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GERMINATION OF THREADLEAF SEDGE (CYPERACEAE: *CAREX FILIFOLIA*)*

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ABSTRACT

Threadleaf sedge (*Carex filifolia* Nutt.) is one of the most important dryland plants native to the grasslands of the northern Great Plains. Low seed production and poor germination are primary reasons making prairie restorations that include this species a challenge. The principal objective of this research was to investigate the influences of warm and cold pretreatments, moisture regime, and substrate type on germination. The work was conducted in a germination chamber with seeds (achenes) collected at two sites and in two years in western Nebraska. Average germination was relatively low with the common pretreatments of cold-wet alone (39% in 1998 and 17% in 1999) and warm-wet plus cold-wet pretreatment (33% in 1998 and 1999). Eight weeks of warm-dry pretreatment produced the greatest average germination (49% in 1998 and 41% in 1999). Warm-dry pretreatment may be a useful tool in preparing threadleaf sedge achenes for planting in prairie restorations.

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Threadleaf sedge (*Carex filifolia* Nutt.) (Fig. 1) is an important perennial, dryland plant native to grasslands of the northern Great Plains of the United States and adjacent Canadian provinces (Coupland 1961, Weaver and Albertson 1956). It occurs on dry plains and hills as far south as Texas and as far west as California (Stubbendieck et al. 1997). Threadleaf sedge grows in association with needle-and-thread, *Hesperostipa comata* (Trin. & Rupr.) Barkw., and blue grama, *Bouteloua gracilis* (Willd. *ex* Kunth) Lag. *ex* Griffiths, but it thrives on dry ridges where little other vegetation survives (Weaver and Albertson 1956). It provides excellent spring forage for wildlife and livestock, and its tough, wiry roots make it invaluable for soil stabilization (Stubbendieck and Foster 1978, Tichota 2000).

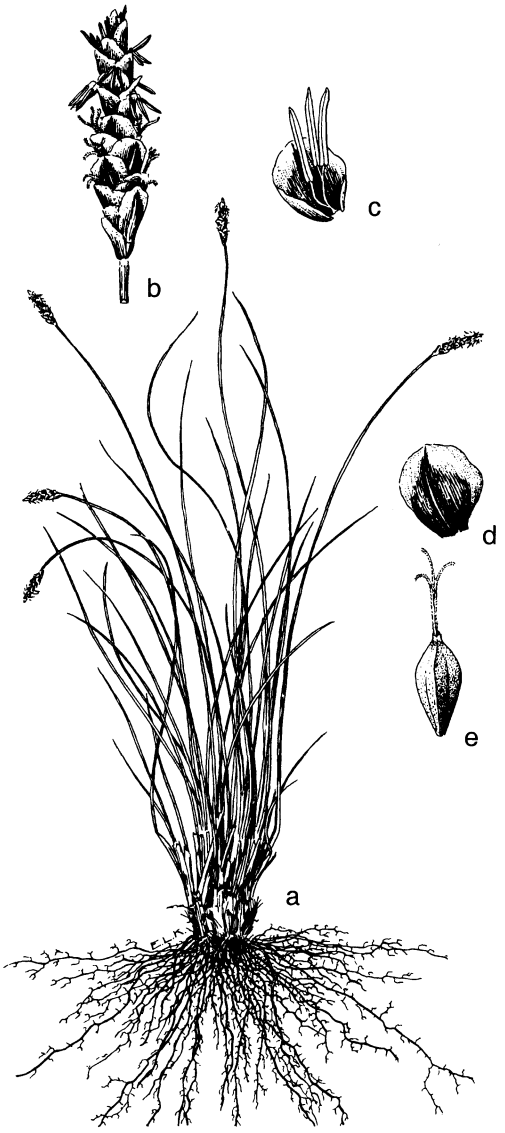

Figure 1. *Carex filifolia*. **a**. habit, × 0.7; **b**. flowering spike, × 2; **c**. staminate flower with subtending scale, × 3.5; **d**. pistillate scale, × 3.5; **e**. achene in enclosing perigyium, × 3.5 (drawing by "E.L.K.," from Hermann 1970).

Many sedge species (but not threadleaf sedge) reproduce by rhizomes, thereby making production of

seedlings much less important in nature (Amen and Bonde 1964, Steinger et al. 1996). While production of threadleaf sedge seedlings may not be critical in established natural communities, understanding germination for the purpose of prairie restoration is crucial. A combination of limiting factors such as low seed production, absence of a commercial seed source, rarity of natural seedling establishment, low levels of germination in laboratory research, and slow recolonization makes reintroduction of this species a challenge.

The objective of this research was to evaluate methods to increase germination through warm and cold pretreatment, substrate type, and moisture regime. Various pretreatment methods and temperature regimes have been tested with other sedge species, but data are not available on germination of threadleaf sedge (Fulbright et al. 1982, Johnson et al. 1965, McDonough 1969, Schutz and Rave 1999, Weisner et al. 1967). A compilation of published records for meadow, dryland, and wetland sedges in the western United States revealed that germination data were unavailable for many species, and the majority of species, especially dryland sedges, exhibited low germination (Link 1993). Researchers have explored the requirement for relatively high germination temperatures for sedges (Schutz, 1997, Schutz and Milberg 1997), but high germination pretreatment temperatures have not been addressed.

MATERIALS AND METHODS

Threadleaf sedge achenes (Fig. 1e) were harvested from late May to early June, 1998 from native mixed grass prairie at Scotts Bluff National Monument (SB) and Wildcat Hills State Recreational Area (WH) in Scotts Bluff County, Nebraska. Achenes were collected by hand stripping with a modified laundry detergent container with comb-like teeth cut out on one side to facilitate "raking" the plants. New achenes were collected from the same locations in 1999, and the experiment was repeated.

Achene appearance and size were generally uniform throughout the collection areas. Achene maturity was a few days later, and, on average, more achenes were produced per inflorescence at WH compared to SB. Achenes were air dried in paper bags at room temperature and stored at 5°C in sealed plastic containers. After storage for 6 months, achenes were separated from the inflorescences on a rub board and placed in an air column to remove debris and empty achenes. Damaged, immature, or smut-infected achenes were removed by hand and discarded. The perigynia surrounding the achenes were not removed. Mean weights of lots of 100 seeds from each location and year were determined.

As a precaution to prevent mold, achenes were surface sterilized with a 5% v:v sodium hypochlorite (household bleach) solution in distilled water for 5 min with agitation. The achenes were placed on either blotter paper or unpasteurized washed river sand in 9-cm plastic petri dishes, with 50 achenes per petri dish, and randomly assigned to treatments. Achenes were not touching one another. Stacks of three petri dishes were sealed inside plastic bags to reduce possible sources of fungal contamination and to retain moisture. Treatments included nine pretreatment temperatures, two achene sources (SB or WH), and two germination substrates (blotter paper or sand) (Table 1). Within each substrate type, wet and dry conditions were compared. Each of the nine pretreatments differed; some were exposed to warm temperature, others to cold temperature, and others to a combination of warm followed by cold pretreatment. Germination of one set of control achenes was initiated at the beginning of the second 8 wk of the study. Germination of the second set of control achenes was initiated at the beginning of the third 8 wk of the study. Two starting periods were used for the control treatment to ensure that there was no difference in total germination from the effects of afterripening or aging during an 8-wk period.

Preparation of petri dishes began by filling each to a depth of 0.5 cm with washed river sand or with two layers of blotter paper. Moist pretreatment dishes were wetted with 10 ml distilled water (pH 5), and excess water was drained from each dish. Dry pretreatment dishes did not receive water. After pretreatment, distilled water was added to all dishes for the germination tests.

Each phase of pretreatment continued for 8 wk. For example, in pretreatment 1, the achenes were placed in a warm-wet pretreatment for 8 wk, followed by a cold-wet pretreatment for 8 wk, and then placed in the germination test during the final 8 wk (Table 1). The temperature and light environments were (1) warm pretreatment with alternating 35/25°C temperatures with 8 hr of light during the high temperature, and (2) cold pretreatment with alternating 10/5°C temperatures with 8 hr of light during the high temperature. Pretreatment temperatures were selected to approximate spring and late summer air temperatures in western Nebraska.

The germination portion of the test was conducted with alternating 25/15°C with 8 hr of light during the high temperature. A cool-white 110-watt fluorescent tube provided light inside the germinator. Light and alternating temperatures are commonly required for germination of sedges (Hurd and Shaw 1992, Thompson and Grime 1983). While there are no records of temperatures required for threadleaf sedge in nature,

Table 1. Schedule of threadleaf sedge pretreatments showing temperature, light, and length of treatment on both paper and sand media. Moisture regimes are designated by wet and dry. All germination tests were at 25/15° C in 8 hr light.

Pretreatment	Warm 35/25° C 8 hr light	Cold 10/5° C 8 hr light
warm-wet+cold-wet	wet	wet
warm-dry+cold-dry	dry	dry
warm-dry+cold-wet	dry	wet
warm-wet	wet	
warm-dry	dry	
cold-wet		wet
cold-dry		dry
no stratification (2nd 8-wk. period)		
no stratification (3rd 8-wk. period)		

temperature levels selected for the germinator were based on local seasonal observations for western Nebraska. Petri dishes were checked for germination every 8 da, at which time additional distilled water was added, if necessary, and germinated achenes were counted and removed. An achene was considered germinated following emergence of the radicle and coleoptile, which usually occurred simultaneously.

Petri dishes were arranged on the shelves of the germinator in a completely randomized design. Each stack of three randomized petri dishes equaled one experimental unit. There were three replications of 50 achenes in each treatment. The treatment design was a $9 \times 2 \times 2$ factorial arrangement, which allowed testing for interactions as well as for individual treatment effects (Dowdy and Wearden 1991). Percent germination values were arcsin transformed for analysis, and back-transformed for presentation in tables. Data were analyzed using a general linear model procedure (PROC GLM), with the Fisher's protected LSD mean separation test ($p < 0.05$) (SAS 1990). The 3 independent variables incorporated in this study were pretreatment, substrate type, and collection location. Germination was the response or dependent variable. Differences in means were considered significant at the 5% level.

RESULTS AND DISCUSSION

Germination

Mean weights for lots of 100 achenes (0.25 g ± 0.002) were the same for each seed source and collection date. Germination levels from the control treatments, initiated in both the second and third 8 wk of the study, were not significantly different from one another, except for SB achenes on paper in 1998 (Table 2). Since this discrepancy for SB achenes was unique and no other control values varied so substantially, the high value for percent germination of control achenes was removed from the analysis. It was likely caused by error in recording or calculation. Since no statistical difference occurred between the two control treatments, their values were averaged and compared to the germination results following the pretreatments. An additional 8 wk of cold storage at 5°C did not influence the germination of achenes in the second control group.

Germination from each year was analyzed separately because of environmental variation between years. A preliminary analysis to determine variation in germination between 1998 and 1999 showed that germination of 1998 achenes from both sources was higher ($p < 0.05$) than germination of 1999 achenes. Because laboratory germination procedures were uniform each year, it is probable that germination differences observed between years were related to different environmental conditions acting on the parent plants during achene development and maturation. At the time of achene collection, daily spring temperatures and seasonal precipitation were higher in 1999 than in 1998 (Tichota 2000). Soil moisture and precipitation can influence seed quality (McDonald and Copelend 1997). In addition, collection location may affect the quality of seeds. Aspect is a characteristic of collection sites that was not held constant between years or sources, and it is a factor that could have influenced germination. For example, Wagner and Reichegger (1997) found that achenes of other species of *Carex* collected from sunlit southern slopes with little snow cover exhibited greater laboratory germination than achenes from northern slopes. Mean germination of WH achenes was similar in 1998 and 1999, although it averaged higher at SB (48%) than at WH (35%) in 1998. Variation in germination between two different achene sources can be related to factors such as stage of maturity or a difference in environmental conditions during the year of collection (Weisner et al. 1967). In 1999, achenes from SB and WH had similar germination rates of 41% and 42%, respectively.

Pretreatment

Percent germination for achenes collected in both 1998 and 1999 showed a similar pattern with significant ($p > 0.05$) pretreatment × substrate interactions, although the specific values for each year differ (Table 2). Comparisons of the germination response of a species can be confounded by differences in dormancy from one year to the next (Schutz and Rave 1999). These differences may be related to changes in environmental conditions acting on the plants during the seed development period (Gutterman 1993, Schutz and Rave 1999). Environmental conditions at SB and WH were different in 1998 and 1999 and may explain the different values obtained. The warm-wet plus cold-wet pretreatment of

Table 2. Mean germination percentage of threadleaf sedge from Scotts Bluff National Monument (SB) and Wildcat Hills (WH) on paper or sand medium as influenced by nine different pretreatments in 1998 and 1999.

	1998				1999			
	Scotts Bluff		Wildcat Hills		Scotts Bluff		Wildcat Hills	
	Paper	Sand	Paper	Sand	Paper	Sand	Paper	Sand
Pretreatment	%				%			
1) warm-wet+cold-wet	32	37	21	42	24	37	30	41
2) warm-dry+cold-dry	55	55	43	37	27	25	38	31
3) warm-dry+cold-wet	47	61	44	45	35	39	45	42
4) warm-wet	52	60	39	50	33	47	35	37
5) warm-dry	65	67	33	31	41	47	33	43
6) cold-wet	45	53	27	29	14	21	15	18
7) cold-dry	51	46	27	36	26	36	23	39
8) no stratification[1]	41	47	24	35	33	29	27	33
9) no stratification[2]	64	40	25	34	29	26	29	23
Least significant difference (LSD 0.05)	11	12	11	9	13	11	7	19

[1]Germination test conducted during second 8 wk of study
[2]Germination test conducted during third 8 wk of study

achenes failed to produce higher germination than the control for SB or WH achenes on either paper or sand in 1998 and 1999 (Table 2). This pretreatment tended to produce the lowest mean percent germination. One explanation is that subjecting achenes to constant moisture for 24 wk was not an accurate representation of the natural environment where alternate dry and wet periods would have occurred.

For warm-dry plus cold-dry pretreatment, WH achenes on paper and SB achenes on sand produced greater germination than the control treatment in both 1998 and 1999 (Table 2). All other WH achenes and SB achenes (on both sand and paper for both years) subjected to this pretreatment failed to produce higher germination than the control. In both years, the SB achenes had lower germination following the warm-dry plus cold-dry pretreatment than following the warm-dry pretreatment alone, suggesting that the cold-dry period may have been responsible for inducing a secondary dormancy.

The warm-dry plus cold-wet pretreatment produced higher germination of SB achenes on sand and WH achenes on sand and paper in 1998 and 1999 compared to the control. No significant difference occurred in germination between the warm-dry plus cold-dry pretreatment and the warm-dry plus cold-wet pretreatment for achene sources, substrate types, or years, except for 1999 SB achenes on sand and 1999 WH achenes on paper. In these two examples, the warm-dry plus cold-wet percent germination was greater than the warm-dry plus cold-dry percent germination. They each had an 8-week warm-dry period in common, but this period was followed by either 8 weeks of cold-wet or 8 weeks of cold-dry pretreatment. The results suggest that the moisture in the cold-wet period increased germination while the cold-dry period inhibited germination.

The warm-wet pretreatment produced greater germination of SB achenes on sand and WH achenes on sand or paper than the control in 1998. In 1999, the SB achenes on sand and WH achenes on paper had greater percent germination when compared to the control. The success of this treatment suggests that if achenes fell from the parent plant in spring and were exposed to warm, moist summer temperatures, they could germinate during the cooler temperatures of fall if sufficient moisture were present.

The warm-dry pretreatment produced higher germination for SB achenes on sand or paper in 1998 and SB achenes on sand in 1999. No germination differences were detected between the warm-wet and the warm-dry pretreatments in 1998 or in 1999, except between achenes from SB on paper in 1998. Here, the warm-dry pretreatment resulted in greater germination than from the warm-wet pretreatment. This comparison would suggest that the effects of warm pretreatment may not be influenced by the addition of moisture. These results suggest achenes that fall from the parent plant in spring and remain on the soil during warm, dry summer conditions, may germinate if cool, moist conditions occur during the fall season. The warm-dry period may be necessary for afterripening.

The cold-wet pretreatment did not produce different total germination in 1998 for SB or WH achenes on sand or paper when compared to the control. In 1999, however, SB and WH achenes on paper had lower germination than the control, although those on sand did not. When compared to the warm-wet pretreatment, the cold-wet pretreatment produced lower germination in SB and WH for both substrate types and both years, with the exception of SB achenes on sand or paper in 1998. This suggests that the warm temperature is at least partially responsible for higher germination. Thus, achenes exposed to cool, wet spring and summer conditions would most likely not germinate as well as those undergoing exposure to warmer conditions.

The cold-dry pretreatment did not produce different germination in either year, location, or substrate type (Table 2). All results were statistically similar to the control treatment. Further, there was no significant difference between the cold-wet and cold-dry pretreatments in 1998. In 1999, however, the cold-dry pretreatment had higher germination than the cold-wet pretreatment for WH achenes on sand (39% and 18%, respectively) and paper (23% and 15%, respectively) and for SB achenes on sand (36% and 21%, respectively). This suggests that in certain years, addition of moisture to the cold pretreatment may increase germination, though it is not certain what environmental characteristics may be directly influencing this requirement.

Germination substrate

Sand substrate produced higher germination than paper substrate, except in the warm-dry plus cold-dry and control treatments. In contrast, Capon and VanAsdall (1966) did not observe a difference in seed germination of desert annuals on sand versus blotter paper. Threadleaf sedge is not a desert annual, it is a dryland species that is found in a semiarid environment. Threadleaf sedge achenes are found in an environment where summers are relatively hot and dry and the substrate is sandy to loamy. The washed river sand that was used in this germination study more closely resembled the substrate found in the natural environment of threadleaf sedge.

General observations

About 60% of the ungerminated achenes were firm and undamaged in appearance. Several types of fungi were observed on the achenes during the treatments, but there did not appear to be a difference in germination between infected or non-infected achenes. Most of the achenes were infected with at least one type of fungus. Similar to the findings of Kirkpatrick and Bazzaz (1979), the presence of the fungi on infected achenes was lower during the final 8-wk germination test at 25/15° C. Fungal growth was also less after seedling emergence, likely because of a decrease in density of viable spores or availability of an adequate nutrient source.

CONCLUSIONS

Germination of threadleaf sedge was highest with warm pretreatment and sand substrate. Lowest germination occurred with cold pretreatment and alternating warm-wet and cold-wet pretreatment. These results indicate warm pretreatment could be a useful tool in preparing threadleaf sedge achenes for germination in prairie restorations. It is most likely a combination of factors that led to the relatively high germination of achenes following warm pretreatment, but this period may have allowed or stimulated the process of afterripening. The use of heat as a pretreatment is similar to the exposure of achenes to the warm summer temperatures in their natural environment. Based on these results, germination would most likely follow during cool, moist conditions in the fall. If the achenes failed to germinate in fall, the cold temperatures of winter may induce a secondary dormancy in some achenes.

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