Plant Ecol (2007) 193:157–169
DOI 10.1007/s11258-006-9255-8

ORIGINAL PAPER

Tools for *Carex* revegetation in freshwater wetlands: understanding dormancy loss and germination temperature requirements

Karin M. Kettenring · Susan M. Galatowitsch

Received: 25 May 2006 / Accepted: 5 December 2006 / Published online: 11 January 2007

Abstract *Carex* is a globally distributed genus with more than 2000 species worldwide and *Carex* species are the characteristic vegetation of sedge meadow wetlands. In the mid-continental United States, *Carex* species are dominant in natural freshwater wetlands yet are slow to recolonize hydrologically restored wetlands. To aid in *Carex* revegetation efforts, we determined the dormancy breaking and temperature germination requirements of 12 *Carex* species. Seeds were cold stratified at 5/1°C for 0–6 months and then incubated in light at 5/1°C, 14/1°C, 22/8°C, 27/15°C, or 35/30°C. We found that all *Carex* species produced conditionally dormant seeds. The optimal temperature for germination for all but three species was 27/15°C. As is the case in other species with physiological dormancy, cold stratification increased germination percentages, broadened the temperature range suitable for germination, and increased germination rates for most species, but the magnitude of the effects varied among species. Many species germinated to 80% at 27/15°C without cold stratification and at 22/8°C with ≤1 month of stratification but required much longer stratification (up to 6 months depending on the species) to germinate to 80% at 14/1°C and 35/30°C. Our findings illustrate how a stratification pretreatment can greatly benefit *Carex* seed sowing efforts by triggering rapid germination to higher percentages. We recommend that cold stratification be targeted towards species with strong dormancy or used across a wider range of species when seed supplies for restoration are limiting. For *Carex* revegetation, establishing *Carex* canopies rapidly may help to prevent the invasion of undesirable species such as *Phalaris arundinacea*.

Keywords Cold stratification · Prairie pothole wetlands · Sedge · Seed ecology · Wetland restoration

K. M. Kettenring (✉) · S. M. Galatowitsch
Department of Horticultural Science, University of Minnesota, 305 Alderman Hall, 1970 Folwell Ave., St. Paul, MN 55108, USA
e-mail: KettenringK@si.edu

Present Address:
K. M. Kettenring
Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA

Introduction

Restoring native plant diversity to degraded ecosystems is a fundamental goal of many restoration projects. However, the natural recolonization of many plant species in restorations is constrained by seed availability, especially because of seed dispersal limitations in increasingly fragmented landscapes (e.g., Holl et al. 2000; Holmes and

Richardson 1999; Wijdeven and Kuzee 2000; Zanini and Ganade 2005). Today, successful restoration of diverse plant communities in most ecosystems requires sowing at least some of the target species. Seed dormancy may limit the success of seed sowing efforts but with an understanding of the seed ecology of target species, seed pretreatments can be applied to improve restoration success. For instance, to ensure rapid germination of species with physical dormancy, a seed pretreatment such as acid or mechanical scarification is required to break the water impermeable seed coat structure (Baskin and Baskin 1998). For species with physiological dormancy, the most common type of seed dormancy in plants, seed stratification (often cold, moist conditions) increases the rate of germination, maximum germination percentages, and the range of temperatures suitable for germination in many species (Baskin and Baskin 1998).

Carex is a globally important genus with more than 2000 species worldwide (Bernard 1990) and has been the focus of restoration research in regions such as temperate North America and the Arctic (e.g., Budelsky and Galatowitsch 2004; Forbes 1993; Galatowitsch and Biederman 1998; Hurd and Shaw 1992; Jones et al. 2004; Martin and Chambers 2002; Steed and DeWald 2003; van der Valk et al. 1999). In the prairie pothole region of the mid-continental United States, thousands of acres of wetlands have been restored since the mid-1980s, but many native *Carex* species common to natural prairie pothole wetlands are rare or absent in unplanted restorations, even 12 years after reflooding (Galatowitsch and van der Valk 1996a, c; Mulhouse and Galatowitsch 2003). A recent study found that *Carex* recolonization is limited by a lack of seeds but when seeds of five *Carex* species (*C. comosa*, *C. cristatella*, *C. hystericina*, *C. stipata*, and *C. vulpinoidea*) were sown into restorations, they germinated readily (Kettenring 2006). More attention to sowing *Carex* seeds to restore the native plant diversity to these wetlands is needed. Approximately 60 species of wetland *Carex* are native to the prairie pothole region of the United States (Barkley 1986), and establishment of even a fraction of them in restorations would greatly improve overall diversity of restored plant communities.

As is the case for revegetation efforts in many parts of the world, in freshwater wetland restoration in the mid-continental US, commercially available seed supplies are very limited (Bohnen and Galatowitsch 2005; Burton and Burton 2002; Galatowitsch et al. 1999). A better understanding of the dormancy and germination ecology of *Carex* will help practitioners to make the most of available seed supplies and improve the propagation of seed source plants. It is particularly important that the range of genetic diversity with respect to seed dormancy and germination is represented in stock supplies (Baskin 2006 Personal communication); knowing how to achieve high germination percentages across a range of dormancy breaking and germination requirements is essential for preserving this diversity.

The dormancy breaking and germination requirements of only a few *Carex* species from this region have been determined (e.g., Budelsky and Galatowitsch 1999; van der Valk et al. 1999). Many European *Carex* species are known to have physiological dormancy (Schütz 2000) but whether this type of dormancy is as prevalent in *Carex* from freshwater wetlands in the mid-continental US is unknown. Specific information is lacking on the length of cold stratification (=moist at 0–10°C, hereafter called stratification) required for dormancy break, germination temperature requirements of seeds as they transition from total or conditional dormancy to nondormancy, and the effect of cold stratification on germination rates of *Carex* seeds from this region.

Seed stratification is potentially a powerful restoration tool for limiting the establishment of undesirable species by more quickly establishing the target native species. In the case of wetland restoration in the mid-continental US, a major threat to native plant establishment in sedge meadows is invasion by *Phalaris arundinacea* (reed canary grass). *Phalaris* is a perennial invasive species that outcompetes native species in freshwater wetlands under a wide range of conditions (Budelsky and Galatowitsch 2000; Green and Galatowitsch 2001, 2002; Maurer and Zedler 2002; Perry et al. 2004). In hydrologically restored prairie pothole wetlands, *Phalaris* is not present or present with low cover in wetlands 2–3 years post-restoration but becomes predominant within

12 years (Galatowitsch and van der Valk 1996b; Mulhouse and Galatowitsch 2003). Thus, there is a ''window of opportunity'' before *Phalaris* establishes when *Carex* can be seeded and established. An established *Carex* canopy creates a light environment unsuitable for *Phalaris* seed germination and hence, invasion (Lindig-Cisneros and Zedler 2001). Seed ecology studies of *Carex* can be used to develop effective seed pretreatments for quickly establishing target communities.

To aid in *Carex* restoration efforts, we studied the seed dormancy and germination ecology of 12 *Carex* species. We chose a multi-species approach to see if there are generalizations that emerge about seed dormancy that could be used to make predictions for a broader array of *Carex* species. We asked if any of the 12 study species produced dormant seeds and if so, whether cold stratification has any effect on breaking dormancy and promoting germination. Our specific goals were to determine (1) temperatures suitable for germination of fresh, mature *Carex* seeds and seeds after different periods of cold stratification, (2) the effect of cold stratification on germination percentages, (3) the minimum period of cold stratification required to achieve at least 80% seed germination at a given temperature regime, and (4) the effect of cold stratification on germination rates. The specific results on *Carex* dormancy and germination can be used to develop optimal guidelines for *Carex* revegetation efforts in the study region and elsewhere.

Methods

Seed collection and viability tests

The 12 *Carex* species used in this study are found throughout the United States and Canada and occupy a range of wetland habitats (Table 1). We chose to focus on a suite of *Carex* species from wetland habitats in order to evaluate genus-level patterns in seed dormancy and germination. Seeds were collected at maturity from wetlands in Minnesota during the 2004 growing season (Table 1). All seeds were allowed to air dry in paper bags at room temperature for approximately 2 weeks prior to the start of the cold stratification treatments. We minimized the amount of time between seed collection and the start of the stratification treatment to ensure that little dormancy loss occurred before the actual stratification treatment began. Seed viability was tested concurrently on a subset of seeds ($n = 200$) for each species using standard tetrazolium procedures (Grabe 1970). For a few species (*C. lacustris*, *C. stricta*, *C. utriculata*), initial seed viability was <1% and seeds were sorted by hand or with an air column separator to remove empty seeds (i.e., unfilled achene in perigynia) in order to have a higher proportion of viable seeds in the experiments.

Seed stratification

Seeds were cold stratified for 0 weeks (control), 2 weeks, or 1, 2, 3, 4, 5, or 6 months. We focus here on cold, moist stratification rather than dry, warm storage (i.e., after-ripening) because the latter is often less effective at breaking dormancy in wetland *Carex* species (Budelsky and Galatowitsch 1999). Prior to the start of stratification, seeds were counted into batches of 25 seeds and each batch was wrapped in filter paper. Seed packets were buried in containers of moist, sterilized, wetland soil for stratification (1 container per species). We took a number of measures to ensure uniform stratification conditions among species to minimize the likelihood that germination responses were due to factors other than different stratification durations: (1) soil for all containers was from a single, well-mixed source, (2) each container was watered regularly to maintain a constant moisture level, and (2) all containers were frequently rearranged within the chamber. All seeds for a species entered the stratification treatment at the same time to ensure that seeds had similar dormancy states at the start of the experiment. The commencement of stratification treatments varied among species, however, because of differences in species-specific seed maturation times. Stratification containers were placed in a 5/1°C growth chamber (10:10 hours of high and low temperature with a 2-h linear transition between temperature changes) and watered weekly to saturation. We chose 5/1°C as a stratification temperature because it is effective for breaking dormancy in many species with physiological dormancy (Baskin and

Table 1 Seed viability, seed collection location and date, geographic range, and habitat of the 12 *Carex* species used in this study

Species	Seed viability (%)	Collection location	Collection date (2004)	Geographic range in North America[b]	Habitat[b]
C. brevior (Dewey) Mackenzie.	82	MVSP	19 July	Quebec (QC) to Virginia (VA), w. to the Pacific	Usually in dry soil
C. comosa F. Boott.	95	MLA	9 Sept	QC to Minnesota (MN), s. to Florida (FL) & Louisiana; Washington (WA) to California (CA) & n. Idaho	Swamps & wet meadows
C. cristatella Britton.	93	MLA	18 Aug	New Hampshire to w. QC to VA, w. to North Dakota, Nebraska (NE), & e. Kansas	Open swamps, wet meadows, & shores
C. cryptolepis Mackenzie.	80	PPF	27 Oct	Newfoundland (NL) to MN, s. to New Jersey, Ohio, & Indiana	Wet meadows & shores in calcareous districts
C. granularis Muhl.	56	MVSP	16–19 July	QC & Maine to Saskatchewan (SK), s. to FL, Oklahoma, & ne. Texas (TX)	Wet meadows & swales, chiefly in calcareous districts
C. hystericina Muhl.	89	MLA	14–21 July	New Brunswick & QC to WA, s. to VA, Kentucky, TX, & CA	Swamps, wet meadows, & shores
C. lacustris Willd.[a]	14	CCNHA	11 Aug	QC to VA, w. to SK & NE	Swamps & marshes
C. scoparia Schk.	90	CCNHA	11 Aug	NL to FL, w. to British Colombia (BC), Oregon, & New Mexico (NM)	Open swamps, wet meadows, & shores
C. stipata Muhl.	71	MLA	28–30 June	NL to Arkansas, s. to FL, NM, & CA	Wet low ground
C. stricta Lam[a]	3	CCNHA	11 Aug	QC & Nova Scotia to MN & Manitoba, s. to VA & TX	Swales & marshes, especially where seasonally flooded
C. utriculata Stokes.[a]	9	CCNHA	11 Aug	Boreal America, s. to Delaware, Indiana, NE, NM, and CA	Wet soil or shallow water
C. vulpinoidea Michx.	82	MLA	18 Aug	NL to FL, w. to BC, WA, & Arizona	Marshes & other wet low places

Seed viability was based on initial tetrazolium tests and performance in germination trials. Seeds were collected from wetlands in central and southern Minnesota. The study species include widespread and more restricted *Carex* species, and species from a variety of wetland habitats with different growth forms

MVSP, Minnesota Valley State Park in Scott County (44°40′ N, 93°37′ W); MLA, Minnesota Landscape Arboretum in Carver County (44°51′ N, 93°36′ W); PPF, Pioneer Park Fen in Anoka County (45°9′ N, 93°8′ W); CCNHA, Cedar Creek Natural History Area in Anoka County (45°24′ N, 93°12′ W)

[a] For a few species initial seed viability was <1% and seeds were sorted by hand or with an air column separator to remove empty seeds (i.e., unfilled achene in perigynia) in order to have a higher proportion of viable seeds in the experiments

[b] Gleason and Cronquist (1991)

Baskin 1998). In a previous germination study with these same species, 5/1°C effectively broke dormancy. At the end of a cold stratification period, the seed packets were removed from the containers for the germination trials.

Seed germination

After stratification, seeds were incubated on a light/dark schedule at one of five diurnal temperature regimes—5/1°C, 14/1°C, 22/8°C, 27/15°C, or 35/30°C—representing winter, late fall/early spring, early fall/late spring, summer, and extreme summer temperatures. One growth chamber per germination temperature was used. All combinations of stratification periods and germination temperatures were tested except for some species when seed supplies were limiting and only some of the stratification treatments could be tested: *C. cryptolepis* (0, 1, 2, 3 months), *C. lacustris* (0, 1, 3, 6 months), *C. stricta* (0, 3 months), and *C. utriculata* (0, 1, 2, 3, 6 months). The high and low temperatures for each temperature regime persisted for 10 h a day with a 2-h linear transition between temperatures. The first four temperature regimes were based on monthly long-term temperature data (1961–1990) taken near Lake Park, Dickinson County, IA (43.45° N 95.31° W), archived by the National Climatic Data Center (acquired at www.worldclimate.com). To represent a temperature a seed might experience on an exposed mudflat in a wetland or in future global warming conditions, 35/30°C was also included.

Seeds were incubated in growth chambers (Model GCW-15, Environmental Growth Chambers, Chagrin Falls, OH) on saturated white quartz sand in Petri dishes. Light was provided by cool white fluorescent bulbs at PAR (photosynthetically active radiation, total irradiance between 400 and 700 nm) = 200 μmol m^{-2} s^{-1} at seed level as measured with the LI-189 quantum sensor (LICOR, Lincoln, Nebraska, USA). Light levels were monitored regularly to ensure that comparable irradiance levels occurred within and among chambers. In addition, Petri dishes were rearranged weekly within the growth chambers to mitigate the effects of any slight variation in environmental conditions on seed germination. These measures should minimize the likelihood that species-specific germination responses are due to factors other than germination temperature. The lights turned on as the temperature began to increase to the high temperature and remained on for the 14 h until the temperature decreased to the low temperature. A single pulse of white light (PAR = 200 μmol m^{-2} s^{-1}) ≤14 h is known to trigger germination in the *Carex* study species (Kettenring et al. 2006), so we assume that light did not limit germination in this study.

We used the initial viability values (Table 1) to ensure that each treatment combination (stratification period × germination temperature) for each species had a sample size of approximately 150 viable seeds. We used either 6 cm or 10 cm Petri dishes for each species with either 25 or 50 seeds $dish^{-1}$, respectively. Thus, the number of Petri dishes required per species and number of seeds per dish was different: *C. brevior* (5 dishes × 50 seeds $dish^{-1}$), *C. comosa* (7 dishes × 25 seeds $dish^{-1}$), *C. cristatella* (7 dishes × 25 seeds $dish^{-1}$), *C. cryptolepis* (4 dishes × 50 seeds $dish^{-1}$), *C. granularis* (6 dishes × 50 seeds $dish^{-1}$), *C. hystericina* (4 dishes × 50 seeds $dish^{-1}$), *C. lacustris* (20 dishes × 25 seeds $dish^{-1}$), *C. scoparia* (4 dishes × 50 seeds $dish^{-1}$), *C. stipata* (5 dishes × 50 seeds $dish^{-1}$), *C. stricta* (12 dishes × 50 seeds $dish^{-1}$), *C. utriculata* (9 dishes × 50 seeds $dish^{-1}$), and *C. vulpinoidea* (8 dishes × 25 seeds $dish^{-1}$). Seeds were checked for germination every 2 weeks for 8 weeks. Germination was defined as root radicle or cotyledon emergence when visible without magnification.

Analyses

We graphically compared *Carex* germination percentages across species by stratification duration and germination temperature using means and standard errors (Statistix; Analytical Software 2003). To graph maximum potential germination, germination percentages for each species were corrected by dividing by its seed viability (Table 1). We also used the mean germination percentages to determine the minimum length of stratification required for seeds to germinate to 80%—a potential restoration goal.

Germination rate was assessed by comparing seed germination percentages after 4 and 8 weeks across different stratification treatments. These time periods were chosen to represent approximately 25% and 50% of the 4 month growing season in the prairie pothole region of northern US. Seeds germinating within 4 weeks potentially have an advantage over those germinating within 8 weeks by establishing roots and rhizomes before the onset of winter. Germination values within these time periods thus give restoration practitioners an indication of the likelihood of successful plant establishment.

Using a two-factorial ANOVA, we compared germination proportions after 4 weeks and after 8 weeks for each species (two separate tests), with stratification duration and germination temperature as the independent variables and germination proportion as the dependent variable. All nominal *P*-values were multiplied by a factor of two to account for the multiple testing (i.e., two separate ANOVAs). Resulting *P*-values of <0.05 were considered significant. All germination proportions were arc-sine square root transformed prior to analyses to standardize the variance.

Results

All the *Carex* species in this study germinated >5% under at least one of the temperature regimes without stratification except *C. granularis* (Fig. 1). After stratification, however, seeds of most species germinated over a wider range of temperatures, to higher percentages, and more quickly than unstratified seeds. Thus, the 12 species produced conditionally dormant (rather than totally dormant) seeds at maturity and seed germination was enhanced by stratification. For instance, nine of the *Carex* species did not germinate at 14/1°C without stratification but germinated at 22/8°C and/or 27/15°C without stratification. With stratification, these species all gained the ability to germinate at 14/1°C.

Temperature significantly affected the germination of the 12 *Carex* species (Table 2). The optimal temperature for maximum germination without stratification was 27/15°C for all species except *C. stricta* and *C. utriculata* (Fig. 1). The latter two species had highest germination at 35/30°C when seeds were unstratified. This pattern also held true after stratification although most of these species then germinated to high percentages at 22/8°C as well. Still, *C. stricta* and *C. utriculata* had highest germination at 35/30°C after stratification. In contrast, *C. granularis* is the only species that had highest germination at 14/1°C after stratification, and was the only species that never gained the ability to germinate at 35/30°C even after 6 months of stratification. No species gained the ability to germinate at 5/1°C after any stratification duration.

Stratification significantly increased germination percentages for all species under at least one of the temperature regimes (Fig. 1; Table 2). This effect was greatest for the six species that failed to germinate at 14/1°C without stratification (*C. brevior*, *C. comosa*, *C. granularis*, *C. hystericina*, *C. stipata*, *C. vulpinoidea*) but germinated to >80% after some stratification. Similarly, at 35/30°C, *C. brevior*, *C. cryptolepis*, *C. hystericina*, *C. scoparia*, and *C. stipata* seeds germinated <40% without stratification, but germinated >80% after stratification. At the more moderate temperature regimes of 27/15°C or 22/8°C, stratification improved germination percentages from <40% to >80% for *C. comosa*, *C. cryptolepis*, *C. granularis*, *C. lacustris*, and *C. vulpinoidea*.

The period of stratification required for germination to 80% varied by species across different germination temperatures (Fig. 1; Table 3). In general, for all species longer stratification was required for 80% germination at 14/1°C and 35/30°C compared to 22/8°C and 27/15°C. For instance, *C. brevior* did not require stratification for 80% germination at 22/8°C and 27/15°C but required 2 and 3 months stratification for germination at 35/30°C and 14/1°C, respectively. Interestingly, *C. vulpinoidea* required no stratification for 80% germination at 27/15°C and 35/30°C, and after just 0.5 months of stratification seeds germinated to 80% at 22/8°C. Six months of stratification were required for 80% germination at 14/1°C. Still, a few species did not germinate to 80% at 35/30°C (*C. comosa*, *C. granularis*, *C. lacustris*) and 14/1°C (*C. lacustris*, *C. utriculata*) with 6 months of stratification.

Stratification increased the rate of germination for all species under at least one of the germination temperature regimes (Fig. 1). For example, when *C. scoparia* seeds were stratified for 1 month, 87% of the seeds germinated at 35/30°C within 8 weeks but only 28% had germinated within 4 weeks. As the stratification treatment increased to 2, 3, and 4 months, more of the total germination occurred within 4 weeks (44% of 86%, 58% of 89%, and 72% of 96%, respectively). Interestingly, although *C. comosa* germinated to high percentages at 14/1°C, none of the seed germinated within the first 4 weeks of the germination trial.

Discussion

This is the first comparative study characterizing dormancy loss and germination temperature requirements of temperate wetland *Carex* from the mid-continental US. All 12 study species

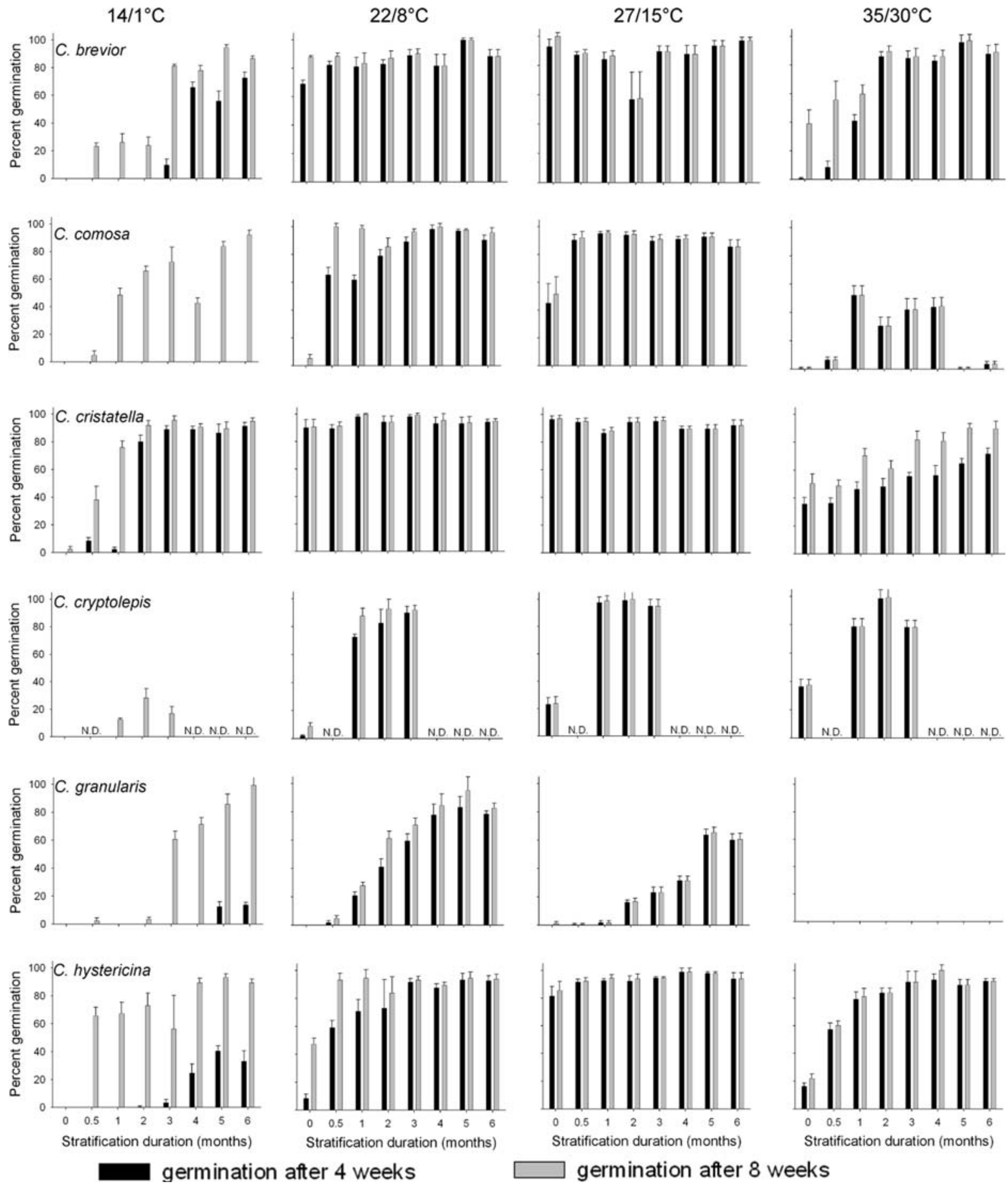

Fig. 1 The effects of cold stratification and temperature on *Carex* germination after 4 and 8 weeks. *N.D.* indicates no data available because condition was not tested. The effects of both factors on *Carex* germination after 4 weeks and 8 weeks were significant (Table 2)

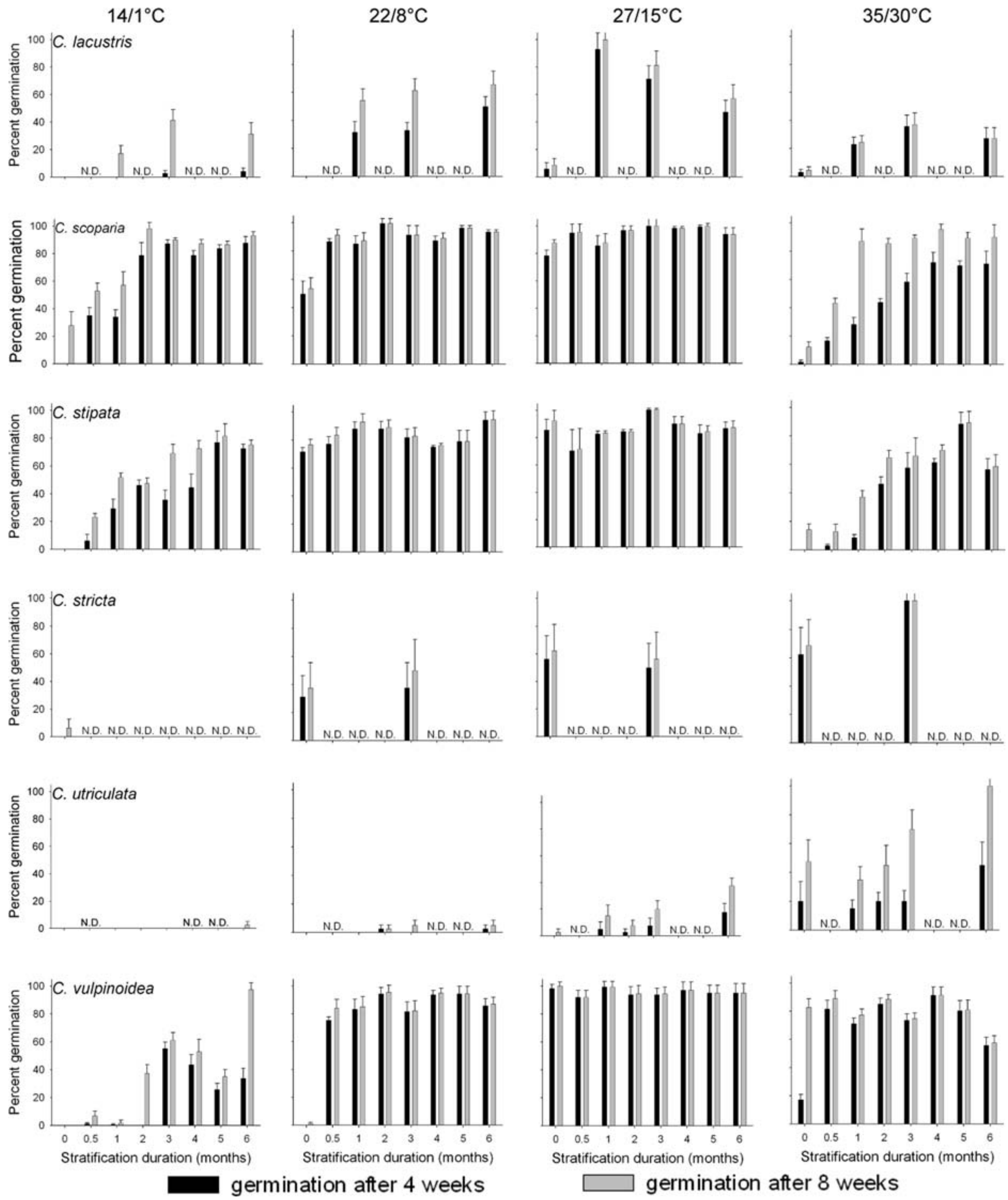

Fig. 1 continued

produced conditionally dormant seeds. Although seeds of many species germinated well at warm temperatures (especially 27/15°C) without stratification, stratification increased the speed of germination and germination percentages for all species across a wider range of temperature

Table 2 Results of the ANOVA of the effects of stratification duration (Strat) and germination temperature (Temp) on *Carex* germination after 4 weeks and 8 weeks

Species	4 weeks			8 weeks		
	Strat	Temp	Strat × Temp	Strat	Temp	Strat × Temp
C. brevior	***	***	***	***	***	***
C. comosa	***	***	***	***	***	***
C. cristatella	***	***	***	***	***	***
C. cryptolepis	***	***	***	***	***	***
C. granularis	***	***	***	***	***	***
C. hystericina	***	***	***	***	***	***
C. lacustris	***	***	***	***	***	***
C. scoparia	***	***	***	***	***	***
C. stipata	***	***	***	***	***	***
C. stricta	*	***	N.S.	N.S.	***	N.S.
C. utriculata	*	***	N.S.	***	***	N.S.
C. vulpinoidea	***	***	***	***	***	***

N.S. = not significant

*** $P < 0.001$,
* $P < 0.05$

Table 3 Cold stratification period required for 80% germination within 4 weeks (top value) and 8 weeks (bottom value) at 14/1°C, 22/8°C, 27/15°C, or 35/30°C

Species	Length of cold stratification required for >80% germination (months)			
	Germination temperature			
	14/1°C	22/8°C	27/15°C	35/30°C
C. brevior	NG	0.5	0	2
	3	0	0	2
C. comosa	NG	3	0.5	NG
	5	0.5	0.5	NG
C. cristatella	2	0	0	NG
	2	0	0	3
C. cryptolepis[a]	>3	2	≤1	2
	>3	≤1	≤1	2
C. granularis	NG	5	NG	NG
	5	4	NG	NG
C. hystericina	NG	3	0	1
	4	0.5	0	1
C. lacustris[a]	NG	NG	≤1	NG
	NG	NG	≤1	NG
C. scoparia	3	0.5	0.5	NG
	2	0.5	0	1
C. stipata	NG	1	0	5
	5	0.5	0	5
C. stricta[a]	>0	>3	>3	≤3
	>0	>3	>3	≤3
C. utriculata[a]	NG	NG	NG	NG
	NG	NG	NG	≤6
C. vulpinoidea	NG	1	0	0.5
	6	0.5	0	0

NG (no germination) denotes when even after 6 months of stratification germination to 80% did not occur

[a] Not all stratification treatments were tested for *C. cryptolepis*, *C. lacustris*, *C. utriculata*, and *C. stricta* so more general estimates are presented

regimes. We suggest that this phenomenon should be harnessed for improving *Carex* and other revegetation efforts worldwide. In our study system, seed stratification can be used to promote the rapid establishment of *Carex* canopies and minimize invasion windows for exotic species (Johnstone 1986).

Our findings mirror what other studies in other regions of the world have found regarding *Carex* dormancy. Jones et al. (2004) looked at the effects of stratification for 0, 7, 30, or 150 days on *C. nebrascensis* and *C. utriculata* germination from riparian systems in Utah. The effects of stratification varied with species and germination temperature but resulted in increased germination for both species in light at the spring temperature regime (11/4°C). Similarly, we found that stratification increased germination percentages at spring temperature regimes (14/1°C and often 22/8°C) for all 12 species. Schütz and Rave (1999) compared germination of fresh seeds of 32 *Carex* species from northern Europe and those stratified for 6 months. They found a strong response of *Carex* germination to stratification, with many species requiring stratification to germinate at many of the temperature regimes. Similarly, in our study, we found that nine species required stratification to germinate at 14/1°C and three species required stratification to germination at 22/8°C.

Carex species are purported to have a high minimum temperature requirement for germination (Schütz 2000), like many wetland plants

(Baskin and Baskin 1998; Grime et al. 1981), compared with many plant species in general. Schütz and Rave (1999) found that germination was generally greatest at 20°C or 25°C (versus 10°C, 15°C, and 30°C) in a study of 15 wetland *Carex* from Northern Europe; the upper limits of germination for *Carex* species are estimated to occur between temperatures of 32°C and 37°C (Schütz 2000). The results of our study also support the claim of a high minimum temperature requirement for germination. We found that *Carex* germination was generally greatest at 27/15°C with unstratified seeds. In addition, after stratification many species also germinated to high percentages at 35/30°C (and 22/8°C). Unstratified and stratified seeds of *C. stricta* and *C. utriculata* had greatest germination at 35/30°C. No species gained the ability to germinate at the lowest temperature regime 5/1°C and some species germinated poorly at 14/1°C as well.

Although generalities about *Carex* dormancy and germination emerge from our study, we found the variability within the genus to be striking given that they occupy similar habitat within one region. This variability does not appear to correspond to phenology or other traits, such as seed maturation times, seed size, or plant growth form (see Gleason and Cronquist 1991 for *Carex* growth forms). For instance, *C. stipata* seeds ripen the earliest of all the study species (late June) and germinate well without stratification at both 22/8°C and 27/15°C. This germination syndrome is the same as those species whose seeds ripen in mid-July (*C. brevior*) and mid-August (*C. cristatella*, *C. scoparia*). On the other extreme, *C. cryptolepis* seeds ripen in late October, the latest of all the study species and germinate to moderate levels at 27/15°C and 35/30°C without stratification, like those of *C. stricta* and *C. utriculata* that ripen in mid-August (and *C. stricta* can also germinate to moderate levels at 22/8°C). However, *C. comosa* seeds, which ripen in early September, germinate only to moderate levels at 27/15°C (but not 35/30°C) without stratification. Finally, *C. granularis* seeds ripen in mid-July but unlike all other study species that ripen during this time period, this species requires long periods of stratification before germinating readily at 27/15°C.

Seed size was a better predictor of seed dormancy and germination patterns than species' habitat preferences, seed maturation times, or plant growth form. In general, most of the smallest seeded species (*C. brevior*, *C. cristatella*, *C. scoparia*, *C. vulpinoidea*) germinated well without stratification at 22/8°C and 27/15°C. The exception to this rule was *C. stricta* seeds, which did not germinate to high percentages at these temperatures (interestingly, this species also had very low seed viability). Still, some of the medium and large seeded species also germinated well at these temperatures with little to no stratification (*C. comosa*, *C. cryptolepis*, *C. hystericina*, *C. stipata*) while the remainder of our study species (*C. granularis*, *C. lacustris*, *C. utriculata*) had stronger dormancy and/or more limited temperature germination requirements (as well as low/moderate seed viability). Schütz and Rave (1999) did not find a strong relationship between seed weight and germination for 18 European wetland *Carex* species. Since these comparisons to plant and habitat characteristics reveal no clear patterns, generalization of our results to unstudied wetland *Carex* species is not possible. This is particularly problematic for restoration practitioners who may be dealing with a much wider array of *Carex* than we studied. Other species of *Carex* must then be added to the long list of native species that require study for restoration purposes to understand their dormancy breaking and germination requirements (Clewell and Rieger 1997).

Our findings indicate that seed stratification is not a one-size-fits-all solution for revegetation and that this treatment is important for some but not all species, depending on the germination temperature. Diboll (1997), in his chapter on designing seed mixes for grassland restoration, suggests that paying attention to species specific biology is important to the success of seeding efforts. Our findings support this statement. We recommend that restoration practitioners consider stratifying seeds of species with stronger dormancy and/or when seed supplies are limited (especially for species with low viable seed production). For instance, unstratified *C. granularis* seeds did not germinate readily at 14/1°C, 22/8°C and 27/15°C (Fig. 1) but 5 and 4 months of stratification increased germination to 80% at 14/

1°C and 22/8°C (Table 3), respectively. For some species, just 2 weeks to 1 month of stratification had a large effect by increasing germination from <20% to >80% (*C. comosa*, *C. cryptolepis*, and *C. vulpinoidea* at 22/8°C; *C. lacustris* at 27/15°C; *C. hystericina*, and *C. scoparia* at 35/30°C). For other species at some germination temperatures, stratification will have little to no effect (e.g., *C. brevior*, *C. cristatella*, *C. hystericina*, *C. scoparia*, *C. stipata*, and *C. vulpinoidea* at 27/15°C). Since stratification did not reduce germination for these species, a stratification pretreatment poses little risk for species where seed ecology studies are lacking (e.g., many of the 250 wetland *Carex* species native to the United States have not been studied) (USDA NRCS 2005). The only drawback to stratifying these species will be increased time and resources required to add a stratification step to a wetland restoration.

The degree of seed dormancy within a species can vary within and among individual plants (Baskin and Baskin 1998), a phenomenon that drives non-uniform emergence from the seed bank (Leck 1989). Within a restoration context, however, it is initially desirable to have rapid, uniform establishment of native plant species, especially to prevent invasive species colonization. In our study, using knowledge of the seed dormancy and germination ecology of *Carex* to maximize its establishment may be important for minimizing the invasion of *P. arundinacea* into sedge meadow communities. *Phalaris* seed germination is inhibited by low red:far red light ratios (Lindig-Cisneros and Zedler 2001)—a condition present under dense plant canopies. Thus, early development of canopies of *Carex* (or other species) in restoration projects may be important in controlling or preventing the establishment of *Phalaris*. As with other species with physiological dormancy, we found that stratification increased seed germination rates and the range of temperatures at which seeds germinated after dormancy was broken (Baskin and Baskin 1998). Thus, stratifying seeds of target native species and sowing them at the appropriate time for maximum germination may allow restoration practitioners to more rapidly establish diverse sedge meadow communities and potentially minimize *Phalaris* invasion. Further study is warranted to determine the benefit of using stratified *Carex* seeds to limit *Phalaris* invasion. In addition, seed stratification should be evaluated more broadly to determine its usefulness in limiting or preventing invasions by exotic species in other restored systems.

By using a stratification pretreatment to minimize the time between seed sowing and seed germination, restoration practitioners can maximize their seed supplies and limited budgets. Restoration practitioners usually accomplish seed stratification through a dormant seeding (i.e., sowing seeds in the fall so that seeds can stratify naturally under winter conditions) but the hazards to seeds prior to germination in the field reduce the efficacy of this approach. Hazards to seeds include seed burial (especially critical in restored wetlands where excess sedimentation can occur), seed predation, loss of seed viability over time, and seedling mortality if a seed germinates prematurely during an unseasonably warm period in winter (Budelsky and Galatowitsch 1999; Jones et al. 2004; van der Valk et al. 1999). In a comparison of germination of seeds of four *Carex* species sown unstratified in autumn (i.e., a dormant seeding) versus sown stratified in the spring, germination percentages were significantly lower for two species (Kettenring 2006). Other studies have shown that *Carex* seed viability declines sharply within 6–18 months after seed maturation (Budelsky and Galatowitsch 1999; van der Valk et al. 1999). Effective stratification procedures under controlled conditions prior to seed sowing will minimize seed mortality in the field. Our findings illustrate how seed stratification can be used to obtain rapid and high germination at different growing season temperatures. The next step will be to develop suitable techniques for stratification pretreatments of the large volumes of seed most restoration practitioners work with before a stratification pretreatment can reliably replace the more widespread practice of dormant seeding.

Acknowledgments We thank Carol Baskin, Albert Markhart, Carrie Reinhardt Adams, and two anonymous reviewers for comments on earlier drafts of this manuscript. This study would not have been possible without field and lab assistance from Stacey Olszewski, growth chamber support from Mike Emerick, statistical advice from Christopher Bingham, and funding from a graduate student grant from Applied Ecological Services, a Brand Fellowship from the University of Minnesota Graduate School, a Delta Waterfowl graduate student fellowship, a Dayton Fellowship from the Bell Museum of Natural History, and the University of Minnesota Agricultural Experiment Station to the first author. This is a publication of the University of Minnesota Agricultural Experiment Station.

References

Analytical Software (2003) Statistix 8.0. Analytical Software, Tallahassee, FL

Barkley TM (ed) (1986) Flora of the Great Plains. University Press of Kansas, Lawrence, KS

Baskin CC, Baskin JM (1998) Seeds – ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego

Bernard JM (1990) Life history and vegetative reproduction in *Carex*. Can J Bot 68:1441–1448

Bohnen JL, Galatowitsch SM (2005) Spring Peeper Meadow: revegetation practices in a seasonal wetland restoration in Minnesota. Ecol Restor 23:172–181

Budelsky RA, Galatowitsch SM (1999) Effects of moisture, temperature, and time on seed germination of five wetland *Carices*: implications for restoration. Restor Ecol 7:86–97

Budelsky RA, Galatowitsch SM (2000) Effects of water regime and competition on the establishment of a native sedge in restored wetlands. J Appl Ecol 37:971–985

Budelsky RA, Galatowitsch SM (2004) Establishment of *Carex stricta* Lam. seedlings in experimental wetlands with implications for restoration. Plant Ecol 175:91–105

Burton PJ, Burton CM (2002) Promoting genetic diversity in the production of large quantities of native plant seed. Ecol Restor 20:117–123

Clewell A, Rieger JP (1997) What practitioners need from restoration ecologists. Restor Ecol 5:350–354

Diboll N (1997) Designing seed mixes. In: Packard S, Mutel CF (eds) The tallgrass restoration handbook for prairies, savannas, and woodlands. Island Press, Washington, DC, pp 125–150

Forbes BC (1993) Small-scale wetland restoration in the high Arctic: a long-term perspective. Restor Ecol 1:59–68

Galatowitsch SM, Biederman LA (1998) Vegetation and seedbank composition of temporarily flooded *Carex* meadows and implications for restoration. Int J Ecol Environ Sci 24:253–270

Galatowitsch SM, van der Valk AG (1996a) Characteristics of recently restored wetlands in the prairie pothole region. Wetlands 16:75–83

Galatowitsch SM, van der Valk AG (1996b) Vegetation and environmental conditions in recently restored wetlands in the prairie pothole region of the USA. Plant Ecol 126:89–99

Galatowitsch SM, van der Valk AG (1996c) The vegetation of restored and natural prairie wetlands. Ecol Appl 6:102–112

Galatowitsch SM, Budelsky RA, Yetka LA (1999) Revegetation strategies for northern temperate glacial marshes and meadows. In: Streever W (ed) An international perspective on wetland rehabilitation. Kluwer Academic Publishers, The Netherlands, pp 225–241

Gleason HA, Cronquist A (1991) Manual of vascular plants of the northeastern United States and adjacent Canada. The New York Botanical Garden, New York

Grabe DF (ed) (1970) Tetrazolium testing handbook for agricultural seeds. Contribution no. 29 to the handbook on seed testing. Association of Official Seed Analysts, Lincoln, Nebraska

Green EK, Galatowitsch SM (2001) Differences in wetland plant community establishment with additions of nitrate-N and invasive species (*Phalaris arundinacea* and *Typha xglauca*). Can J Bot 79:170–178

Green EK, Galatowitsch SM (2002) Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities. J Appl Ecol 39:134–144

Grime JP, Mason G, Curtis AV, Rodman J, Band SR (1981) A comparative study of germination characteristics in a local flora. J Ecol 69:1017–1059

Holl KD, Loik ME, Lin EHV, Samuels IA (2000) Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. Restor Ecol 8:339–349

Holmes PM, Richardson DM (1999) Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African fynbos. Restor Ecol 7:215–230

Hurd EG, Shaw NL (1992) Seed technology for *Carex* and *Juncus* species of the intermountain region. In: Landis TD (ed) Intermountain Nurseryman Association annual meeting. USDA, Forest Service, Rocky Mountain Forest and Range Experiment Station, Park City, UT, pp 74–83

Johnstone IM (1986) Plant invasion windows: a time-based classification of invasion potential. Biol Rev Camb Philos Soc 61:369–394

Jones KL, Roundy BA, Shaw NL, Taylor JR (2004) Environmental effects on germination of *Carex utriculata* and *Carex nebrascensis* relative to riparian restoration. Wetlands 24:467–479

Kettenring KM (2006) Seed ecology of wetland *Carex* spp. – implications for restoration. Applied Plant Sciences. University of Minnesota, St. Paul, 133 pp

Kettenring KM, Gardner GM, Galatowitsch SM (2006) Effect of light on seed germination of eight wetland *Carex* species. Ann Bot 98:869–874

Leck MA (1989) Wetland seed banks. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press, Inc., New York, pp 283–305

Lindig-Cisneros R, Zedler JB (2001) Effect of light on seed germination in *Phalaris arundinacea* L. (reed canary grass). Plant Ecol 155:75–78

Martin D, Chambers JC (2002) Restoration of riparian meadows degraded by livestock grazing: above- and belowground responses. Plant Ecol 163:77–91

Maurer DA, Zedler JB (2002) Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. Oecologia 131:279–288

Mulhouse JM, Galatowitsch SM (2003) Revegetation of prairie pothole wetlands in the mid-continental US: twelve years post-reflooding. Plant Ecol 169:143–159

Perry LG, Galatowitsch SM, Rosen CJ (2004) Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. J Appl Ecol 41:151–162

Schütz W (2000) Ecology of seed dormancy and germination in sedges (*Carex*). Perspect Plant Ecol Evol Syst 3:67–89

Schütz W, Rave G (1999) The effect of cold stratification and light on the seed germination of temperate sedges (*Carex*) from various habitats and implications for regenerative strategies. Plant Ecol 144:215–230

Steed JE, DeWald LE (2003) Transplanting sedges (*Carex* spp.) in southwestern riparian meadows. Restor Ecol 11:247–256

USDA NRCS (2005) The PLANTS Database, Version 3.5. National Plant Data Center, Baton Rouge, LA. http://plants.usda.gov

van der Valk AG, Bremholm TL, Gordon E (1999) The restoration of sedge meadows: seed viability, seed germination requirements, and seedling growth of *Carex* species. Wetlands 19:756–764

Wijdeven SMJ, Kuzee ME (2000) Seed availability as a limiting factor in forest recovery processes in Costa Rica. Restor Ecol 8:414–424

Zanini L, Ganade G (2005) Restoration of *Araucaria* forest: the role of perches, pioneer vegetation, and soil fertility. Restor Ecol 13:507–514