treatment performed the best having the greatest germination, reproductive success, and biomass, followed by the summer treatment, with the fall planting period performing the worst, which did not support my first hypothesis. The treatments receiving ground cover performed better than those without it, supporting my second hypothesis. The interaction showed ground cover's impact on the plant performance each season. The analysis also showed a statistically significant interaction between planting season and litter for the number of plants that emerged. This was not detected for the number of plants that reached their reproductive life stage or biomass. With the dwindling prairie distribution, this research allowed our team to make recommendations to increase diversity and expand pollinator habitat in shortgrass prairies in a changing climate.

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INTRODUCTION

Temperate grasslands are productive and diverse ecosystems globally recognized for their contributions to ecological stability and human society, such as carbon storage, agricultural production, water purification before aquifer recharge, and pollinator support (Alstad et al. 2016). Grasslands are the largest ecosystem worldwide covering ~40% of the planet's ice-free land area (Anderson, 2006). Less than 40% of North America's 550 million acres of historical grasslands remain today (NPS, 2018). Prairies are native grasslands across North America that are home to over 100 species of lichens and liverworts, over 70 species of grasses, about 300 forb species, and numerous woody trees and shrubs (Jarchow et al., 2020; National Park Service, 2021). Prairies can be separated into tallgrass, mixed grass, and shortgrass (Jarchow et al., 2020). Before European colonization, Tallgrass prairies covered 170 million acres of North America. Currently, it is estimated at less than 4%, NPS (2018), making them one of the Earth's most endangered ecosystems (Bassett, 2017). Mixed-grass prairie is the transition zone between the tallgrass and the shortgrass prairies. Historically mixed-grass prairies covered 140 million acres, 21% remains today. Shortgrass prairie covered 265 million acres of the Western Great Plains before European settlement. Today, only half of that remains (Audubon, 2022).

Plant group diversity plays critical roles in ecosystems, such as supporting primary productivity, resisting invasive species, and maintaining nutrient cycling (Alstad et al., 2016). The presence of flowering forb species is of value to pollinating insects as well. Flower-dependent insects that use reconstructed prairies have been shown to improve crops and native plant performance within the restored prairie (Drobney et al., 2020). Restored prairies tend to have lower species richness, native forb densities, and more exotic species than remnant native prairies (Hillhouse et al. 2011). When comparing the two ecosystems, native and restored prairies both tend to be dominated by grasses, with native prairies having a subdominant forb community while restored do not (Polley, 2005). The loss of forb diversity seen in restored prairies results from small patch sizes, fragmentation, and conversion to shrubland due to the absence of natural disturbances, especially fire (Alstad et al., 2016). Compacted soils, low soil nutrient availability and moisture, and a lack of soil biota make forb establishment difficult (Barrera, 2021).

Fifteen families of angiosperms have physical dormancy requiring scarification or stratification to break (Baskin et al., 2008). Seed scarification is nicking, breaking, softening, or weakening a seeds coating to speed up germination. Scarification can occur naturally with the freezing and thawing of the soil in winter and often is the key to fall plantings. Another way to break physical dormancy is stratification, the process of treating seeds to stimulate natural conditions for germination to occur (Statwick, 2016).

Cover crops have been used as a natural pest control tool in agriculture and have been shown to result in increased soil moisture (Lando-Monserrat et al., 2014). Tuure et als. (2021) study showed that mulching will increase soil moisture in the effective root zone in a semi-arid environment. In addition, it has been used as an effective erosion control tool and enhances the microbial response to rewetting (Lando-Monserrat et al., 2014). Decomposing plant biomass also releases nitrogen and phosphorous, shaping the microbial and, in turn, the plant community structure (Chen et al., 2020).

This project assessed the impact of planting season and the use of crop residue on germination, reproductive success, and above-ground biomass forb species commonly used in grassland restoration projects in Kansas. My main objectives for this research were to determine the best planting season for forb seed additions in restorations and the necessity of including leaf litter in restorations of forb species. My first hypothesis was that the fall treatment would have significantly higher germination rates, reproductive success, and biomass than the spring and summer plantings. My second hypothesis was that the treatments with litter would have higher germination reates, reproductive success, and above-ground biomass than those without.

MATERIALS AND METHODS

I conducted an experiment using mesocosms to determine the best planting season and the effects of litter in a greenhouse at the Fort Hays State University campus. Seeds and soil came from Smokey Valley Ranch, an 18,000-acre cattle and bison operation owned by the Nature Conservancy. The ranch is located in northwest Kansas within a semi-arid landscape outside of Oakley, KS. The ranch has an average summer high temperature of 32.8°C and a low of 17.2°C, a winter high of 5.6°C and a low of -8.9°C. This area receives an average of 51cm of precipitation annually. This area is made up of shortgrass prairie. Shortgrass prairies are dominated by grasses that are 0.3-0.5m tall, including buffalo grass (*Buchloe dactyloides*), blue grama (*Bouteloua gracilis*), side oats (*B. curtipendula*) and hairy grama (*B. hirsute*) (Anderson, 2006). Subdominant species seed are needleleaf sedge (*Carex* duriuscula), western wheatgrass (*Pascopyrum smithii*), scarlet globemallow (*Sphaeralcea coccinea*), prairie sagewort (*Artemisia* *fridgida*), and plains prickly pear (*Opuntia polycantha*) (Wilcox et al., 2020). Smokey Valley plants to restore 760 acres of cropland located on the ranch and enhance an additional 5,200 acres of CRP and intact prairie surrounding the site to attract pollinators. My study was done to help them collect data on the season planting occurs and the use of crop residue's effect on flowering forb species.

I created mesocosms to scale down the size of the field and only test for the variables of interest (planting time and cover). The mesocosms were made of 18-gallon totes (58.72 x 46.12 x 37.80cm) with drainage holes drilled into the bottom. Topsoil (top 30 cm) from the cropland to be restored was collected and sieved to homogenize it and remove any large debris. Once sieved, I filled the mesocosms 80% full. Soil from the location was used so that the natural soil microbes would not differ between this project and the restoration. The mesocosms were placed in the greenhouse in a randomized block design; these were blocked by replicate with two replicates per block to prevent the treatments being exposed to location variability. (Fig. 1).

Figure 1. This figure represents the spring (left) and summer (right) plantings approximate locations in the greenhouse. The green rectangles represent the mesocosms with ground cover, the grey ones represent the mesocosms without ground cover, and the white ones represent spaces where summer plantings would be added. The blue lines along the outside represent windows. The right figure represents the summer plantings addition to the greenhouse The fall treatment was setup similar to the spring except no spaces were left open for an additional planting.

We had three seasonal plantings, one in the spring, one in the summer, and one in the fall. Planting occurred on April 21st, June 4th, and November 28th in 2021. A watering plan was created using data from a weather station located on Smokey Valley Ranch, this was done to mimic natural rainfall (Table 1). Average total monthly precipitation was determined over ten years, I then determined the acre inches for the proposed restoration and then scaled down to mL based on the size of the mesocosms. Average precipitation occurrences were also determined monthly, including any precipitation event over ¼ in. The spring treatment involved leaving the mesocosms outdoors until the day of planting to imitate cool soil temperatures seen that season. The summer mesocosms were brought inside one week prior to planting to allow the soil to reach 18.33°C. it is important to note that the greenhouse roof broke in a storm while the summer mesocosms were warming up inside, causing flooding, however, the top few inches of the soil had dried before planting. The fall mesocosms were planted in mesocosms and they were left outdoors through the winter and placed beside the greenhouse with the straw-like material bundled around them to shield them from the wind and provide insulation comparable to the soil environment. They were brought inside in the spring after the initiation of green up was observed on campus.

Table 1. This table shows the monthly precipitation average (in) calculated using Smokey Valley's weather system, the total amount of water (mL) each mesocosm received monthly, and the number of monthly precipitation occurrences for our greenhouse study conducted 2021-2022.

Month	**Monthly precipitation (in)**	**Water (mL)**	**Precipitation occurrences**
April	1.61	7,619.47	3
May	2.57	12,115.65	4
June	2.50	11,802.89	4

July	2.86	13,473.51	4
August	2.43	11,481.98	4
September	2.43	11,463.10	3
October	2.45	11,566.50	3

The NRCS Plants Materials Lab provided seeds for the nine study species to be planted in the mesocosms. The species used are described in Table 2. Five of each seed were planted into each of the mesocosms; the seeds were randomly placed into five rows of nine to replicate broadcast seeding done at the ranch. Toothpicks were colored to represent the nine seeds (one color for each species), increasing the accuracy of data collection. Sixteen mesocosms were seeded for the spring, sixteen for the summer, and sixteen for the fall. To determine the effects of litter on germination, eight of the sixteen mesocosms were covered in sorghum stocks each season. The litter covered 80% of the soil surface uniformly along all the blocks. This was done to replicate the crop residue at Smokey Valley Ranch. Eight mesocosms were not covered in sorghum stocks to test the performance of plants without ground cover.

Table 2. This table lists the species used in this greenhouse study conducted 2021-2022, their family, and whether they require scarification or stratification. * indicates that cold stratification may result in better germination rates (USDA-NRCS, 2011).

Species	Family	Requires scarification or stratification

Asclepias speciosa	*Apocynaceae* (Dogbane) (UDSA, NRCS, 2022)	Yes
Coreopsis tinctoria	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	Yes
Desmanthus illinoensis	*Fabaceae* (Legume) (UDSA, NRCS, 2022)	No*
Echinacea angustifolia	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	Yes
Engelmannia persistenia	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	Yes
Echinacea purpurea	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	Yes
Liatris punctata	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	Yes
Ratibida columnifera	*Asteraceae* (Daisy) (UDSA, NRCS, 2022)	No*
Salvia azurea	*Lamiaceae* (Mint) (UDSA, NRCS, 2022)	No

Data collection occurred during each watering occurrence after initial emergence; collection continued until the plants finished their lifecycle. The data collected during watering were the total number of plants germinated, the number of each species germinated, and the number that reached sexual maturity. Sexual maturity was determined by the presence of flowers or buds. After the plants ended their lifecycle, they were clipped at the bottom of their stem, dried in an oven for 72 hours, and the total dry mass for each mesocosm was taken in grams.

R version 4.1.1 “Kick Things” was used for data analysis. A significance level of 0.05 was used. A MANOVA test was run using the “mvnormtest” package (Jarek, 2012). The following packages were used to test the assumptions of the MANOVA: “mvoutlier” (Filzmoser, Gschwandtner, 2021), to check for outliers, “energy” (Rizzo and Szekely, 2022) to check for multivariate normality, and “heplots” (Fox et al., 2021) to check for homogeneity of variance. The MANOVA test was followed by three Tukey HSD tests to compare differences within seasons and treatments in emergence rates, reproductive rates, and dry biomass. I used “ggplot2” (Wickham, 2016) and “tidyverse” (Wickham et al., 2019) were used to create graphs to help with data visualization.

RESULTS

For the spring treatment, all nine species were observed to have germinated (Fig. 2A), and four reached sexual maturity (Fig. 2A): *C. tinctoria* (5 plants), *D. illinoensis* (3 plants), *E. persistenia* (3 plants), and *S. azurea* (20 plants). The species that had the most individuals germinate were *C. tinctoria* (17 plants), *E. persistenia* (25 plants), and *S. azurea* (30 plants). For the summer treatment, all nine species were observed to have germinated (Fig. 2B) and three reached sexual maturity (Fig. 3B): *C. tinctoria* (1 plant), *E. persistenia* (1 plant), and *S. azurea* (13 plants). The top germinating species were *A. speciosia* (8 plants), *E. angustifolia* (15 plants), and *S. azurea* (16 plants). Three species were observed to have germinated for the fall treatment (Fig. 2C), *A. speciosa* (7 plants), *C. tinctoria* (12 plants), and *E. purpurea* (6 plants). *C. tinctoria* (9 plants) was the only species that reached sexual maturity (Fig. 3C).

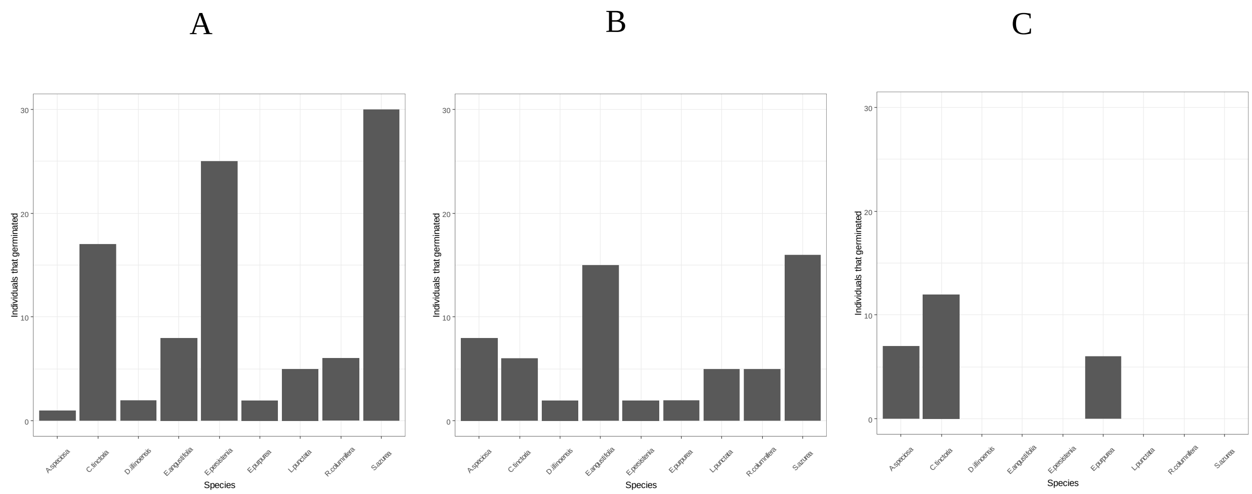

Figure 2. A, the number of individuals within each species that germinated in the spring treatment. **B,** the number of individuals within each species that germinated in the summer treatment. **C,** the number of individuals within each species that germinated in the fall treatment.

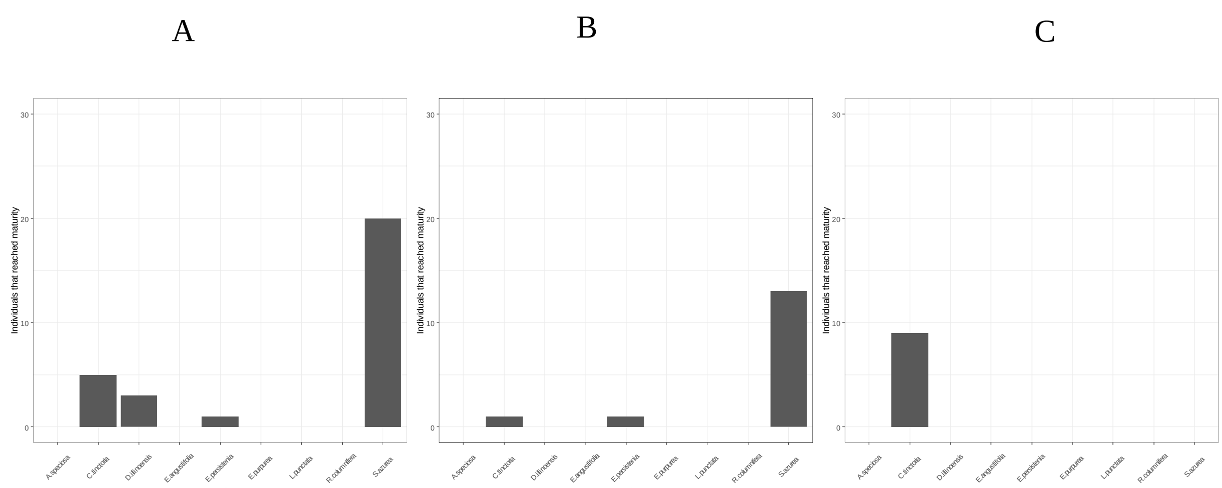

Figure 3. A, the number of individuals within each species that reached their reproductive stage in the spring treatment. **B,** the number of individuals within each species that reached their reproductive stage in the summer treatment. **C,** the number of individuals in each species that reached their reproductive stage in the fall treatment.

Planting season

Planting season had a significant ($p<0.001$) effect on the number of plants that germinated (Fig. 4A). The spring treatment had significantly more plants (6 plants ± .83 (Mean ± SE)) germinate than the summer (3.81 plants ± .90; $p=0.029$) and fall (1.56 plants ± .43; $p<0.001$) treatments. Planting season also had a significant ($p=0.018$) effect on the number of plants that achieved reproductive success (Fig. 4B). The spring planting had significantly ($p=0.016$) more plants (1.81 plants ± 0.40) reach their reproductive life stage than the fall planting (0.63 plants ± 0.29). Planting season had a significant ($p<0.001$) effect on the biomass as well (Fig. 4C). The spring planting produced plants that were significantly ($p<0.001$) heavier (9.99g ± 1.46) than the fall planting (063g ± 0.29).

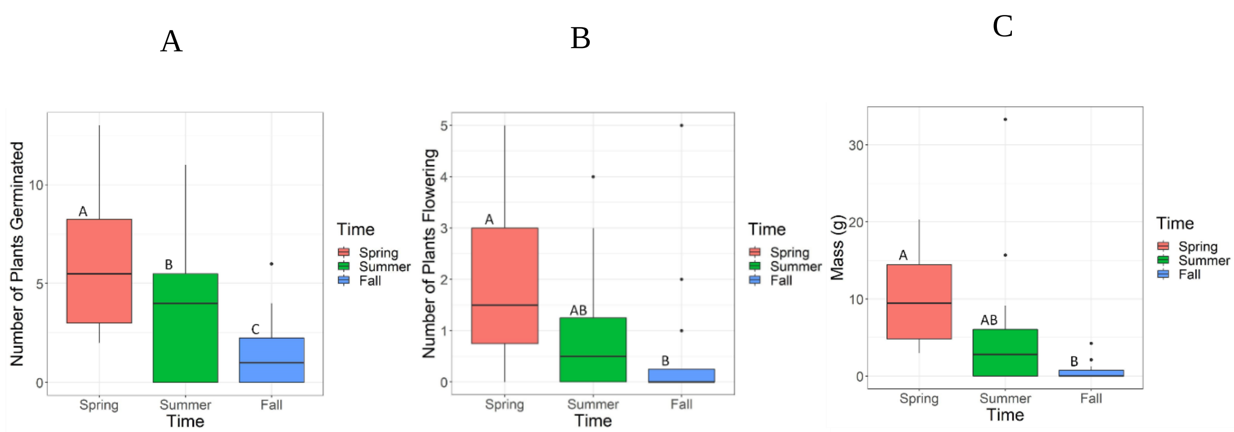

Figure 4. A, the total number of plants germinated. B the total number of plants that reached sexual maturity. C the above-ground biomass for spring, summer, and fall. Letters by the plots represent whether or not the treatments are different; different letters represent statistical differences, while the same letters represent similarities. In the box plot, the bar through the middle of the box represents the median, the lower half of the box represents the second quartile (1/4 the dataset), and the upper half of the box is the third quartile (1/4 the dataset). The whiskers represent the last half of the dataset. The dots appearing represent outliers.

Ground cover treatment

The cover treatment had significantly more plants (5.46 plants ± 0.75) that germinated than the treatment without cover (2.13 plants ± 0.47; Fig. 5A). The cover treatment had significantly (p=0.001) more plants (1.7 plants ± 0.35) reach sexual maturity than the treatment without litter (0.5 plants ± 0.16; Fig. 5B). The cover treatment did not significantly (P=0.054) affect biomass (Fig. 5C).

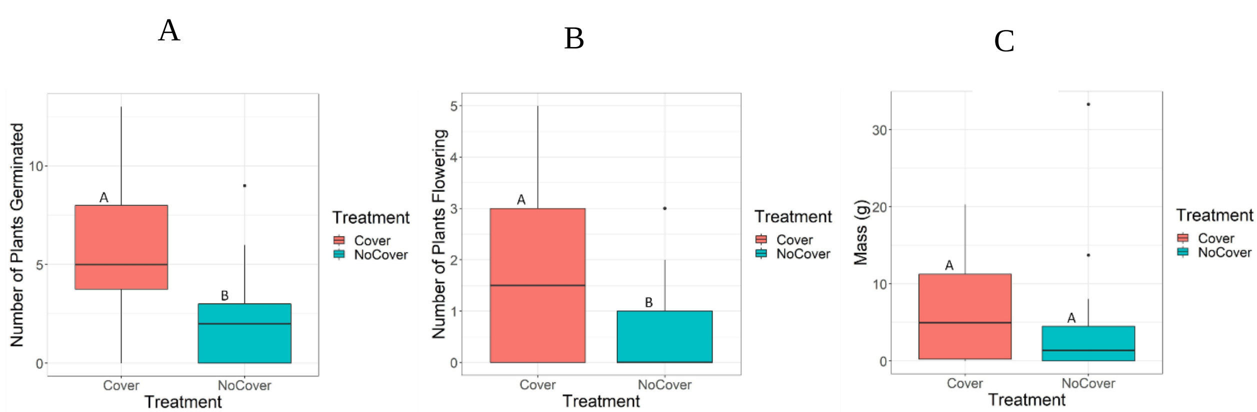

Figure 5. A, the total number of plants germinated. **B,** the total number of plants that reached sexual maturity. **C**, represents the biomass..

Treatment interactions

The interaction between planting season and the cover treatment had a significant (p=0.024) effect on the number of plants that germinated (Fig. 6A). For the spring (p=0.002) planting the cover treatment had significantly better germination (8.38 plants ± 1.02) than the treatment without cover (.3.63 plants ± 0.56). The summer planting had significantly better (p=0.003) germination in the treatment with ground cover (6.13 plants ± 0.85) than the one without (1.5 plants ± 1.10) as well. In the fall planting, there was no statistical difference (p=0.994) between the emergence of the treatments with (1.88 plants ± 0.79) and without (1.25 plants ± 0.37) ground cover. Across all seasonal plantings, cover did not significantly (p=0.103) affect the reproductive success of the plants (Fig. 6B). The plantings that had the most plants reach their reproductive growth phase were the spring and summer plantings with ground cover. Within the spring planting, the cover (2.75 plants ± 0.56) treatment had significantly (p=0.041) greater reproductive success than the treatment without (0.88 plants ± 0.35) ground cover. The cover treatment did not impact the summer (p=0.105) and fall (p=0.999) plantings' reproductive rates. The interaction between planting season and litter use did not have a significant (p=0.125) impact on the mass of the plants (Fig. 6C). There was no statistical difference in mass within the seasonal plantings based on cover treatment for spring (p=0.073), summer (p=0.994) or fall (p=0.999).

A B C

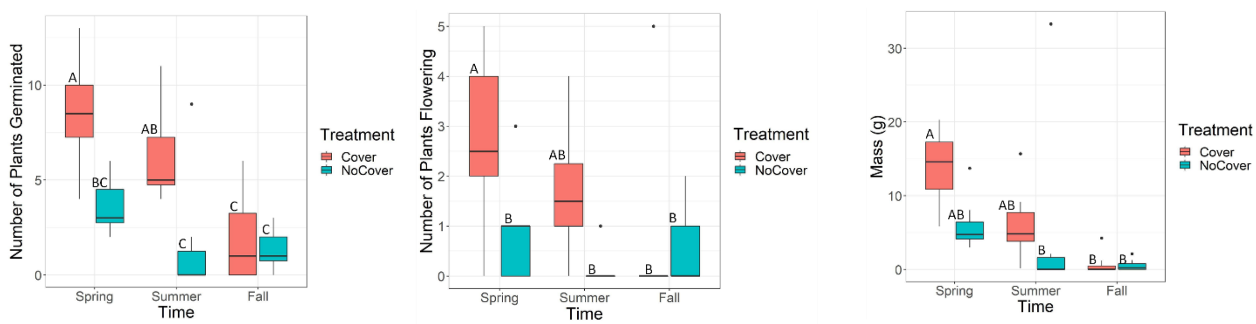

Figure 6. **A**, the left panel represents the number of plants that germinated. **B**, the center panel represents the number of plants that reached their reproductive life stage. **C,** the right panel represents the biomass in spring, summer, and fall with and without litter.

DISCUSSION

The first hypothesis stated was that the fall planting would have greater germination, reproductive success, and mass than the spring and summer plantings. This was not supported (Fig 4). The spring planting performed the best out of all three seasons for germination, reproductive success, and above-ground biomass. The fall planting was the lowest performing in all three categories. My second hypothesis was that the litter treatment would have greater germination, reproductive success, and mass than the treatment with no ground cover. This hypothesis was supported with the exception of biomass, which was marginally significant (Fig. 5).

Planting season

The spring treatment had more plants germinate than the summer and fall treatments did. This could be due to the seeds likely being stored in a cooler before being sent to us, giving them the required stratification, as six of the nine species required cold stratification for germination to occur (Table 1). Other studies have seen the best success with warm-season plantings and cold-stored seeds as well. Hockenberry Meyer et als.' (2019) study found that the grass prairie dropseed had the best germination results with 4.44°C dry storage followed by direct seeding into a 23.89°C greenhouse. Additionally, the best-performing plant *S. azurea,* does not require nor benefit from cold stratification. The spring treatment also had more plants achieve reproductive success, followed by the summer treatment, then the fall planting. This is likely due to the spring planting receiving more water than the summer planting (~3in; Table 2). The spring planting also had the largest above-ground biomass; this is due to the largest amount of plants being present seen in this planting. Although we did not see success with the fall planting, I believe it is due to outside reasons, as other studies have viewed increased forb establishment rates in sites planted in the fall (Lukens et al., 2020) The fall plantings performance can be attributed to a lack of soil biota due to how the soil was stored. The soil was stored in a pile on top of a tarp in front of the greenhouse; it is thought that over the summer, there was a death of some microbes due to high heat. An additional reason for the low fall performance could be the potential of seed death while overwintering.

Cover treatment

Crop residue has been shown to increase agricultural crop yields (Lu, 2020). The cover treatment performed significantly greater in germination rates and reproductive success, likely due to the increased moisture retention in the soil, as crop residue can increase soil water content and water use efficiency (Lu, 2020; Fu et al., 2021). Increased moisture content allows for greater success when moisture is a limiting factor as it is in western Kansas. Crop residue improves soil quality, increasing total porosity in soil, aggregate stability, cation exchange capacity, organic carbon, phosphorous, and potassium (Fu et al., 2021). Nitrogen, a limiting nutrient, is also increased in the soil during decomposition (Chen et al., 2020). Increased soil organic matter has also been shown to increase the entomopathogenic fungi group. This group of fungi serves as biological control for insect pests (Vukicevich et al., 2019). Above-ground biomass was moderately significant; this could partially be due to the low performance in the fall treatment skewing the results. Additionally, the high greenhouse temperatures could have been too hot for the biomass to lower soil water evaporation.

Treatment interactions

There was an interaction between the planting season and the presence of ground cover for the number of plants that germinated. The spring treatment with cover was the best performing and was statistically different from all other treatments for germination, reproductive success, and above-ground biomass (Fig. 6). This is likely due to this treatment receiving more water than the summer treatment while not having as high of temperatures during its early growth phases. The summer treatment without cover and both fall treatments were the lowest performing across the board, and their germination, reproductive success, and masses were not statistically different from one another. The summer planting without cover likely performed poorly due to the high temperatures mitigating the water retention ability of the soil with coverage. When looking at the use of groundcover across the different seasons, it increased germination rates. The spring treatments with and without cover and the summer treatment were different from one another. This study showed that both planting season and crop residue can significantly impact the performance of these forb species.

Future directions

My first recommendation for replicating this project would be to collect seeds by hand. This would remove the effect of storing seeds in a refrigerator. I would expect different results with that factor removed. This project could also be performed outside using two fields with and without sorghum stocks, blocked into three sections for spring, summer, and fall. Using this method, this research could be followed long-term in a large-scale setting. I would expect the utilization of natural prairie disturbances such as fire and grazing to give different results than seen in this small-scale project. This would also provide beneficial knowledge for future prairie restorations focused on increasing diversity.

CONCLUSION

This research showed the importance of the season of planting and use of ground cover on the success of forb species in a small-scale environment. When planning a restoration, it is important to consider the origin of the seeds, the plant hardiness zone of the location to be restored, and the type of plants being used. This project showed that seeding season significantly impacts the performance of these forb species, making it an important variable. Another factor that must be considered is using a cover crop or crop residue. It has been shown that cover crops increase the performance of plant productivity (Abdalla et al., 2019), and this project has not shown a difference. While continued research would need to be done at a larger scale, recommendations can be made based off this project achieving our goal. Ultimately, this data can be used to recommend a spring planting with crop residue left on the field before planting. This recommendation could change if the seeds are not purchased, but if they are kept in a cooler, they should perform well in the spring.

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