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Effect of overwinter hydration, seed storage time, temperature, and photoperiod on germination of some C*arex, Juncus*, and A*lopecurus* species

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ABSTRACT

Factors affecting seed germination of select wetland and aquatic plants were investigated in two experiments. In the first, seeds of four *Juncus* species, *Alopecurus aequalis,* and *Carex nebrascensis* were stored overwinter at 3–4 °C either dry or wet. In spring, these groups were further divided into four constant temperature treatments (15, 20, 25, or 30 °C) and 3 fluctuating temperature treatments (low, moderate, high). In the second experiment, the duration of cold-wet storage, photoperiod, and temperature were further evaluated. In the first experiment, cold-wet seed storage led to higher and faster germination for all but *C. nebrascensis*; Fluctuating temperatures produced the highest germination of *A. aequalis*, *C. nebrascensis, Juncus tenuis*, and *J. ensifolius*. In Experiment 2, seed storage time effects varied with species. Photoperiod effects (12 v. 14 h light) were generally insignificant. Diel temperature fluctuations of 15–27 °C produced higher seed germination than 32–38 °C for all four *Juncus* species. The highest germination percentages of *J. balticus* (64–69%) and *J. ensifolius* (61–73%) occurred after at least 2 months of cold-wet storage and incubation at 15–27 °C, whereas *J. tenuis* and *J. torreyi* had 51–58% germination both at the start of the study and after a year of wet vernalization. The conditions for germination of the species studied still needs further optimization, but the effects of temperature, temperature fluctuation, overwinter storage conditions, as well as the days to germinate at a given temperature, have been at least partly elucidated for the species studied. The data can now be used for initiating seed pretreatment for habitat projects.

1. Introduction

In an effort to develop habitat in irrigation reservoirs, where large water level fluctuations are common, the Utah Division of Wildlife Resources is interested in using wetland and aquatic plants to fill the need. Wetland plants provide food, shade, and shelter for waterfowl, songbirds, aquatic invertebrates, fish (especially juveniles), and other animals (O'Neill, 1972). Aquatic macrophytes can also play a role in the control of algal blooms, moving the nutrients from the water column to macrophyte tissue (Hasler and Jones, 1949; Passarge et al., 2006; Ibelings et al., 2007). Also, establishing desirable aquatic macrophytes can reduce the spread of undesirable weedy species (Smart et al., 1994). An additional benefit is that some aquatic macrophytes can also remove heavy metals from sediment, assuming vegetation is removed (Santos-Díaz and Barrón-Cruz, 2011).

Establishing aquatic and wetland plants relies on either transplantation of rhizomes and tubers or upon seeds sown directly or used to produce container stock. Seed germination of aquatic and wetland plants has been studied for a number of years (Schaumann, 1926; Muenscher, 1936; Grime et al., 1981), but has proven to be much more difficult than typical crop plants. These germination difficulties have led to transplantation and container stock propagation programs, which have been successful at establishment of aquatic plants for fisheries and wildlife in the southeastern U.S. (Fowler and Maddox, 1974; Smart et al., 1996, 1998; Fleming, 2010; Webb et al., 2012). Transplanting of container stock is very labor intensive and also increases the risks of transferring unwanted organisms such as fish and plant pathogens and undesirable plants. Given the difficulties of plant propagation and dormancy of wetland plant species, efforts in western U.S. reservoirs have largely focused on artificial habitat rather than wetland plants (Uberuaga and Bizios, 1991; Rogers and Bergersen, 1999).

Assuming high germination percentages, sown seeds would require much less labor and have a better chance of survival in arid western environments. Research on wetland plant germination to date has indicated that cold-wet storage for at least 4–6 weeks is helpful in improving germination of many wetland plants (Clevering, 1995; Schűtz, 1997b; van der Valk et al., 1999; Hock et al., 2006). Some species may require longer cold storage; e.g., *Polygonum pensylvanicum* had 92% germination after 30 weeks, compared to 35% after 15 weeks (Jordan et al., 1982). The question of whether to store seeds dry or wet is still being answered for each species, but studies to date have shown that storing wet within closed containers decreases germination (Harris and Marshall, 1960; Willis and Mitsch, 1995), likely due to anoxia.

Soil and water depth effects on germination have also been studied. Sowing seeds at the soil surface, or just below (≤ 1–2 cm), has provided the highest germination (Galinato and van der Valk, 1986; Clevering, 1995; Seabloom et al., 1998). Water depth requirements for germination have been studied in some species and results generally favor very moist exposed soil for germination rather than inunda-

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tion (Willis and Mitsch, 1995; Kellogg et al., 2003). However, some species such as *Carex stipata* have had higher germination when inundated (van der Valk et al., 1999). Temperature appears to be a key factor for germination in several studies and temperature fluctuations in particular may be required for breaking dormancy and inducing germination (Grime et al., 1981; Clevering, 1995; Thompson and Grime, 1983; Schűtz, 1997b; Seabloom et al., 1998; Martinez-Sánchez et al., 2006).

Recent development of a process called Solid Matrix Priming, in which seeds are partially hydrated and brought closer to the point of breaking dormancy, has been used for commercial seed production to shorten germination times and improve germination percentages (see review by Eskandari, 2013). The only research to date on priming of wetland species we are aware of is by Hock et al. (2006) with *Polygonum pensylvanicum*.

So, germination of aquatic and wetland plants is dependent on a variety of variables (Baskin and Baskin, 1998). These include initial viability percentage (Lacroix and Mosher, 1995); seed moisture content (Kovach and Bradford, 1992); seed storage conditions (wet/dry)(Comes et al., 1978); exposure to cold (simulating winter; Schűtz, 1997b); seed oxygen requirements (Baskin and Baskin, 1998); seasonal dormancy patterns, including seed age effects (Jones et al., 2004); effects of light, shading and photoperiod (Wetzel and McGregor, 1968; Schűtz, 1997b); water depth (van der Valk et al., 1999); planting depth (Galinato and van der Valk, 1986); temperature and temperature fluctuation (Grime et al., 1981); nutrient effects (Wetzel and McGregor, 1968); and scarification (Hoag et al., 2001). For this study, six species found in Utah were chosen that could potentially provide fish and invertebrate habitat, but were not considered weedy (e.g., *Phragmites australis,* which has been the target of removal efforts*;* Kettering and Mock, 2012; Mykleby, 2012). While the germination of some of the species discussed in this article have been studied, treatments in this study have not been evaluated and upper and lower thresholds for the variables remain to be determined.

The objective of the current study was to improve understanding of how cold-wet storage, seed storage duration, constant or fluctuating temperature, and photoperiod influence the germination of seeds from six wetland/aquatic plant species found in Utah. The resulting data is to be applied to aquatic habitat improvement projects to improve fishing, but could have broader application to wetlands restoration and other similar projects.

2. Methods

2.1. Experiment 1

Seeds of foxtail grass *Alopecurus aequalis* were obtained in September 2013 from ditch banks in the vicinity of Park City, Summit County, Utah, USA. These were air dried for about 1–2 weeks at room temperature, then stored in a refrigerator (4–7 °C). Nebraska sedge *Carex nebrascensis,* Baltic rush *Juncus balticus,* daggerleaf rush *J. ensifolius,* poverty rush *J. tenuis,* and Torrey's rush *J. torreyi* seeds were purchased from Granite Seed, Lehi, Utah County, Utah, USA. The commercially purchased seeds of *J. balticus, J. ensifolius, J. tenuis,* and *J. torreyi* were obtained by the seed company from a third party in November 2012, November 2013, July 2012, and August 2012, respectively. The time from harvest to the start of the experiment is unknown for these purchased seeds, as well as storage conditions (other than they were stored dry and the seed company warehouse is not temperature controlled). Treatments evaluated in the factorial design were simulated winter storage conditions (wet or dry) and temperature. The temperature treatments included four fixed temperatures (15, 20, 25, and 30 °C), as well as three fluctuating temperature treatments with similar daily lows (15–16 °C), but with three different daily maximum temperatures (19, 23, 27 °C; low, moderate, and high fluctuating, respectively). Temperatures were based on previous research by Grime et al. (1981). There were four replicate plates per treatment and species, containing 25 seeds in each.

On 10 December 2013, the seeds were split into two groups, one stored dry in the refrigerator in manila coin envelopes and the other stored wet. Wet storage consisted of putting seeds into 100 mm diameter Petri plates containing 8 mL well water (pH = 7.6, hardness and alkalinity = 200 mg/L) and sphagnum moss (Hoag et al., 2001; 1.2-1.4 g/plate) and holding all the plates in the same refrigerator. Periodic water additions kept the dish hydrated over time.

Germination tests generally followed the protocol of Boscaiu et al. (2011). A layer of sterile sand (20 mL) was put in 100 mm diameter Petri plates and de-ionized water was added to hydrate, but not immerse, the seeds. Prior to transfer, wet and dry seeds of both *C. nebrascensis* and *A. aequalis* were chemically treated to reduce fungal problems. This treatment consisted of 70% ethanol for 5 min, followed by 5 min with 0.05% sodium hyprochlorite (from 8.3% Clorox® bleach stock in de-ionized water) containing 0.05% Triton X-100, followed by thorough rinsing with de-ionized water. *Juncus* seeds were rinsed with de-ionized water, but not otherwise chemically treated because of their small size and a fear of killing embryos with the treatment.

The plates, with 25 seeds each, were put in germination chambers on 21 May 2014. The germination chambers consisted of plastic containers (80.6 cm × 50.8 cm × 43.2 cm; Contico, Bridgeton, MO) placed on closed cell insulation board to insulate them from the concrete floor. Within the container, water was added and an aquarium bayonet immersion heater used for producing the desired temperature. The high fluctuating temperature treatment had a recirculating heater (Polyscience Inc., Niles, Illinois 60714-4516 USA) instead of the bayonet heater, which provided more power and better temperature control. A small pump was used to circulate the water within each tote. Within the larger container, another smaller clear plastic container with a lid (60.3 cm x 42.2 cm x 27.9 cm, Bella Storage Solutions, Leominster, MA) was used to house all the Petri plates. The plates were put on strips of insulation board to elevate the dishes above the bottom which was in contact with the water. Above the clear lid of the interior container, a full spectrum fluorescent light (46 cm, 9325 K, General Electric F15T8, for spectrum details see commercial.gelighting.com/catalog/p/22910) was elevated about 10–12 cm above the lid. This fluorescent light was controlled by timer switches to produce a 12 h photoperiod. Measurements with a light meter indicated that light intensity ranged from 24 μmol m^{-2} s^{-1} at the bottom of the chamber to 105 μmol m^{-2} s^{-1} at the top; so plates were exposed to light intensities within this range. Preliminary adjustments of heaters, outside tote insulation, and outer lid manipulations prior to the germination test were made to achieve and maintain the target chamber temperature regime. Temperature data loggers (Hobo Pendant Temp, Onset Corporation, Bourne, Massachusetts, USA) were used to record internal tote temperatures. Fluctuating diel temperatures were achieved with on/off timer switches controlling power to the immersion heaters (day) and fans (night). Actual temperatures recorded during the study are shown in Table 1.

The plates were examined for germination every 2 days and sprouted seeds (green shoot visible) were removed and counted. Well water was added as needed to keep the plates hydrated. The plates were rotated at each check to transfer the plate at the bottom of the stack of 4 to the top; plate treatment groups were also rotated to preclude any bias in position in relation to the light source. During the

Table 1
Comparison of mean percent germination ($n = 4$, ± SD) for six plant species whose seeds were stored either wet or dry overwinter and then exposed to one of seven temperature regimes. A different letter following the mean indicates significant differences among temperature treatments within a seed storage treatment. The highest percent germination observed for each species is in bold type.

Seed storage	Nominal Temperature (Actual mean temperature °C ± SD)	Actual daily low and high temperature °C, mean (± SD)	*Alopecurus aequalis*	*Carex nebrascensis*	*Juncus balticus*	*Juncus ensifolius*	*Juncus tenuis*	*Juncus torreyi*
Dry	15 (14.8 ± 0.7)	13.9 (0.6) 16.3 (0.8)	6.0 ± 4.0ab	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 2.0a	6.0 ± 12.0a	0.0 ± 0.0a
	20 (20.0 ± 1.2)	18.4 (0.7) 21.9 (0.9)	5.0 ± 3.8ab	1.0 ± 2.0a	7.0 ± 6.8ab	1.0 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a
	25 (23.9 ± 1.5)	21.8 (1.5) 25.3 (1.3)	1.0 ± 2.0a	16.0 ± 10.8b	28.0 ± 21.9abc	3.0 ± 6.0a	0.0 ± 0.0a	0.0 ± 0.0a
	30 (28.8 ± 1.9)	24.6 (3.8) 30.2 (1.2)	3.0 ± 2.0a	8.0 ± 0.0ab	33.0 ± 10.5bc	3.0 ± 6.0a	0.0 ± 0.0a	4.0 ± 4.6a
	Fluctuating Low (19.6 ± 1.6)	17.2 (0.8) 22.0 (1.0)	6.0 ± 4.0ab	0.0 ± 0.0a	7.0 ± 11.5ab	0.0 ± 0.0a	2.0 ± 2.3a	7.0 ± 8.2a
	Moderate (20.8 ± 2.6)	17.4 (1.0) 24.3 (2.0)	16.0 ± 3.3b	30.0 ± 4.0c	21.0 ± 11.5abc	1.0 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a
	High (22.5 ± 3.6)	18.1 (2.4) 26.6 (1.6)	**40.0 ± 9.8c**	**71.0 ± 3.8d**	38.0 ± 14.0c	25.0 ± 42.1a	1.0 ± 2.0a	1.0 ± 2.0a
Wet	15 (14.8 ± 0.7)	13.9 (0.6) 16.3 (0.8)	10.0 ± 6.9a	4.0 ± 3.3ab	10.0 ± 5.2a	41.0 ± 27.8ab	10.0 ± 2.3a	**10.0 ± 8.3a**
	20 (20.0 ± 1.2)	18.4 (0.7) 21.9 (0.9)	5.0 ± 3.8a	10.0 ± 5.2ab	15.0 ± 6.8a	21.0 ± 7.6ab	0.0 ± 0.0b	**10.0 ± 10.1a**
	25 (23.9 ± 1.5)	21.8 (1.5) 25.3 (1.3)	17.0 ± 11.0a	16.0 ± 3.3abc	34.0 ± 21.8ab	1.0 ± 2.0a	1.0 ± 2.0b	3.0 ± 2.0ab
	30 (28.8 ± 1.9)	24.6 (3.8) 30.2 (1.2)	20.0 ± 10.8a	18.0 ± 9.5abc	51.0 ± 19.4b	11.0 ± 8.9ab	0.0 ± 0.0b	0.0 ± 0.0b
	Fluctuating Low (19.6 ± 1.6)	17.2 (0.8) 22.0 (1.0)	18.0 ±10.0 a	7.0 ± 6.0ab	23.0 ± 8.2ab	3.0 ± 2.0ab	0.0 ± 0.0b	0.0 ± 0.0b
	Moderate (20.8 ± 2.6)	17.4 (1.0) 24.3 (2.0)	24.0 ± 11.8a	21.0 ± 7.6bc	37.0 ± 17.1ab	**46.0 ± 26.2b**	**13.0 ± 10.0a**	0.0 ± 0.0b
	High (22.5 ± 3.6)	18.1 (2.4) 26.6 (1.6)	23.0 ± 13.2a	29.0 ± 6.0c	**52.0 ± 9.8b**	41.0 ± 31.7ab	8.0 ± 11.3ab	0.0 ± 0.0b

last 3 weeks of the study, the plates were examined weekly, since germination had largely stopped. The final observation was made on 17 July 2014 (57 d). This duration was based on a germination study of 403 species by Grime et al. (1981).

2.2. Experiment 2

The effect of seed storage time (defined herein as the duration in Petri dishes in cold [4–7 °C] wet conditions), was evaluated at four time periods: 0 months (November 2014), 2 months (January 2015), 6 months (May 2015), and 12 months (November 2015). Four *Juncus* species (*J. balticus, J. ensifolius, J. tenuis,* and *J. torreyi*) were exposed to three treatments: 1) fluctuating temperature of 15–27, 12 h photoperiod, 2) fluctuating temperature of 32–38 °C, 12 h photoperiod, and 3) fluctuating temperature of 32–38 °C, 14 h photoperiod. The longer photoperiod would be typical of spring conditions here in Utah. The 32–38 °C range was based on the upper limits to germination observed by Grime et al. (1981). Seeds were rinsed with de-ionized water, but not chemically treated prior to addition to the Petri plates, which were prepared as in the previous experiment.

In this experiment, based on low germination percentages in Experiment 1, the numbers of seeds per plate in the first time period was increased to 5000 seeds/plate for *J. ensifolius* and 2500 seeds/plate for *J. balticus*, *J. tenuis*, and *J. torreyi*. Based on germination percentages observed in the first time period, 100 seeds per plate were used in the subsequent 2, 6, and 12 month periods. For counting in the first time period, 2500 or 5000 seeds were hand counted for the first replicate and the weight of this used to weigh (to a thousandth of a gram) seed quantities for the other replicates. The seeds for the 2, 6 and 12 month period were all hand counted with the aid a microscope. The Petri dishes or beakers of all seeds were kept in the germination chambers described in the first experiment. Data loggers within each germination chamber recorded actual temperatures during the experiment (see Table 3).

2.3. Statistical analysis

All analyses were made using NCSS (Number Cruncher Statistical Software, 2007 version; J. Hintze, Kaysville, Utah, USA). An alpha of 0.05 was used as the level of significance for all tests. Corrections for experiment-wise error rate were made with the Bonferroni correction (0.05/number of tests; Kuehl, 2000). For example, mean contrasts of interest resulted in 41 separate tests for some species in Experiment 1, reducing the level of significance to 0.0012. This correction adjusts significance levels to account for the correlation between the number of tests and the probability of getting a significant difference (Kuehl, 2000).

Analyses were made separately for each species, except for tests evaluating differences among species. A saturated general linear model (GLM) was used to analyze all the treatment effects on percent germination or days to 50% germination for each species. In Experiment 1, treatments were overwinter storage (wet or dry) and temperature (7 levels). For the response variable ‘days to 50% germination' (t_{50}), only plates on which growth occurred were included in the analyses. Tests of normality were made for each species and if results were significant, data were rank transformed for the GLM test. The Tukey-Kramer multiple comparison test was used to compare means within a significant treatment.

If any interaction terms were significant, the response variable was analyzed by one-way ANOVA or rank-transformed ANOVA (if

Table 3
Mean percent germination (± SD, $N = 4$) of seeds of four *Juncus* species after being stored cold, wet, and dark: comparison of the effects of storage time, photoperiod, and daily fluctuating temperature regime. A different letter following the mean indicates significant differences among temperature treatments within a seed storage treatment. The highest percent germination observed for each species is in bold type.

Storage (months)	Nominal Temperature (°C)	Actual daily temperature, mean ± SD (°C)	Actual mean daily low and high temperature, mean ± SD (°C)	Light (h)	*J. balticus* (%)	*J. ensifolius* (%)	*J. tenuis* (%)	*J. torreyi* (%)
0	15–27	21.9 ± 4.6	14.7 ± 2.2 26.4 ± 2.6	12	38.9 ± 6.6a	26.5 ± 5.3a	**58.4 ± 15.8a**	**54.1 ± 6.5a**
	32–38	33.3 ± 4.8	25.8 ± 3.8 38.2 ± 2.4	12	22.1 ± 7.9b	0.4 ± 0.5b	9.6 ± 18.5b	0.0 ± 0.0b
	32–38	33.4 ± 4.5	24.8 ± 4.9 38.2 ± 3.5	14	6.3 ± 5.6c	<0.01 ± 0.0b	27.7 ± 19.7b	0.1 ± 0.2b
2	15–27	22.0 ± 4.5	14.9 ± 1.7 26.9 ± 1.6	12	68.2 ± 1.7b	61.5 ± 4.9a	30.7 ± 4.5a	17.0 ± 2.2a
	32–38	32.9 ± 5.4	26.8 ± 3.1 36.7 ± 3.4	12	54.5 ± 8.5b	0.0 ± 0.0b	6.0 ± 10.1b	0.8 ± 1.5b
	32–38	31.9 ± 4.6	27.4 ± 4.3 38.4 ± 3.0	14	32.7 ± 10.0a	0.2 ± 0.5b	2.5 ± 3.1b	0.2 ± 0.5b
6	15–27	23.4 ± 2.7	19.3 ± 0.9 26.6 ± 0.9	12	**69.5 ± 4.5b**	**73.2 ± 12.1a**	41.0 ± 17.4a	44.7 ± 12.9a
	32–38	35.3 ± 2.5	30.7 ± 1.7 37.9 ± 0.9	12	30.5 ± 9.1a	0.2 ± 0.5b	1.5 ± 3.0b	0.0 ± 0.0b
	32–38	34.4 ± 2.7	30.8 ± 2.5 37.6 ± 1.9	14	58.5 ± 16.1b	0.5 ± 0.6b	0.0 ± 0.0b	0.5 ± 0.6b
12	15–27	21.9 ± 4.6	18.2 ± 1.4 25.7 ± 1.2	12	63.7 ± 9.6a	37.2 ± 2.9c	51.2 ± 7.6a	51.0 ± 10.5a
	32–38	33.3 ± 4.8	27.5 ± 4.0 36.9 ± 3.8	12	31.7 ± 18.2a	0.0 ± 0.0a	0.7 ± 1.5b	0.0 ± 0.0b
	32–38	33.4 ± 4.5	27.1 ± 4.3 35.4 ± 3.5	14	42.0 ± 25.9a	13.0 ± 10.4c	9.5 ± 4.8b	20.7 ± 16.6b

equal variance assumption was not met and log transformations failed to correct it) separately for each level of winter storage, or temperature treatment. Similarly in Experiment 2, to evaluate effects of seed storage time, a separate one-way ANOVA was used for each temperature-photoperiod treatment. To analyze temperature effects, just the 12 h photoperiod treatments were used for *t*-tests conducted separately for each storage time treatment. Similarly, photoperiod effects were analyzed by *t*-tests for each seed storage time treatment using only data from the two 32–38 °C treatments.

The correlation between temperature and germination was determined for some species using ordinary least squares regression. For the fluctuating temperature treatments, temperatures of 19, 23, and 27 °C were used in combination with the fixed temperature treatments (15, 20, 25, 30 °C) for the x-axis of the correlation.

3. Results

3.1. Experiment 1: germination

Germination percentages for each of the treatments and species are given in Table 1. The statistical analyses of percent germination are given in Table 2. The germination percentage for seeds stored wet exceeded those of seeds stored dry for *J. balticus, J. ensifolius*, and *J. tenuis* (Fig. 1). However *C. nebrascensis* had higher germination after dry storage ($F_{1,56} = 4.2$, $p = 0.047$). *J. torreyi* had poor germination regardless of storage method.

For *A. aequalis*, winter storage and incubation temperature significantly affected germination (Table 2). For dry-stored seeds, germination in the high fluctuation treatment (40.0%) was significantly higher than the percentages in the other temperature treatments (1–16%). Similar benefits of fluctuating temperatures were noted among the latter: germination in the moderate fluctuation treatment (16%) was significantly higher than in the 25 and 30 °C treatments (1–3%). For seeds stored wet overwinter, germination was also greatest in the moderate and high fluctuating temperature treatments (23–24%), but the difference among temperature treatments was not significant (Table 2). During cold storage, the sphagnum moss helped keep fungal growth from establishing in the Petri plates, but some fungal growth was developing by late April in all plates. In some plates, seeds germinated in the dark refrigerator prior to starting the study (*A. aequalis*,105 seeds; *C. nebrascensis,* 1 seed). Effects of cold-wet storage on germination varied among the temperature treat-

Table 2
Summary of statistical analyses for Experiment 1. Probabilities and F statistics (with degrees of freedom subscripts) are given for the 2-factor general linear model comparing percent germination between two storage methods (wet or dry) and temperature treatments (four fixed and three daily fluctuating temperatures). The effects of temperature analyzed separately for wet-stored and dry-stored seeds is also given.

		Two-factor GLM analysis			One-way analysis of variance	
Species		Storage method	Temperature	Interaction term	Wet-stored temp effect	Dry-stored temp effect
Juncus balticus	*p*	0.001	<0.001	0.96	0.001	0.002
Juncus ensifolius	$F_{d.f.}$	$12.4_{1,56}$	$10.8_{6,56}$	$0.2_{6,56}$	$5.5_{6,28}$	$5.5_{6,28}$
	p	<0.001	0.006	0.083	0.008	0.341
Juncus tenuis	$F_{d.f.}$	$15.1_{1,56}$	$3.6_{6,56}$	$2.0_{6,56}$	$3.9_{6,28}$	$0.3_{6,28}$
	p	0.025	0.010	0.095	0.011[a]	0.318[a]
Juncus torreyi	$F_{d.f.}$	$5.4_{1,56}$	$3.2_{6,56}$	$2.0_{6,56}$	16.5_{6}	7.0_{6}
	p	0.19	0.14	0.001	0.014	0.076
Carex nebrascensis	$F_{d.f.}$	$1.1_{1,56}$	$1.7_{6,56}$	$4.5_{6,56}$	$3.6_{6,28}$	$2.3_{6,28}$
	p	0.047	<0.001	<0.001	<0.001	<0.001
Alopecurus aequalis	$F_{d.f.}$	$4.2_{1,56}$	$76.3_{6,56}$	$22.5_{6,56}$	$7.9_{6,28}$	$122.6_{6,28}$
	p	<0.001	<0.001	<0.001	0.133	<0.001
	$F_{d.f.}$	$18.9_{1,56}$	$10.8_{6,56}$	$4.7_{6,56}$	$1.9_{6,28}$	$32.3_{6,28}$

[a] Kruskal-Wallis one-way ANOVA and corresponding χ^2 statistic.

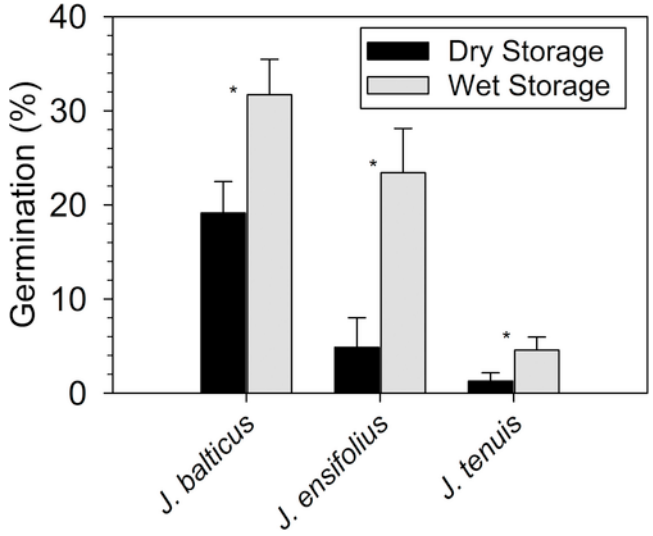

Fig 1. Comparison of the percent germination between seeds stored wet or dry at 4–7 °C overwinter (~161 d); data pooled across temperature treatments).

ments. Germination generally increased after this pre-treatment at most temperatures, but the difference was significant only at two fixed temperatures, 25 and 30 (Fig. 2).

For *C. nebrascensis*, germination was significantly higher for seeds stored dry. Fluctuating temperatures significantly increased the percentage of seeds that germinated. E.g., germination for dry-stored seeds in the high fluctuation treatment (71.0 ± 3.8%, mean ± SD) was significantly higher than in the moderate fluctuation treatment (30.0 ± 4.0%) which in turn was significantly higher than the low fluctuation and fixed temperature treatments (0–16%; Table 1). Similarly for seeds stored cold and wet, germination was highest in the moderate (21.0 ± 7.6%) and high (29.0 ± 6.0%) fluctuation treatments, which were significantly higher than for the 15 °C treatment (4.0 ± 3.3%; Table 1).

For *J. balticus*, winter storage and temperature effects on germination were significant (Table 2). For seeds stored dry, the highest germination (21 to 38%) was in the highest temperature treatments

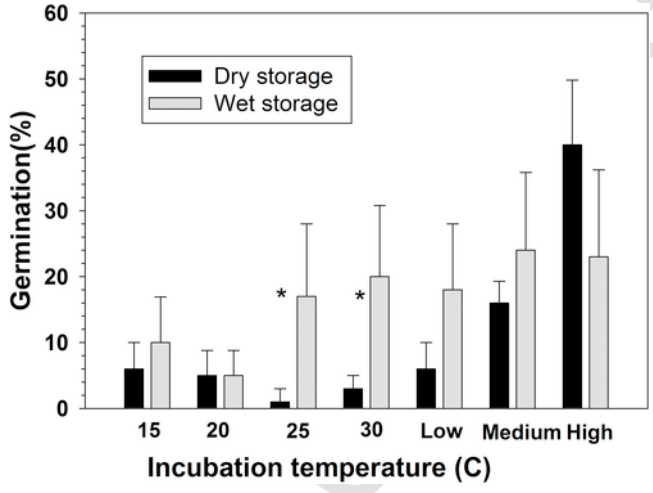

Fig. 2. Comparison of the percent germination of *Alopecurus aequalis* seeds stored wet or dry at 4–7 °C overwinter for each incubation temperature treatment. Fluctuating temperature treatments are labeled low (17–22 °C), medium (17 to 24 °C), high (18 to 27 °C).

(25 & 30 °C, plus moderate and high fluctuation treatments; Table 1). Similarly for seeds stored wet, the highest germination (51–52%) was achieved with seeds exposed to maximum temperatures of 27 to 30 °C. There was a general trend in both dry ($r = 0.74$, $p < 0.001$) and wet ($r = 0.74$, $p < 0.001$) storage treatments for germination to increase as temperature increased (Table 1).

For *J. tenuis*, germination was significantly augmented by cold-wet storage (Table 2). For dry-stored seeds, the highest germination (6%) was observed at 15 °C, but there were no significant differences among temperature treatments. For the wet-stored seeds the highest germination was in the moderate and high temperature fluctuation treatments (8–13%) and at 15 °C (Table 1).

For *J.ensifolius,* germination was significantly affected by winter storage and temperature. The highest germination for seeds stored dry was for the high fluctuation treatment (25%), whereas at other temperatures, germination was ≤3%. For seeds stored wet, there were significant differences among temperature treatments (Table 2), but only between the extremes of the germination range (i.e., between 1% germination at 25 °C and 46% germination in the moderate fluctuation treatment). The highest germination was achieved at 15 °C and in the moderate and high fluctuation treatments (41 to 46%; Table 1).

For *J. torreyi,* germination was generally low and not significantly affected by storage method. Germination differed significantly among temperatures for wet-stored seeds, but not for seeds stored dry (Table 2). For seeds stored dry, germination ranged from 0 to 7%. For wet-stored seeds, mean germination was highest (10%) in the constant 15 and 20 °C treatments whereas other temperature treatments only had 0–3% germinated.

3.2. Experiment 1: time to 50% germination

Cold-wet storage significantly reduced the time required for germination for several species, including *A. aequalis* ($F_{1,50} = 6.8$, $p = 0.013$), *C. nebrascensis* ($F_{1,44} = 13.4$, $p < 0.001$), and *J. torreyi* ($F_{1,14} = 53.0$, $p = 0.018$; Fig. 3). For some species, t_{50} was significantly affected by temperature as well. For *A. aequalis*, t_{50} was significantly longer in the high fluctuation treatment (19.5 days) than in the other temperature treatments (8-9 days, $F_{6,50} = 4.0$, $p = 0.003$), except the 25 °C treatment (12 days), which did not significantly differ from any treatment. For *J. torreyi*, t_{50} was significantly different among temperature treatments ($F_{5,14} = 254$, $p = 0.003$). Values ranged from 6 days (25 °C) to 57 days (19 °C), but did not follow any trend with temperature (Fig. 3). For *C. nebrascensis*, t_{50} was not significantly affected by temperature ($F_{6,44} = 1.8$, $p = 0.133$) and averaged 9.7 days for wet-stored seeds and 14.9 days for dry-stored seeds.

For dry-stored seeds of *J. balticus*, there was a trend for fewer days to reach 50% germination as temperature increased. Values ranged from 8 (high temperature fluctuation treatment) to 31 days (low fluctuation treatment) for dry-stored seeds and from 6 (at 30 °C) to 27 days (moderate temperature fluctuation treatment) among seeds stored wet. Mean values were 21.0 days for dry-stored seeds and 11.5 for wet-stored seeds.

For *J. tenuis*, t_{50} was 24 days for dry-stored seeds (range, 22-28 days) and 16 days for wet-stored seeds (range, 10-21 days). For *J. ensifolius*, t_{50} was similar between wet- (5.0 days) and dry-stored (8.5 days) seeds ($F_{1,32} = 0.9$; $p = 0.34$) and among temperature treatments (Fig. 3). For *J. torreyi,* t_{50} was highly variable, ranging from 6 days for wet-stored seeds at 25 °C to 57 d for dry-stored seeds at 19 °C. Wet-stored seeds averaged fewer days to 50% germination (12.9 days) than dry-stored seeds (37.2 days).

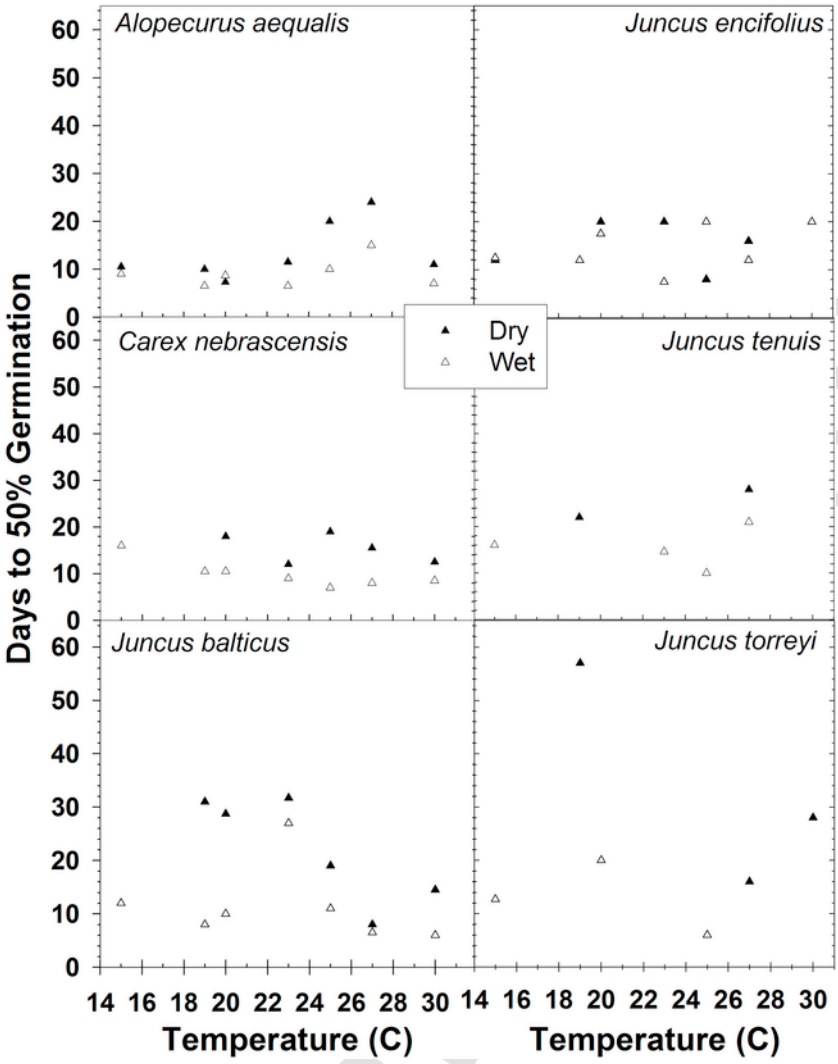

Fig. 3. Days to 50% germination for germinated seeds that were stored cold overwinter either wet (open triangle) or dry (solid triangle) and then given a 12 h photoperiod at various fixed and fluctuating temperatures. Data points for 19, 23, and 27 °C represent values for the low, medium, and high temperature fluctuation treatments.

3.3. Experiment 2: germination

Germination percentages for each of the treatments and species are given in Table 3. The statistical analyses of percent germination are given in Table 4. In general, germination was greater for seeds of all four *Juncus* species exposed to 15–27 °C than to 32–38 °C at either a 12 or 14 h photoperiod (Tables 3, 4). Photoperiod effects on germination at 32–38 °C were not significant ($p > 0.007$, Bonferroni correction) for any of the four *Juncus* species (Tables 3, 4).

Effects of cold-wet storage time varied with species. For *J. balticus,* cold-wet storage for at least two months significantly increased mean percent germination at 15–27 °C from 38.9% (no storage) to 63.7 to 69.5% (Table 3). Similar trends were observed in the 32–38 °C −14 h treatment. For *J. ensifolius,* germination was also significantly greater after at least 2 to 6 months of cold-wet storage (61.5 to 73.2%) than for seeds not stored cold and wet (26.5%; Table 3). However, percent germination for *J. tenuis* was significantly higher in month 0 (31.9%) than after 2 (13%) or 6 (14%) months of cold-wet storage. At 15–27 °C, germination of *J. torreyi* at 2 months (simulated winter) was significantly lower (17%) than in the other storage time treatments (45–54%), but cold-wet storage did not significantly improve germination.

The highest germination percentages for all four *Juncus* species occurred for seeds incubated at 15–27 °C under a 12 h photoperiod (Table 3). For *J. balticus,* maximum germination (64–69%) occurred after at least 2 months of cold-wet storage. The highest germination percentage of *J. ensifolius* (62–73%) occurred after 2–6 months of wet vernalization. For *J. tenuis*, the highest percent germination (58%) occurred in the 15–27 °C–12 h treatment at the start of the study (Table 3). For *J. torreyi*, the highest germination was in the 15–27 °C–12 h treatment and it was not improved by cold-wet storage (e.g., similar germination, 45 to 54%, at months 0, 6, and 12).

3.4. Experiment 2: time to 50% germination

For *J. balticus*, the time to 50% germination (t_{50}) was not significantly affected by seed storage time ($F_{3,48} = 0.6$, $p = 0.59$), but was significantly shorter ($F_{3,48} = 5.5$, $p = 0.008$) in the 15–27 °C–12 h treatment (6.7 ± 1.1 {SE} days) than in the 32–38 °C–14 h treatment (11.7 ± 1.1 days). Analysis of just the 32–38 °C treatments, pooling

Table 4
Summary of statistics (two-tailed *p*, F statistic with degrees of freedom subscripted) from general linear model analysis of percent germination for four *Juncus* species evaluating the effects of seed storage time (ST) and three temperature-photoperiod treatments (T-Ph). Also shown are results of one-way analysis of variance tests evaluating effects of ST separately for each of the T-Ph treatments and effects of T-Ph treatment for each ST treatment. Photoperiod effects were compared with *t*-tests, only between the two 32–38 °C treatments. Temperature effects (15–27 v. 32–38 °C) were compared between the two 12-h photoperiod treatments (t-tests).

		Juncus balticus		*Juncus ensifolius*		*Juncus tenuis*		*Juncus torreyi*	
		p	$F_{d.f.}$	*p*	$F_{d.f.}$	*p*	$F_{d.f.}$	*p*	$F_{d.f.}$
2 factor GLM analysis									
	ST	<0.001	$16.4_{3,48}$	<0.001	$20.3_{3,48}$	<0.001	$7.1_{3,48}$	<0.001	$13.4_{3,48}$
	T-Ph	<0.001	$23.1_{2,48}$	<0.001	$464.5_{2,48}$	<0.001	$62.7_{2,48}$	<0.001	$163.5_{2,48}$
	Interaction term	0.006	$3.6_{6,48}$	<0.001	$28.0_{6,48}$	0.199	$1.5_{6,48}$	<0.001	$8.9_{6,48}$
ST effects by T-Ph									
	15-27C, 12 h	<0.001	$20.6_{3,16}$	<0.001	$35.5_{3,16}$	0.043	$73.7_{3,16}$	<0.001	$314.2_{3,16}$
	32-38C, 12 h	0.012	$5.6_{3,16}$	0.366	$1.2_{3,16}$	0.627	$0.6_{3,16}$	0.430	$1.0_{3,16}$
	32-38C, 14 h	0.005	$7.2_{3,16}$	0.010	$36.0_{3,16}$	0.010	$76.0_{3,16}$	0.009	$6.1_{3,16}$
T-Ph effects by ST									
	Month 0	<0.001	$23.2_{2,12}$	<0.001	$96.7_{2,12}$	0.012	$7.4_{2,12}$	<0.001	$278.9_{2,12}$
	Month 2	<0.001	$21.7_{2,12}$	<0.001	$612.9_{2,12}$	<0.001	$21.6_{2,12}$	<0.001	$152.1_{2,12}$
	Month 6	0.002	$13.3_{2,12}$	<0.001	$144.0_{2,12}$	<0.001	$20.8_{2,12}$	<0.001	$47.7_{2,12}$
Month 12		0.105	$2.9_{2,12}$	<0.001	$37.1_{2,12}$	<0.001	$104.7_{2,12}$	<0.001	$20.5_{2,12}$
Photoperiod effects by ST									
		p	t	*p*	t	*p*	t	*p*	t
	Month 0	0.017	3.3	0.194	1.5	0.229	1.3	0.259	1.2
	Month 2	0.016	3.3	0.356	1.0	0.532	0.7	0.550	0.6
	Month 6	0.023	3.0	0.537	0.7	0.356	1.0	0.134	1.7
	Month 12	0.541	0.6	0.0.46	2.5	0.013	3.5	0.046	2.5
T effects by ST									
	Month 0	0.017	3.3	<0.001	9.8	0.007	4.0	<0.001	16.7
	Month 2	0.020	3.1	<0.001	24.9	0.004	4.5	<0.001	12.4
	Month 6	<0.001	7.6	<0.001	12.0	0.004	4.5	<0.001	6.9
	Month 12	0.021	3.1	<0.001	25.9	<0.001	13.0	<0.001	9.7

data across all storage times, indicated no significant difference in t_{50} between the 12 and 14 h photoperiods ($F_{1,32} = 1.5$, p = 0.23, Table 5).

For *J. ensifolius*, effects of storage time on the days to 50% germination were analyzed separately for each temperature-photoperiod treatment since poor germination led to several treatments that had no germination (see Table 5). For the 15–27 °C–12 h treatment, the t_{50} was significantly affected by storage time ($F_{3,16} = 21.4$, p < 0.001); decreasing from time 0 (11.7 ± 0.6 days) through 2, 6, and 12 months (respectively: 6.0 ± 0.6, 6.0 ± 0.6, 5.5 ± 0.6 days). Temperature effects (controlling for photoperiod) in the age-0 treatment and were not significant (p = 0.69, t = 0.42; Table 5).

For *J. tenuis*, effects of storage time on t_{50} were significant ($F_{3,35} = 52.8$, p < 0.001) as well as the interaction term 'ST x T-Ph' ($F_{6,35} = 5.0$, p = 0.002). For the 32–38 °C treatments, photoperiod did not significantly affect t_{50} at time 0 (p = 0.38, t = −0.96) or at 2 months (p = 1.0, t = 0.00; Table 5). Pooling across photoperiods, t_{50} was significantly shorter in the 15–27 °C treatment than in the 32–38 °C at month 0 ($F_{1,11} = 20.5$, p = 0.001) and 2 months ($F_{1,8} = 48.6$; p < 0.001), but not at 12 months ($F_{1,9} = 2.8$, p = 0.13; Table 5). Effects of storage time analyzed separately for each temperature indicated that, for seeds incubated at 15–27 °C, t_{50} at month 0 (Fall 2014) was significantly longer than in subsequent time periods ($F_{3,16} = 54.0$; p < 0.001), which did not significantly differ among them. For seeds incubated at 32–38 °C, t_{50} was similarly significantly higher in the initial time period (28.1 ± 1.7 days) than in later periods (617 days; $F_{3,19} = 26.3$, p < 0.001; Table 5).

For *J. torreyi*, t_{50} for seeds incubated at 15–27 °C was significantly longer ($F_{3,16} = 14.9$, p < 0.001) in the 0 month treatment (26.0 ± 2.4 days) than in subsequent time periods (6.5 to 8.5 days; Table 5). Regarding temperature effects, t_{50} was significantly lower in the 15–27 °C treatment than the 32–38 °C treatment in both month 0 (p = 0.04, t = −3.1) and month 12 (p < 0.001, t = −9.9; Table 5).

Table 5
Mean days to 50% germination for seeds of four *Juncus* species after being stored cold, wet, and dark: comparison of the effects of storage time, photoperiod, and daily fluctuating temperature regimen. Treatments in which there was no germination or just a single seed are indicated with a '-'.

			Days to 50% germination			
Storage (months)	Temperature (C)	Light (h)	*J. balticus*	*J. ensifolius*	*J. tenuis*	*J. torreyi*
0	15–27	12	9.0 ± 2.0	11.7 ± 2.4	18.0 ± 1.1	26.0 ± 9.6
	32–38	12	7.0 ± 2.0	15.0 ± 15.6	26.3 ± 5.0	–
	32–38	14	16.0 ± 10.4	–	29.5 ± 3.8	48.0 ± 0.0
2	15–27	12	6.0 ± 0.0	6.0 ± 0.0	8.0 ± 0.0	8.5 ± 1.0
	32–38	12	8.0 ± 1.6	–	17.0 ± 4.2	20.0[a]
	32–38	14	12.0 ± 3.6	–	17.0 ± 1.4	–
6	15–27	12	6.0 ± 0.0	6.0 ± 0.0	9.0 ± 2.0	6.5 ± 1.0
	32–38	12	13.7 ± 6.4	–	6.0[a]	–
	32–38	14	8.5 ± 2.5	–	–	–
12	15–27	12	6.0 ± 1.1	5.5 ± 1.0	9.5 ± 1.0	7.0 ± 0.0
	32–38	12	9.0 ± 1.6	–	7.0[a]	–
	32–38	14	10.5 ± 5.7	12.5 ± 1.9	16.5 ± 4.7	16.5 ± 1.9

[a] only 1 plate had any germination in this treatment.

4. Discussion

4.1. Effects of seed storage conditions and duration

The benefit of pre-treating seeds with cold-wet storage varied with species. Cold-wet storage of seeds led to significantly higher germination for *J. balticus* and *J. ensifolius*. *J. tenuis* and *J. torreyi* however, germinated equally well before and after 12 months of cold-wet storage. A database of the Royal Tasmanian Botanical Gardens (RTBG, 2016) similarly noted that cold pre-treatment was not necessary for *J. tenuis* germination and for 17 other *Juncus* species, but cold storage was necessary for germination of *J. antarcticus* and improved germination of *J. curtisae* and *J. falcatus*. Grime et al. (1981) similarly noted high germination (73–99%) for freshly col-

lected seeds of *J. articulatus, J. effusus, J. squarrosus, J. conglomeratus,* and *J. inflexus* in Great Britain. However, seeds of *J. bufonius* had only 3% germination when freshly collected, but germination reached 99% for seeds stored dry at 20 °C for 12 months (Grime et al., 1981).

The benefits of cold-wet storage have been reported for other wetland plant species (Muenscher, 1936; Harris and Marshall, 1960). Grime et al. (1981) observed higher germination after cold-wet storage for four *Carex* species, *Eleocharis palustris*, and three *Polygonum* species. van der Valk et al. (1999) similarly noted improved viability of *Carex* seeds stored wet and cold, although freezing reduced viability by 66%. In some studies, cold-wet seed treatment was required to break dormancy. For example, Isely (1944) noted that germination for four *Schoenoplectus* species was only obtained after seeds were stored in water at low temperature. Leck (1996) observed that *Alisma subcordatum* germinated only after storage underwater at 1–3 °C. Germination has been observed for *Juncus tenuis, Polygonum* species, and other species after being submerged for over 4 years (Shull, 1914), indicating the duration of wet storage can be prolonged and yet still provide viable seeds.

For wet storage, it is important that seeds have enough oxygen to maintain the embryo (Harris and Marshall, 1960; Holm, 1972). Tilley (2013) noted significantly higher germination for aerated seeds of *Carex nebrascensis*. Relative humidity can play a role in the viability of seeds stored 'dry' (Leck, 1996), which, considering the generally low humidity of Utah, could have influenced the seeds stored dry in this study.

Seasonal effects on dormancy have been reported for several wetland species, so the time of year can influence germination (Gerritsen and Greening, 1989). For example, Baskin et al. (1994) found the wetland annual *Leucospora multifida* was conditionally dormant in late summer and autumn, but non-dormant in spring and early summer. Similarly, some *Carex* species have lower germination percentages for freshly collected seeds or for seeds in the following summer compared to late winter or spring (Baskin et al., 1996; Schűtz, 1997b). *Carex* species can vary substantially in their life history and adaptations (Schűtz, 1997a), so species can differ in their response to cold-wet storage. In this study, seed storage time effects were significant for several species. For *Juncus balticus* and *J. ensifolius*, the germination for seeds at month 0 was significantly lower than in subsequent periods for seeds stored wet overwinter. This fall-dormancy trait would favor plants that might have high mortality during the winter.

Both *C. nebrascensis* and the grass *A. aequalis* had comparable germination percentages after dry storage (1–40%) and after wet storage (5–24%). Both germinated in darkness in the refrigerator, indicating that cold-wet storage is not needed and that both would germinate under a variety of environmental conditions. Grime et al. (1981) also noted that most grasses in their study similarly were able to germinate relatively quickly under a variety of temperature and light conditions.

The time required for germination (not including storage time) was typically less for seeds stored wet overwinter than for dry-stored seeds. This phenomenon is part of the strategy of solid matrix priming, in which seeds are hydrated to a point short of germination, reducing germination time (Eskandari, 2013).

4.2. Fluctuating temperature effects

Seed adaptation to fluctuating temperatures is thought to provide a depth sensing mechanism, i.e., greater fluctuation occurs at or near the surface or when mudflats are exposed (Thompson and Grime, 1983). These fluctuations provide the cue that conditions are favorable for germination. It is also conceivable that daily temperature fluctuations lead to expansion and contraction of the embryo which may aid in weakening or rupturing the pericarp, leading to water infiltration and germination.

Species vary in their requirement for temperature cues, some requiring no fluctuation and others requiring a change of 8–12 °C (Grime et al., 1981; Thompson and Grime, 1983; Clevering, 1995). Thullen and Eberts (1995) noted that for *Schoenoplectus acutus*, daily temperature fluctuations of 15 °C (from 10 to 25 °C) led to 92–97% germination, but lower amplitude fluctuations (18–22 °C) gave significantly lower germination. Of 403 species examined by Grime et al. (1981), 16 required diurnal fluctuations in temperature to germinate. The cattail *Typha latifolia* (Morinaga, 1926) and the rice field weeds *Schoenoplectus juncoides* and *Fimbristylis littoralis* (Pons and Schröder, 1986) also germinated in response to fluctuating temperatures.

In this study, the best germination for C. *nebrascensis* (71 ± 3.8%) occurred after dry cold storage and incubation at fluctuating temperatures of 15–27 °C. Similarly for seeds of *C. canescens* stored dry, fluctuating temperatures of 12 °C magnitude (maximum 22 °C) led to higher germination (78%) than fixed temperatures of 15 or 25 °C (20–34%; Schütz and Milberg, 1997). Baskin et al. (1996) noted that fluctuating the temperature between 20 and 35 °C increased the germination of *Carex comosa* and *C. stricta*. *C. psuedocyperus, C. remota, C. paniculata, C. elongata,* and *C. cespitosa* similarly had higher germination after exposure to daily temperature fluctuations of 10–22 °C than to constant 15 °C (Schűtz, 1997b). In contrast, *C. otrubae* did not require fluctuating temperatures to achieve high germination percentages (Thompson and Grime, 1983).

Fluctuating temperature in this study resulted in higher germination for *A. aequalis* and *J. balticus,* though daily temperature fluctuations from 32 to 38 °C generally reduced germination relative to the 15–27 °C treatment for all species. However, *J. balticus* also germinated at warmer fixed temperatures, so temperature fluctuation was not a requirement to break dormancy. Martinez-Sánchez et al. (2006) similarly observed that fluctuating temperatures were not required for germination of *Juncus acutus;* e.g., germination exceeded 90% at fixed temperatures of 20 or 30 °C or alternating temperatures (15–25 °C, 20–30 °C). In our study, *J. ensifolius* similarly did not require fluctuating temperatures to germinate. *Juncus* species appear to vary in their requirement for fluctuating temperatures. E.g., species such as *J. acuminatus, J. amabalis*, *J. pallidus*, and *J. vaginatus* germinated at both fixed and fluctuating temperatures (RTBG, 2016). However, other species required daily temperature fluctuations (15–27 °C) to achieve high germination percentages (e.g., *J. bassianus, J. curtisiae, J. falcatus*, *J. holoschoenus, J. kraussii, J. pauciflorus,* and *J. tenuis*; RTBG, 2016).

4.3. Carex nebrascensis

Seeds stored dry had the highest germination in this study, but wet storage also led to germination. In contrast, Comes et al. (1978) noted that wet storage significantly improved germination of woolly sedge *C. lanuginosa*. Hoag et al. (2001) noted that perigynium removal improved germination of cold-wet stored seeds of *C. nebrascensis* by 25%. In our study the perigynium was removed and we observed comparable germination percentages (up to 71%) to Hoag et al. (2001; 53–60%). Tilley (2013) found that soaking *C. nebrascensis* seeds in warm water (24–35 °C) up to 10 days improved germination relative to seeds stored dry at 3 °C. *C. stipata* germination was improved by immersion in 1 cm of water compared to 0 cm or lower,

but *C. stricta* germination was not influenced by water depth (van der Valk et al., 1999).

Storage time effects were not tested for this species, but dormancy of freshly harvested seeds has been reported for many *Carex* species (Schűtz, 1997b; Kettering and Glatowitsch, 2007). Jones et al. (2004) noted that *C. nebrascensis* and *C. utriculata* seeds aged 0.5 and 1.5 years had lower germination than seeds aged 2 years. However, in other studies, viability decreased rapidly with age. For example, at one month, *C. lacustris* and *C. stricta* had 44 and 68% germination, respectively, but *C. atherodes*, as well *C. lacustris* and *C. stricta*, had low or no germination after 6–18 months of storage (van der Valk et al., 1999). For *C. canescens* seeds stored dry, Schütz and Milberg (1997) also reported a decrease in germination from one month (78%) to 3 months (43%); however seeds in cold storage had similar germination percentages between 1 (95.1%) and 3 (92.5%) months. These data underscore the fact that seed storage conditions affect long term seed survival and germination.

In this study, the time to 50% germination for *C. nebrascensis* seeds was 7-19 days, which is comparable to other *Carex* species (9–23 days) studied by Grime et al. (1981), although *C. pulicaris* required 47 days. Jones et al. (2004) observed a t_{50} for *C. nebrascensis* and *C. utriculata* that was about 12 to 28 days, depending on temperature treatment. Schűtz (1997b) noted that at 25 °C, t_{50} was 5 days for six *Carex* species, but at 15 °C, *C. canescens* and *C. remota*, germinated earlier (t_{50} = 9, 10, respectively) than *C. elongata*, *C. cespitosa*, or *C. paniculata* (12–13 days).

4.4. Juncus species

Germination of the *Juncus* species in this study ranged from 0 to 73% and was significantly affected by storage time, storage conditions, and incubation temperature. Differences among species were observed, with *J. balticus* typically producing more and larger seedlings over a wider range of temperatures than the other species. Richards and Clapham (1941) reported 97% germination of *J. effusus*, but also noted germination variation among *Juncus* species. Similarly Boscaiu et al. (2011) observed 95% germination for *J. acutus*, but only 48% for *J. maritimus*.

Storage time effects varied with species. *J. balticus* and *J. ensifolius* had higher germination after at least 2 months of cold-wet storage, but *J. tenuis* and *J. torreyi* had high germination at the start of the second experiment. These latter two species also had less germination when only given two months of wet storage (i.e., attempted to germinate during winter, compared to spring or fall of the following year). Lazenby (1955) reported an anecdotal observation that *Juncus* species have a delayed germination, with growth deferred until the second year. The storage history of the seeds in our study varied. The commercially purchased seeds of *J. balticus, J. ensifolius, J. tenuis,* and *J. torreyi* were obtained by the seed company from a third party in November 2012, November 2013, July 2012, and August 2012, respectively. So, most seeds were at least 2–3 years old (dry warehouse storage) when the germination tests began. While the different histories may affect comparisons among species in this study, differences among treatments within a species remain valid observations. Muenscher (1936) observed 96–97% germination of *J. articulatus* seed stored in cold water for 5–7 months, but dried seeds had very poor germination. Germination of *J. effusus* in the field was observed to be only 5% (Lazenby, 1955; Ervin and Wetzel, 2001).

Temperature had a significant effect on all the *Juncus* species studied. In Experiment 1, both *J. balticus* and *J. ensifolius* species had their highest germination percentages after exposure to fluctuations of 8–12 °C magnitude. *J. balticus* germination was significantly correlated with temperature, similar to observations by Seabloom et al. (1998) for several wetland plant species of prairie potholes. Workers at the Kew Royal Botanical Gardens (Kew, 2016) reported 100% germination of *J. balticus* at fixed temperatures of 25 or 26 °C under photoperiods of 8 or 12 h. Kew (2016) reported 85% germination of *J. ensifolius* at 5 °C and an 8 h photoperiod compared to a maximum of 46% germination in our study at 15–23 °C. In their work, seeds were dried to 15% relative humidity and frozen for 25 days, whereas in this study the relative humidity during storage prior to the experiments is unknown. Storage conditions may affect the depth of dormancy and germination response (Baskin and Baskin, 1998).

Grime et al. (1981) noted that *Juncus* species in the U.K. required 5–25 days to reach 50% germination. In Experiment 1, t_{50} for the *Juncus* species generally decreased as temperature rose, ranging from 8 to 57 days for dry-stored seeds and 6–27 days for wet-stored seeds. For wet- stored seeds of J. *balticus*, t_{50} at 30 °C was 6 days, and averaged 11.5 days among all the temperature treatments. Stevens et al. (2012) similarly reported that about a week was needed to germinate *J. arcticus littoralis* (syn. *J. balticus littoralis;* Snogerup et al., 2002). In the second experiment, mean t_{50} ranged from 6 to 16 days for both *J. balticus* and *J. ensifolius*. *J. torreyi* and *J. tenuis* required more time to achieve 50% germination (26–48 d) if not stored cold and wet. For *J. acutus*, t_{50} was 14–17 days at 20–25 °C and 18–22 days under fluctuating temperatures of 15–25 or 20–30 °C (Martinez-Sánchez et al., 2006).

4.5. Conclusions

While the conditions for germination of the species studied still needs further investigation to better determine thresholds, limits, and optima of each variable, the effects of temperature, temperature fluctuation, overwinter storage conditions, and photoperiod are better known for the species studied. These variables can now be optimized for study of additional variables that affect germination. For germination of *A. aequalis* and *C. nebrascensis* seeds, we recommend dry storage overwinter and subsequent exposure to a fluctuating temperature regimen in which temperature amplitudes of 8–12 °C reach maxima of 23–30 °C. For the *Juncus* species studied, fluctuating temperatures between 15 and 27 °C are recommended to optimize germination and ≥8 weeks of cold-wet storage is recommended for *J. balticus* and *J. ensifolius*. The data generated by this study led to sufficient germination percentages that the recommended treatments can be applied to seed pre-treatment for field applications for habitat improvement in wetlands, reservoirs, or other similar habitats. Ideally seeds can be prepared by cold, wet storage overwinter, followed in spring by fluctuating temperature treatments until the first shoots appear. The seeds can then be sown at a time of year that has similar temperature fluctuations using various planting methods which will be the subject of future research.

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