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# Soaking Nebraska sedge seeds in warm, aerated water improves germination

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## ABSTRACT

Nebraska sedge (*Carex nebrascensis* Dewey [Cyperaceae]) is a broadly distributed and locally abundant sedge in western North America. The species is commonly used in wetland and riparian restoration and enhancement plantings. Germination is typically achieved by subjecting the achenes with their perigynia removed (hereafter simply referred to as seeds) to 30-d stratification at 3 °C (37 °F); however, personal observations indicate that Nebraska sedge can be germinated using soaking water treatments without a stratification treatment. Staff at the USDA Natural Resources Conservation Service, Aberdeen Plant Materials Center subjected seeds of Nebraska sedge to a number of soaking treatments and compared average germination and germination rates with those obtained using traditional methods. We found that seeds soaked in warm (24–35 °C [75–95 °F]), aerated water germinated significantly faster (3 to 6 times) than all other treatments. We also determined that, in most cases, soaking Nebraska sedge seeds in warm water significantly increased germination percentages compared with seeds germinated using the traditional 30-d stratification treatment (87% compared with 47%, respectively).

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## KEY WORDS

propagation, riparian, seed dormancy, wetland, Cyperaceae, *Carex nebrascensis*

## NOMENCLATURE

USDA NRCS (2011)

Nebraska sedge (*Carex nebrascensis* Dewey [Cyperaceae]) is a widespread sedge species common throughout western North America from the West Coast to the Great Lakes region, north to Alberta and Saskatchewan and south to Arizona and New Mexico (USDA NRCS 2011). It can be locally abundant in wet to semi-wet meadows, springs, and riparian fringes at lower to mid-elevations throughout its range (Welsh and others 2003). Because of its abundance and its dense root matrix, Nebraska sedge is frequently used for wetland and riparian creation and restoration projects in the West (Manning and others 1989; Hoag and others 2011).

Sedges produce small seed-bearing achenes clothed in a saclike structure referred to as a perigynium. The standard method for germinating seeds of Nebraska sedge involves 1) removing the perigynium from the achene; 2) subjecting the seeds to a 30-d stratification at 3 °C (37 °F); and 3) germinating the seeds at high temperatures with exposure to light (Johnson and others 1965; Shaw and Hurd 1992; Jones 1999; Hoag and others 2001). Staff at the USDA Natural Resources Conservation Service, Aberdeen Plant Materials Center, however, have observed that Nebraska sedge seeds without exposure to a stratification treatment may germinate at high levels under hot greenhouse conditions. We believe that under certain conditions the stratification requirement may be circumvented and allow for faster, more uniform germination of sedge seeds. Our objective was to place seeds in warm, moist environments and examine subsequent germination to determine if such treatments might yield user-friendly germination techniques that can be applied to relatively large quantities of seeds for use in wetland plantings.

## MATERIALS AND METHODS

Seeds were harvested from an established wetland pond located at the Aberdeen Plant Materials Center, Aberdeen, Idaho, in 2000. Seeds were cleaned, including removal of the perigynia, and stored in cold, dry conditions at 4 °C (39 °F) and approximately 35% relative humidity. A sample of the seedlot was sent to the Idaho State Seed Laboratory for a tetrazolium (TZ) test prior to this trial in 2010. The results indicated 79% viable seeds.

The experiment included 3 soaking conditions (constant soaking; changing the water every 3 d with fresh clean water; and constant soaking in aerated water) applied in one of 2 locations (growth chamber or greenhouse) for a total of 6 treatments. The seed-soaking treatments were conducted in 0.47-l (16-oz) glass Mason jars to meet the light requirement of the species (Jones 1999; Kettenring and others 2006). The water-change treatments were drained and refilled with clean water every 3 d after initiation of soaking. Aeration was performed

using a Profile® 1500 aquarium air pump fitted with a 2.5-cm (1-in) bubbling air stone (Figure 1). The Hoffman® growth chamber was kept at a constant 35 °C (95 °F) with 24 h constant light supplied by six, 34-watt lite white fluorescent bulbs, 3 on the door and 3 on the back panel. Greenhouse temperatures were allowed to fluctuate from 24 °C (75 °F) at night to 35 °C (95 °F) during the day under natural midsummer photoperiod. Photosynthetically active radiation (PAR) was measured using a Decagon AccuPAR LP-80 Ceptometer® (Decagon Devices, Pullman, Washington). Greenhouse PAR measured 1300  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  at 13:00 and growth chamber PAR measured 45  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ . A 7th treatment, using methods that resulted in the highest germination in Hoag and others (2001), served as the control. Seeds for the control treatment were mixed with 8 g sphagnum moss in 200 ml water and stratified at 3 °C (37 °F) for 30 d before being germinated on blotter paper in Petri plates sealed with paraffin wax and kept inside the Hoffman® growth chamber described above with diurnal temperatures of 26 °C (78 °F) and 37 °C (98 °F).

For each of the soaking treatments, 1.65 g of seeds (approximately 3000) were placed inside each jar. Germination was



Figure 1. Nebraska sedge seed and germinants 10 d after initiation (DAI) of a greenhouse aerated soaking water treatment. Photo by Derek Tilley

evaluated daily beginning 6 d after initiation (DAI) through 10 DAI. After 10 DAI, seedlings were evaluated at 14, 21, and 28 DAI. At each evaluation, the seeds in the jars were stirred to mix floating and sunken seeds, and then several hundred seeds were randomly removed from the jar with a spoon and placed into a Petri plate for evaluation. Twenty-five seeds were randomly evaluated under a dissecting scope at 10x magnification to determine germination. Seeds were counted as germinated if the seedcoat had split to reveal cotyledon growth. For the control, 4 replications of 25 seeds were evaluated for germination.

Germination rate was determined at 10 DAI using the method described by Maguire (1962). The number of seedlings obtained at each counting (6, 7, 8, 9, and 10 DAI) was divided by the number of days after planting, and the values obtained at each count were summed at the end of the test as follows. Denominators correspondingly were 6, 7, 8, 9, and 10:

$$\text{Germination rate} = \left( \frac{\text{Number of seedlings}}{\text{Days after planting}} \right) + \dots + \left( \frac{\text{Number of seedlings}}{\text{Days after planting}} \right)$$

Each evaluation was replicated 4 times. Germination at 10 DAI, final germination at 28 DAI, and germination rates were analyzed with a one-way analysis of variance with an alpha of 0.05 to determine significance. Means were then separated using a LSD (least significant difference) test. Because the same seeds and seedlings were not observed at each evaluation in the soaking treatments, it was possible for percentage germination to decline in subsequent observations. Evaluation of the aerated treatments was discontinued at 10 DAI, and evaluation of the growth chamber change treatment was similarly discontinued at 21 DAI because seeds had achieved the maximum expected germination (>100% of TZ test).

## RESULTS, DISCUSSION, AND IMPLICATIONS

Aerated seeds, regardless of location, had >80% total germination (>100% of expected) after 7 d, while all other treatments remained <50% germination even after 10 d (Figure 2). At 10 DAI, total germination of aerated seeds averaged 87%, significantly greater than all other treatments, which averaged 26% germination (Table 1). By 28 DAI, the greenhouse soak and greenhouse change treatments had essentially reached the maximum of expected germination and did not differ significantly from the final germination of the aerated treatments. Germination of the control seeds (30-d stratification) appeared to plateau by 28 DAI and had the lowest final germination, significantly lower than all other treatments with the exception of the growth chamber soaking treatment.

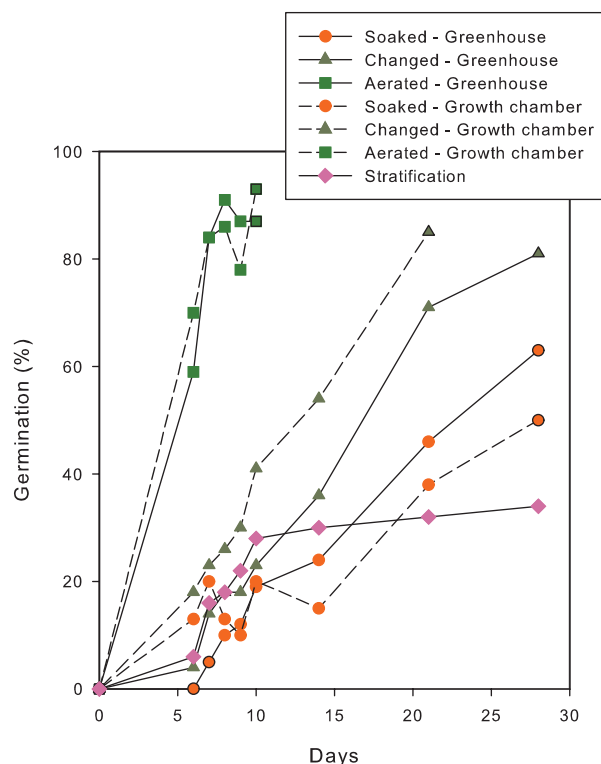


Figure 2. Average cumulative germination of Nebraska sedge under germination treatments. Soaked = constant soaking; Changed = water changed every 3 d; Aerated = soaked with aquarium aerator. Evaluations ended when germination had reached the maximum of the expected 79% based on a TZ test. Aerator evaluations for the greenhouse and growth chamber concluded at 10 DAI; growth chamber evaluations for treatments with changed water concluded at 21 DAI.

At 10 DAI, germination rate for the aerated seeds was about 13, significantly faster than the 2.3 value averaged by the remaining treatments (Table 1). The lowest germination rates were observed in the greenhouse soak and the control (30-d stratification) treatments (about 1.5).

With the exception of the growth chamber soaking treatment, all soaking treatments in concert with warm temperatures yielded significantly higher total germination and faster germination rates than the traditional germination procedure involving a 30-d stratification. Under the warm soaking conditions, no stratification was required to obtain high rates of germination, suggesting that using a warm, aerated soaking treatment can significantly reduce the amount of time required to prepare seeds for planting.

To my knowledge, this study is the first to show this method for overcoming seed dormancy in wetland sedges. Although the highest germination achieved in the control (30-d stratification) was only 34% (compared with a TZ value of 79%), nearly all of the warm soaking treatments evaluated in this trial yielded germination that approached or exceeded the TZ test

TABLE 1

Germination response to soaking treatments and a 30-d stratification treatment at 3 °C (37 °F).

Treatment	Germination (10 DAI) (%)	Final germination (28 DAI <sup>z</sup> ) (%)	Germination rate <sup>y</sup>
Growth chamber, Aerated	93 a <sup>x</sup>	93 a	13.10 a
Greenhouse, Aerated	87 a	87 a	12.90 a
Growth chamber, Changed	41 b	85 a	4.24 b
Greenhouse, Changed	23 c	81 a	2.21 c
Growth chamber, Soaked	20 c	50 bc	2.49 c
Greenhouse, Soaked	19 c	63 b	1.30 d
Control (30-d stratification)	29 c	47 c	1.7 cd
<i>P</i> value	<0.001	<0.001	<0.001
LSD (0.05)	9.8	15.3	0.83

<sup>z</sup> Final percentage germination taken at 10 DAI for aerated treatments and 21 DAI for changed water treatment in the growth chamber.

<sup>y</sup> Germination rate is a comparative value with no associated unit of measure; larger numbers signify faster germination.

<sup>x</sup> Means followed by the same letter are not significantly different ( $P \leq 0.05$ ) using LSD (least significant difference) test.

value. This suggests that soaking this single source of Nebraska sedge seeds in warm, aerated water fully overcame the inherent dormancy mechanisms, while the standard stratification treatment only partially overcame seed dormancy.

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