The effect of temperature and cold-moist stratification on seed germination of temperate climate grass and forb prairie species

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Abstract
Prairies are important biomes which sequester carbon, support pollinators and improve ecosystem services. They also provide biomass for livestock and are used for restoration purposes. Successful establishment of prairie species depends on breaking seed dormancy and reliable germination, particularly during early spring. In order to improve species selection and seeding methods for establishment, the germination of nine temperate grass and forb species was assessed under controlled environment conditions (8ºC and 12ºC) with/without cold stratification treatment. Germination percentage was highest for cold stratified seeds of *Lupinus perennis* germinated at 12ºC. (73%) while germination was null for untreated *Echinacea* sp. seeds. For all species, cold stratification increased germination and in some cases the increase was up to fourfold. Mean germination of stratified seeds at 8ºC was 24%. Increasing to 12ºC improved germination to 47%. Understanding germination requirements is essential to optimize seeding methods for restoration projects and enhance biodiversity.

Keywords
Dormancy; ecosystem; seeding; species mix; grasses.

Introduction
Restoration projects are intended to recover a damaged ecosystem. Determining an ecologically reasonable seed mixture is the key to a successful restoration project (Jiménez-Alfaro et al. 2016) with the intention of establishing a high-diversity, low-maintenance prairie or savanna. Successful establishment of prairie species depends on breaking seed dormancy and reliable germination, particularly during early spring. An excellent seed mixture takes into consideration the time for germination (Schramm 1978) and the appropriate pure live seed percentage for each species (Baskin and Baskin 1998), as both affect the final composition of the stand. Seed germination is an important phase in the plant life-cycle and germination is highly related to environmental conditions (Sanhueza et al. 2017). Different species require different environments for germination and emergence. Cold stratification, which is the process of subjecting seeds to cold and moist conditions (CMS) for a period of time, increases percent germination in most temperate species; therefore, restoration managers can overcome genetic determinants of seed germination timing using CMS as a pre-treatment (Barak et al. 2018). The knowledge of how different species respond to seed dormancy breakage is important to identify optimum seed mixtures. Our goal was to quantify the seed germination response after CMS in plant species typically used for ecological restoration in temperate regions.

Methods
Seed testing

Seeds of one native grass, sand dropseed (*Sporobolus cryptandrus*), and eight native forbs (below) common milkweed (*Asclepias syriaca*), butterfly weed (*Asclepias tuberosa*), lanceleaf tickseed

(*Coreopsis lanceolata*), pale purple coneflower (*Echinacea pallida*), common sneezeweed (*Helenium autumnale*), blanket flower (*Gaillardia grandiflora*), lupine (*Lupinus perennis*), wild bergamot (*Monarda fistulosa*) were used. Seeds of each species were sourced from the Ontario Seed Company (Waterloo, Ontario, Canada) and 25 seeds (CMS and untreated control) were sown in each of four plug trays and incubated at 8°C or 12°C with a 14/10 hour light/dark regime. CMS consisted of seeds placed in Petri dishes containing wet sand and stored at 4°C for one month from February 5 to March 8, 2018 (Eckberg et al. 2015; Voigt 1997). Upon removal from CMS, the seeds were immediately tested for germination.

Measurements and analyses

Germination was recorded when the emerging radicle was 2 mm in length (Wang 2005). Germination was measured after 45 days of test and reported as cumulative germination percentage (G%) and standard error (SE).

Analyses were performed in SAS 9.4 (SAS Institute 2002-2012), using PROC GLIMMIX with a Binomial distribution for germination percentage. PROC PROBIT was used for determining the number of days to reach 50% germination (GT50). ANOVA determined the effects of single factors at a confidence level of 0.05. The normality of the residuals was tested using a Shapiro-Wilk test. GT50 values with 95% fiducial interval from the PROBIT regression analysis for each species are reported. Optimal seed mixtures were identified with a K-means clustering partitioning method with three extract groups using R software.

Results

Seed germination percentage ranged from 0 (wild bergamot control seeds, 8ºC) to 73±1.7% (lupine CMS seeds, 12ºC), with an overall mean of 22% (Figure 1). The average germination percentage increased from 9±1.2% to 36±1.6 by using CMS treatment. Overall, seed germination was 15±1.2% at 8ºC and doubled to 30±1.7% at 12ºC incubation (Figure 1).

Blanket flower without CMS was affected by an increase in temperature, but once subjected to the CMS treatment, which reduced germination time, temperature no longer had an effect. The germination of untreated seeds of common milkweed was higher (23±1.6%).

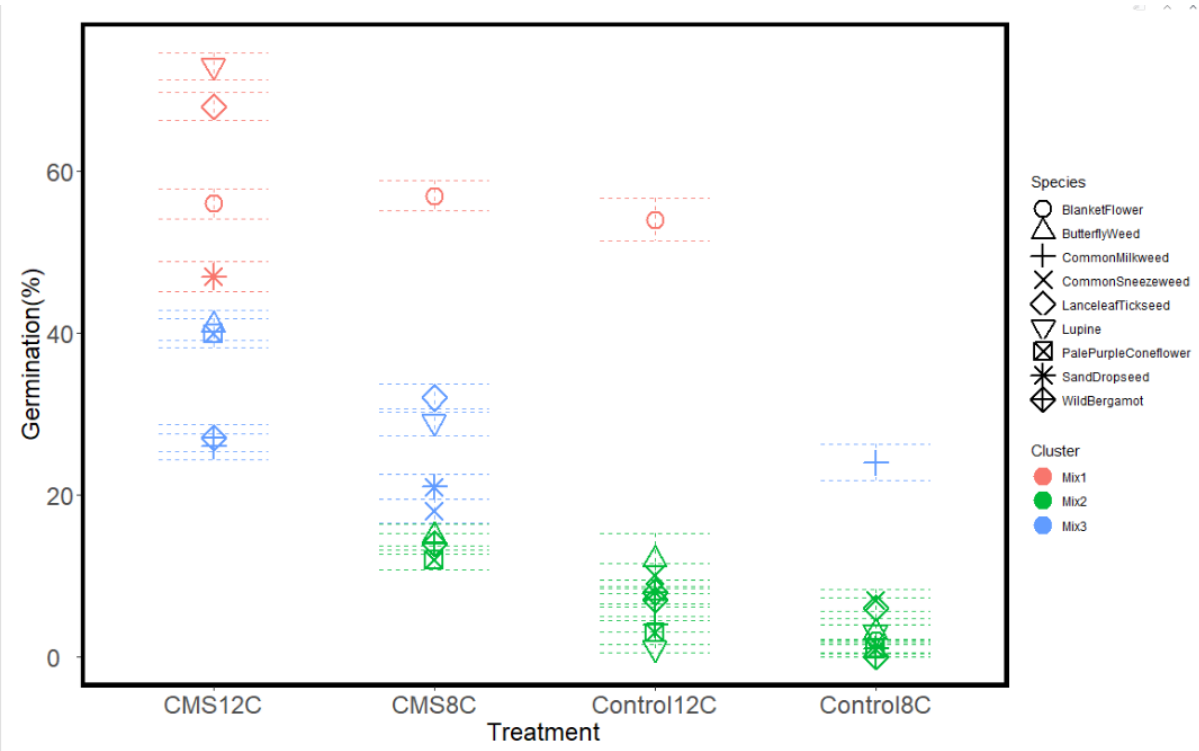

Figure 1. Effects of temperature (8°C and 12°C) and cold moist stratification (CMS) or untreated control on germination percentage of nine species incubated for 45 days. Symbols represent species. Colour (red, green, blue) indicate the optimal group mixtures clusters.

Fast germination and establishment of native species are desired for out-competing invasive species that are normally present in disturbed habitats and tend to have early germination phenology (Barak et al. 2018). In our study, the lowest estimated mean number of days to reach 50% germination (GT50) was 14 (LB=1.6 to UB=15.3) for lupine and lanceleaf tickseed CMS seeds incubated at 12ºC (Table 1). In contrast, GT50 for untreated sand dropseeds incubated at 8ºC was 121 (88.1 to 233.9) days (Table 1). For all species, GT50 was 37 (55.5 to 86.8) days for CMS seeds whereas untreated seeds (control) had a mean GT50 of 65 (55.5 to 86.8) days. An increase of 4ºC on the incubation temperature decreased GT50 by 19 (14 to 39.1) days, which is aligned with the results presented in Figure 1.

Table 1. Effect of temperature (8°C and 12°C) and a cold moist stratification (CMS) treatment or untreated control on days to 50% germination (GT50) of seeds of nine species incubated for 45 days. LB and UB represent lower and upper boundaries of the confidence interval of the model, respectively.

Species	GT50 (days)	LB	UB	GT50 (Days)	LB	UB
	Control 8°C			Control 12°C		
Sand dropseed	121	88	234	37	36	38
Common milkweed	79	66	104	66	60	76
Butterfly weed	88	70	143	57	53	63
Lanceleaf tickseed	79	67	103	83	71	106
Pale purple coneflower	n.s*	n.s	n.s	57	53	63
Common sneezeweed	69	61	81	53	49	58
Blanket flower	68	58	87	63	57	70
Lupine	60	55	67	21	20	23
Wild bergamot	103	79	178	62	57	70

CMS and 8°C				CMS and 12°C		
Sand dropseed	50	45	59	25	24	26
Common milkweed	44	41	48	41	38	44
Butterfly weed	52	47	60	29	27	31
Lanceleaf tickseed	37	34	40	15	14	15
Pale purple coneflower	87	67	141	29	27	31
Common sneezeweed	47	43	52	25	23	27
Blanket flower	20	18	22	21	18	23
Lupine	41	38	47	13	9	16
Wild bergamot	52	47	59	38	36	41

*n.s not significant

Conclusion

A mixture of lupine (untreated) and CMS-treated lanceleaf tickseed seeds is recommended for areas which require quick establishment after springtime seeding. Those two species can be used in combination with CMS-treated blanket flower seeds because their germination percentage and GT50 are similar. Based on GT50, we concluded that for an early winter sowing scenario, the optimal seed mix would consist of untreated seeds of lupine, blanket flower and common sneezeweed in combination with CMS-treated seeds of butterfly weed, wild bergamot and sand dropseed. This mixture would take an average of 58 days to reach 50% germination, but establishment would be even with less sown species competitiveness.

References

Barak RS, Lichtenberger TM, Wellman-Houde A, Kramer AT and Larkin DJ (2018). Cracking the case: Seed traits and phylogeny predict time to germination in prairie restoration species. Ecology and evolution, 8, 5551-5562.

Baskin CC, and Baskin JM (1998). Seeds: Ecology, biogeography, and evolution of dormancy and germination. Lexington, Kentucky: Elsevier.

Eckberg JO, Casler MD, Johnson GA, Seefeldt LL, Blaedow KE, and Shaw RG (2015). Switchgrass population and cold–moist stratification mediate germination. Crop Science 55, 2746-2752.

Jiménez-Alfaro B, Silveira FA, Fidelis A, Poschlod P, and Commander LE (2016). Seed germination traits can contribute better to plant community ecology. Journal of Vegetation Science 27, 637-645.

Sanhueza C, Vallejos V, Cavieres LA, Saez P, Bravo LA, and Corcuera LJ (2017). Growing temperature affects seed germination of the antarctic plant *Colobanthus quitensis* (Kunth) Bartl (Caryophyllaceae). Polar Biology 40, 449-455.

Schramm P (1978). The "do's and don'ts" of prairie restoration. In 5th Midwest prairie conference proceedings, Midwest prairie conference, Ames, IA. Ames, IA: Iowa State Universit, 139-150.

Voigt JW (1977). Seed germination of true prairie forbs. Journal of Range Management 439-441.