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#### RESEARCH ARTICLE

Seed Germination Characteristics of Prairie Dropseed (Sporobolus heterolepis)

# Chad A. Fedewa J. Ryan Stewart<sup>1</sup>

Department of Natural Resources and Environmental Sciences University of Illinois 1201 South Dorner Drive Urbana, IL 61801

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ABSTRACT: Prairie dropseed (*Sporobolus heterolepis* [A. Gray] A. Gray) is a warm-season grass that is an important component of remnant tallgrass prairies of North America, but is rarely found in restorations. We conducted three experiments to determine germination rates of prairie dropseed under different conditions in an attempt to potentially increase its successful establishment under field conditions. In the first experiment, we evaluated the duration of cold-moist stratification on the germination of prairie dropseed. Seeds were cold-moist stratified for 0, 30, or 60-d at 4 °C and then placed in a dark growth chamber at 26 °C. The other two experiments consisted of seeds planted in three different soil media: 1:1:1 soil:peat:perlite mixture, non-compacted field soil, and compacted field soil. They were then exposed to full irradiance or 50% shade. Germination was highest for seeds cold-moist stratified for 30 or 60 d. Seeds planted in either non-compacted or compacted field soil had higher germination rates than the 1:1:1 mixture in both full irradiance and 50% shade. We conclude that prairie dropseed has higher germination percentage after a period of cold-moist stratification. While our results indicate that seeds germinate better in field soil, germination rates were low compared to the amount of viable seed. It also appears that seed of prairie dropseed germinate at relatively higher levels under partial shade.

Index terms: prairie grass, propagation, stratification, tallgrass prairie

# **INTRODUCTION**

The first concentrated and systematic effort to restore a tallgrass prairie to mimic pre-settlement vegetation began at the University of Wisconsin Arboretum in the 1930s (Cottam and Wilson 1966). Efforts since then to duplicate plant species diversity levels found in remnant prairies have not been successful, suggesting that further research into the species dynamics of remnant prairies is still needed (Polley et al. 2005). Higher species diversity in remnant prairies may be partly due to seeds of some species found in remnants not being included in seed mixes used for restorations (Polley et al. 2005). Restorations often include species that establish quickly such as big bluestem (Andropogon gerardii Vitman), Indian grass (Sorghastrum nutans [L.] Nash), Canada wild rye (Elymus canadensis L.), purple coneflower (Echinacea purpurea [L.] Moench), grey-headed coneflower (Ratibida pinnata [Vent.] Barnhart), black-eyed susan (Rudbeckia hirta L.), and wild bergamot (Monarda fistulosa L.), while excluding slow-to-establish species such as lead plant (Amorpha canescens Pursh), gentians (Gentiana spp. L.), and prairie dropseed (Sporobolus heterolepis [A. Gray] A. Gray) (Nuzzo 1978; Diboll 2005). Species that establish relatively quickly are often favored by restoration managers because the results are observed by the public within a short period of time (Nuzzo 1978). Slow-to-establish species, even when included in seed mixes, are often out-competed by the more aggressive, quick-to-establish species (Diboll 2005).

With the recent growing interest in restoring prairies, techniques that maximize biodiversity have become a high priority for restoration efforts (Mutel and Packard 2005). As a result, more information is needed on establishment techniques, germination rates, and growth habits of some of the more difficult-to-establish species in order for them to be included in prairie restorations. Including these species in prairie restorations and limiting the abundance of dominant species is one step towards increasing diversity in restorations (Polley et al. 2005). It may also alter ecological processes, including resource partitioning and dispersal dynamics, which may contribute to the higher diversity in remnants relative to that in restorations (Polley et al. 2005). These difficult-toestablish species are also important food sources for many rare birds (Byre 2005) and insects (Taron 2005).

We attempted to increase the available knowledge related to the propagation of a difficult-to-establish, warm-season prairie grass, prairie dropseed. Prairie dropseed is a perennial bunchgrass native to the tallgrass prairie region of North America, stretching from Canada south to Oklahoma and from Colorado to the Atlantic coast (NRCS 2008). It is generally found in undisturbed mesic sites (Schramm 1978; Nelson and Anderson 1983). It is also listed as threatened or endangered in several eastern states (NRCS 2008). While it appears to be an important component of high-quality remnant prairies (Greene and Curtis 1953; Taft et al. 1997), it is

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<sup>&</sup>lt;sup>1</sup> Corresponding author: rstewart@illinois.edu

not regularly found in prairie plantings or restorations (Schramm 1978; Hitchmough et al. 2004). Reasons for its absence may include its low germination rate, slow establishment (Diboll 2005), and sensitivity to competition (Schramm 1978).

We hypothesized that seeds placed in treatments that replicate natural conditions (i.e., cold-moist stratification and planted in field soil) would have a higher germination response than seeds not exposed to cold and moist conditions. Our objective was to determine the germination response of prairie dropseed to various treatments under controlled conditions with the overall goal of identifying techniques to expedite the germination and growth of this species to improve its success in prairie plantings. Some restorationists have had greater success planting prairie dropseed in the fall rather than in the spring (E. Ulaszek, horticulturist, Midewin National Tallgrass Prairie, pers. comm.). One possible explanation is that fall plantings allow the seeds to go through natural freeze-thaw cycles that break dormancy. Thus, one project focused on cold-moist stratification of seeds to replicate over-wintering conditions. In order to make basic inferences to field plantings to enhance species establishment, two other experiments focused on the effects of soil media on seed germination under different irradiance conditions.

## **METHODS**

Seeds for both experiments were purchased from Prairie Moon Nursery in Winona, Minnesota. One batch, used for both soil experiments, was purchased in February 2007. The other batch was purchased in October 2007 and was used for the coldmoist stratification experiment. Seeds were stored in dry conditions at 4 °C until the projects were initiated. Tetrazolium tests (Peters 2000) were performed on 20 December 2007 on two replications of 100 seeds from each seed batch to get an estimate of their viability.

# **Cold-moist stratification**

This study was set up in a completely randomized design with three treatments:

(1) a control (0-d stratification); (2) 30-d stratification; and (3) 60-d stratification. Each treatment was replicated five times with an overall total of 15 experimental units. Treatments were initiated on 20 December 2007.

Each experimental unit consisted of 50 seeds placed between two pieces of 90-mm-diameter filter paper within a 100-mm x 15-mm plastic petri dish. Each experimental unit then received 2.0 mL of distilled water. The control group was immediately placed in a dark growth chamber at 26 °C. Groups receiving the stratification treatments were placed in a dark growth chamber at 4 °C for the appropriate time period.

Germination was defined as the emergence of a radicle, which could be viewed without magnification. Once germination began, seeds were counted every 2-3 d for 30 d; germination percentage, day of first germination, and mean daily germination were then calculated. Throughout the duration of the experiment, 1.0 mL of distilled water was added to the filter paper when it appeared dry. Filter paper was replaced as needed to prevent fungal growth.

# Data analysis

The effects of time of stratification on seed germination were analyzed by analysis of variance (ANOVA) and mean separation was done with Tukey's honestly significant difference (HSD) option of SAS software, version 9.1 (SAS Institute, Cary, NC) using the general linear models procedure. Stratification treatments were also compared to the control using the least squares means option with the Dunnett adjustment for multiple comparisons.

### Soil treatment

Experimental units were arranged in a completely randomized design for both soil treatment experiments in a greenhouse setting. Each experimental unit consisted of 50 seeds scattered on the soil surface in 2048 cm<sup>3</sup> square pots (height = 12.7 cm, width = 12.7 cm). All seeds were coldmoist stratified for 55 d, similar to the first experiment prior to treatment initiation.

Treatment initiation began on 31 March 2008. Three soil treatments were tested for each experiment: (1) 1:1:1 soil:peat:perlite mixture; (2) non-compacted, sterilized field soil; and (3) compacted, sterilized field soil. The field soil used was a mixture of Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquoll) and Flanagan silt loam (fine, smectitic, mesic Aquic Argiudoll) soils (Endres 2001). These are poorly drained soils common to central Illinois. The compacted field soil treatment was compacted with an initial 3733 g weight to replicate local field soil conditions. Approximately 1.27 cm of soil was added incrementally and compacted as soil was added to the pot to ensure even compaction from bottom to top.

The first soil experiment was conducted at an irradiance level of approximately 660  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> and the second experiment under a 50% shade cloth with an approximate irradiance level of 340  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. Multiple 1000-W, high-pressure sodium lamps provided supplemental irradiance between 0600 and 2200 HR.

Each soil treatment was replicated five times with an overall total of 15 experimental units for each experiment. Experimental units at each irradiance level were set up in three rows of five pots each.

All experimental units were watered as needed with a mist-type nozzle to maintain adequate soil moisture. After planting, seedling numbers were recorded every 7-8 d for a total of 37 d. Germination percentage, mean daily germination, and final seedling total were calculated.

# **Data analysis**

The effects of soil type on seed germination for each soil experiment were analyzed separately by ANOVA, and mean separation was done with Tukey's HSD option of SAS (SAS Institute) using the general linear models procedure.

## **RESULTS**

Tetrazolium tests revealed that the seed batch purchased in October 2007 and used

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for the cold-moist stratification experiment had 46% of the seeds viable, 17% were immature, and 37% non-viable. Tests for the batch purchased in February 2007 and used for both soil experiments revealed that 74% of the seeds were viable, 5% were immature, and 21% were non-viable.

#### **Cold-moist stratification**

Average germination percentage across all treatments was 43% (± 0.03 SE). According to the ANOVA, the germination response differed among treatments (P = 0.03). The germination response of seeds in the 30-d treatment was nearly 16% more than that of seeds in the control treatment (Table 1). There were no differences, however, in germination percentage and mean daily germination between seeds in the 30-d and 60-d treatments (Table 1). Mean daily germination rates also differed among treatments (P = 0.03), with the 30-d treatment yielding the highest non-significant rate (Table 1). There were no differences in mean daily germination between those of the 30-d and 60-d treatments (Table 1).

The average day of first germination was different among the treatment groups (P < 0.0001), with seeds in the control treatment having the most delayed first day of germination (Table 1). The average first day of germination of seeds in the 30-d (P = 0.0014) and 60-d (P < 0.0001) were not different from each other (Table 1).

#### Soil treatment

Average germination percentage, mean daily germination, and seedling totals across all treatments in full irradiance were 9.4%  $(\pm 0.02 \text{ SE})$ , 0.128 %/d  $(\pm 0.03 \text{ SE})$ , and 4.73 seedlings (± 1.01 SE), respectively. Tukey's HSD test showed differences in the responses of seeds planted in the different soil types. Seeds in the compacted field soil treatment had the highest non-significant values for germination percentage, mean daily germination, and seedling total relative to that of the other treatments (Table 2). However, there were no differences in these values between seeds in the compacted treatment and the non-compacted soil treatment (Table 2). There were also

Table 1. Germination measurements for *Sporobolus heterolepis* (prairie dropsed) after different periods of cold-moist stratification at 4 °C. Tetrazolium tests estimated that 46% of seeds were viable prior to the experiment.

Stratification Period	Germination Percentage	Mean Daily Germination (%/day)	Day of First Germination
0 days (control)	34.8 b	0.58 b	8.8 a
30 days	50.4 a	0.84 a	4.4 b
60 days	45.2 ab	0.75 ab	2.2 b

<sup>&</sup>lt;sup>a,b</sup> Stratification periods within the each column with the same letter are not different at  $P \le 0.05$  according to Tukey's honestly significant difference

no differences in these response variables between seeds in the 1:1:1 mixture and non-compacted treatment (Table 2).

In the 50% shade experiment, ANOVA showed no significant differences in germination percentage, mean daily germination, and seedling totals between soil types (P = 0.06). Average germination percentage, mean daily germination, and seedling totals across all treatments in 50% shade were 25.5% ( $\pm$  0.03 SE), 0.344 %/d ( $\pm$  0.04 SE), and 12.73 seedlings ( $\pm$  1.35 SE), respectively. Tukey's HSD test also showed no difference between soil treatments. The non-compacted field soil treatment had the highest non-significant values for germination percentage, mean daily germination, and seedling total (Table 3).

## **DISCUSSION**

Our results indicate that cold-moist stratification not only increased germination of prairie dropseed, but also hastened the initiation of the germination process (Table 1). It was also demonstrated that in comparison to the percentage of seeds that were estimated to be viable by tetrazolium tests, prairie dropseed germinated at relatively low rates when planted in soil under full-irradiance conditions (Table 2) and in 50% shade (Table 3).

Greene and Curtis (1950) found no statistical difference in germination percentage between prairie dropseed seeds that were cold-moist stratified for two months (53%) and seeds that were not stratified (42%). Our data confirm their results, but

Table 2. Germination measurements for *Sporobolus heterolepis* (prairie dropseed) using different soil media under full-irradiance conditions. Tetrazolium tests estimated that 74% of seeds were viable prior to the experiment.

Soil Type	Germination Percentage	Mean Daily Germination (%/day)	Average Seedling Total
1:1:1 mixture*	3.2 b	0.043 b	1.6 b
Field Soil (non-compacted)	10.8 ab	0.146 ab	5.4 ab
Field Soil- (compacted)	14.4 a	0.195 a	7.2 a

<sup>\*</sup> Mixture is soil:peat:perlite.

<sup>&</sup>lt;sup>a,b</sup> Soil types within the each column with the same letter are not different at  $P \le 0.05$  according to Tukey's honestly significant difference test.

Table 3. Germination measurements for *Sporobolus heterolepis* (prairie dropseed) using different soil media under 50% shade. Tetrazolium tests estimated that 74% of seeds were viable prior to the experiment.

Soil Type	Germination Percentage	Mean Daily Germination (%/day)	Average Seedling Total
1:1:1 mixture*	16.8 **	0.227	8.4
Field Soil (non-compacted)	30.8	0.416	15.4
Field Soil (compacted)	28.8	0.389	15.4

<sup>\*</sup>Mixture is soil:peat:perlite.

also indicate that cold-moist stratification enhances germination of prairie dropseed (Table 1).

Germination across all treatments for the cold-moist stratification experiment averaged 43% (± 0.03 SE). This is very close to the percentage of viable seeds (46%) the tetrazolium tests revealed for the batch of seed used in the experiment. Tetrazolium tests for the batch of seeds used for both soil experiments showed that 74% of the seeds were viable. However, germination percentage across all treatments in full irradiance and 50% shade was 9.4% (± 0.02 SE) and 25.5% ( $\pm$  0.03 SE), respectively. These values are much lower than the amount of viable seeds determined by tetrazolium tests. Although tetrazolium tests do not incorporate the effects of abiotic and biotic factors on seed health or vigor under controlled or even field conditions, combined with actual germination assays, they can provide powerful insights into the capability of seeds to germinate (Stewart and Graves 2005).

Based on the difference in the amount of viable seeds and the number of seeds that germinated, it appears physical or chemical properties of the media or biotic factors inhibited prairie dropseed seed germination. Hitchmough et al. (2004) suggested that in a prairie establishment study in England, fungal infection of the

seed coat of prairie dropseed contributed to its low emergence. Also, some seeds in our study may have remained dormant, similar to what is observed in seed banks (Johnson and Anderson 1986; Perez et al. 1998). In fact, a study done in a remnant prairie in central Illinois found that prairie dropseed had the fifth-highest number of viable seeds in the seed bank, making up 5% of the total seed bank (Johnson and Anderson 1986).

In the two soil experiments, both field soil treatments had higher germination totals than the 1:1:1 mixture (Tables 2 and 3). While past efforts to establish prairie dropseed via direct seeding have had poor results (Nuzzo 1978; Schramm 1978), our results could be an indication that seedlings could be established through direct seeding in the field under favorable conditions. To determine these favorable conditions, experiments on appropriate seeding rate levels as well as management techniques to increase seedling survival and reduce competition in the field should be investigated.

The design for our soil experiments did not allow us to statistically compare the results based on irradiance conditions. However, notable differences in germination percentage, mean daily germination, and seedling totals led us to postulate that seedlings will germinate and grow better in partial shade. Indeed, it is reported that prairie dropseed exhibits an intermediate level of shade tolerance (NRCS 2008), indicating it will grow in partial shade. Further irradiance studies are needed to verify this, particularly under field conditions. If these results reflect the field performance of prairie dropseed, we would assume that the species would be present in restorations, particularly in the shade of larger plants. However, prairie dropseed is not regularly found in restored prairies (Schramm 1978; Hitchmough et al. 2004), suggesting that other factors, such as competition, are contributing to the lack of growth and establishment in restorations.

Another reason prairie dropseed may not be found in restorations is that it does not establish well when planted from seed (Diboll 2005). Moreover, sites where prairie dropseed is often observed are prairie remnant sites that have had little or no disturbance other than periodic fires (Johnson and Anderson 1986; Abrams 1988). Thus, prairie dropseed may be well-suited for inclusion in restorations after initial establishment of other species via interseeding (Packard, 2005). Most establishment success has been dependent upon growing seedlings in controlled settings and then transplanting them into the field (Nuzzo 1978; Schramm 1978). After seedlings of prairie dropseed are planted, active management is needed to reduce competition and ensure survival. Once past germination and the seedling stage, where it is sensitive to competition, prairie dropseed will persist indefinitely (Schramm 1978).

As previously mentioned, seeds were stored under dry conditions at 4 °C for several months before the initiation of these experiments. This storage period could have led to after-ripening (Hartmann et al. 2002), leading to higher germination rates than those that might occur naturally. Caution should be exercised in using these data to make management decisions for restoration activities. Further testing should be done in a field setting before additional recommendations are made regarding direct seeding of prairie dropseed.

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<sup>\*\*</sup>Soil types within the each column are not different at  $P \le 0.05$  according to Tukey's honestly significant difference test.

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Chad Fedewa works as a Wildlife Technician for the Michigan Department of Natural Resources. He holds a B.S. in Wildlife Biology from Central Michigan University and a M.S. in Natural Resources and Environmental Sciences from the University of Illinois at Urbana-Champaign.

Ryan Stewart is an assistant professor of horticulture in the Department of Natural Resources and Environmental Sciences at the University of Illinois at Urbana-Champaign. He earned his degrees at Utah State University (B.S., M.S.) and Iowa State University (Ph.D.). His research interests include propagation of difficult-to-germinate native species and risk assessment of species with invasive potential.

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