

Factors affecting germination of endangered northeastern bulrush, *Scirpus ancistrochaetus* Schuyler (Cyperaceae)

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Summary

Knowledge of seed germination requirements is important for conservation and propagation of endangered plant species. In a laboratory study, conditions to maximize germination percentage of seeds of endangered northeastern bulrush, *Scirpus ancistrochaetus* Schuyler, were investigated. Treatments included length of dry storage, length and temperature of cold stratification, seed origin, and addition of gibberellic acid (GA). Germination of seeds subjected to two years of dry storage was higher than germination of freshly collected seeds. Germination of seeds stratified at -3 , 3 or 8°C increased significantly with each increase in treatment temperature. Seeds stratified for eight or 12 weeks had higher germination than seeds stratified for four weeks. However, several interactions between factors were also important. Taking all significant interactions into account, the highest germination of seeds was obtained for seeds stratified at 3 or 8°C for eight or 12 weeks. Addition of GA enhanced germination for freshly collected seeds, yet did not affect germination of seeds dry-stored for two years. We recommend that fresh seeds should first be subjected to a dry storage period or addition of GA, followed by stratification for eight or 12 weeks at 3 or 8°C to obtain maximum germination of this species.

Introduction

A variety of factors affect the germination of seeds and optimal germination conditions are often species-specific. Many seeds require a period of “after-ripening,” after which germination is able to occur or is much enhanced (Simpson, 1990). Seeds of many species after-ripen during storage in moist conditions at low temperatures, a treatment known as “stratification” (Mayer and Poljakoff-Mayber, 1989). Both the temperature and length of stratification can be important factors in the germination of wetland plant species. For example, seeds of *Scirpus robustus* exhibited the highest germination following stratification at 2°C , while freezing at -7°C resulted in the lowest germination (Dietert and Shontz, 1978). Conversely, seeds of *Scirpus atrovirens* had a higher percent germination after being frozen outdoors when compared to seeds kept at $2-4^{\circ}\text{C}$ in the lab (Isely, 1944). Seeds of *Scirpus acutus* had higher germination success when

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stratified for eight or 12 weeks compared to four weeks (Thullen and Eberts, 1995). Gibberellic acid (GA) can also promote seed germination, even in the absence of a stratification period (Deno, 1993; Mayer and Poljakoff-Mayber, 1989).

Another method of conditioning seeds of many species for germination is achieved through a period of dry storage (Larson and Stearns, 1990; Simpson, 1990; Bradbeer, 1988; Mayer and Poljakoff-Mayber, 1989). For example, dry storage increased germination in seeds of *S. robustus* while freshly collected seeds did not germinate (Dietert and Shontz, 1978). In contrast, drying of seeds of either *Scirpus americanus* or *Scirpus validus* did not enhance germination (Muenscher, 1936; Harris and Marshall, 1960; Maguire and Heuterman, 1978).

Northeastern bulrush, *Scirpus ancistrochaetus* Schuyler, is an emergent wetland sedge, typically found in small depressional palustrine seasonal wetlands. It ranges from West Virginia to Vermont, but is limited to approximately 60 extant populations, most of which are in Pennsylvania. It is currently listed as "endangered" by the U. S. Fish and Wildlife Service (1991). Very little information is available on this rare species which was described by Schuyler (1962). A previous study of germination yielded low values of approximately 10–20% using a method of cold stratification at $\approx 2^{\circ}\text{C}$ in soil for 12 weeks (W. Brumback, personal communication).

Within a species, cues for germination may not be exactly the same for all individuals, especially between populations. Unstratified seeds of *S. acutus* from two separate populations subjected to different climates showed small differences in germination, suggesting ecotypic variation in germination requirements (Thullen and Eberts, 1995). Similarly, local adaptation to slightly different habitats in isolated populations of *S. ancistrochaetus* may have caused some ecotypic differences in optimal conditions for germination. Seeds of *S. ancistrochaetus* from two separate and distinct sites were used in the following study to determine if possible variation between sites in germination requirements exist.

Information on germination is critical for nursery propagation and conservation of *S. ancistrochaetus* and can be used towards understanding germination processes under natural conditions. In order to understand the factors that may control seed germination in *S. ancistrochaetus*, two fully factorial germination experiments were conducted. One experiment combined the factors of length of dry storage, length and temperature of stratification, and seed origin, and the other investigated the factors of GA addition, length of dry storage and seed origin.

Materials and methods

Seeds (achenes) were haphazardly collected from about 50 plants in each of two separate, large ($> 50 \text{ m}^2$) natural populations of *S. ancistrochaetus* located approximately 72 km apart, one in Huntingdon County (HC) and the other in Centre County (CC), Pennsylvania. Seeds were collected from HC in October 1994 and 1996 and from CC in October 1994 and November 1996. Seeds were cleaned, placed in small paper envelopes and stored at room temperature until the start of the experiment.

Experiment 1 – Effect of site, length of dry storage, temperature and length of stratification

On 14 November 1996, seeds were placed inside folded paper towels moistened with distilled water which were placed in plastic bags following procedures outlined by Deno (1993). The seed packets were placed in a dark incubator set at -3°C , 3°C or 8°C . Seeds were removed from the incubators after four, eight or 12 weeks of stratification. Three replicates of 20 seeds each for each treatment combination of site, length of dry storage, temperature and length of stratification were used. In addition, another group of seeds that had not been subjected to cold stratification was immediately placed under the lights and tested for germination. When seeds were removed from the incubators after stratification, they were placed exposed on new paper towels moistened with distilled water and placed in new plastic bags. Seeds were placed under fluorescent light on a 12 h light/dark cycle in a growth chamber set at 23°C . The conditions for germination were chosen based on earlier successes in preliminary germination studies under similar conditions. The light intensity was $250 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when measured with a quantum radiometer/photometer (Li-Cor Model LI-185B, Lincoln, NB). Seeds were checked daily for germination, which was indicated by coleoptile emergence. Seeds were removed from the paper towels as they germinated and distilled water was added to the paper towels as needed throughout the experiment.

Experiment 2 – Effect of gibberellic acid, length of dry storage and site

The effect of GA on germination was investigated three months after the start of the first experiment, using a subset of untreated seeds from the previous experiment. Addition of GA alone does not significantly enhance germination in *S. ancistrochaetus*, so GA was added in conjunction with a cold stratification period in this study (Lentz, unpublished data). Seeds were placed on paper towels moistened with distilled water or with a 500 ppm aqueous solution of GA (Carolina Biological Supply Company, Burlington, NC), and placed in a 4°C incubator for stratification. Three replicates of 10 seeds each were used in each treatment combination of site, length of dry storage, and presence/absence of GA. Seeds were removed from cold stratification after four weeks, placed on paper towels moistened with distilled water, placed under lights at 23°C as in above experiment and examined for germination.

At the conclusion of both experiments, seeds were visually assessed for viability. Non-viable seeds are easily identified by the transparency of the seed. Final percent germination was calculated based on the percentage of viable seeds germinated. A four-way Analysis of Variance (ANOVA) with fixed factors was used to examine the effect of site, length of dry storage, temperature and length of stratification on total percent germination (SAS Institute, Inc., 1989). A two-way ANOVA with fixed factors of site and length of dry storage was used for the unstratified seeds. For the second experiment, a three-way ANOVA with fixed factors was conducted to examine the effect of site, length of dry storage and presence of GA. Data were arcsine transformed to meet model assumptions of normality and equality of error variance. When significance was found in a main factor, Tukey's test was used to determine significant differences be-

tween means. Significant interactions were interpreted by graphing the data. The alpha level used for all tests was 0.05.

Results

Experiment 1 – Effect of site, length of dry storage, temperature and length of stratification

No significant difference in seed germination between sites was found (Figure 1). Seeds subjected to about two years of dry storage had significantly higher germination than seeds subjected to < one month of dry storage. Seeds stratified at 8°C had significantly higher germination than seeds stratified at 3°C, which in turn had significantly higher germination than seeds stratified at -3°C. Seeds stratified for four weeks had significantly lower germination than seeds stratified for eight or 12 weeks. Several two-way interactions were significant (Figure 2). Seeds from HC had a greater increase in germination from the < one month dry-stored to the two years dry-stored seeds than those from CC (Figure 2A). Seeds subjected to two years dry storage had a constant germination over all three lengths of stratification, while germination of seeds dry stored < one month increased sharply from four to eight weeks of stratification, then remained constant (Figure 2B). The interaction of site by temperature was also significant; in general, germination of seeds from both sites increased with increasing temperature, although seeds collected from CC responded more favorably to stratification at 8°C (Figure 2C). In general, germination increased steadily with temperature for seeds stratified for four weeks, while germination of seeds stratified for eight and 12 weeks reached a plateau at 3°C (Figure 2D). Two three-way interactions were significant (Figure 3). Germination of seeds strati-

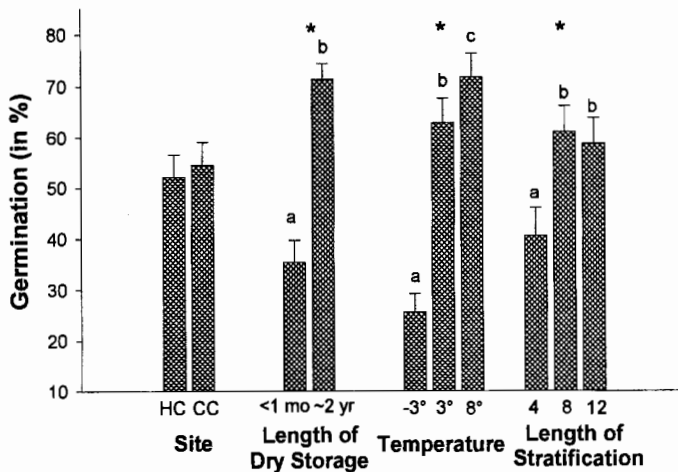


Figure 1. Average percentage germination \pm SE for main factors. Asterisks indicate significant differences at $p = 0.0001$. Within each main effect, means with the same letter are not significantly different from each other. HC = Huntingdon County, CC = Centre County.

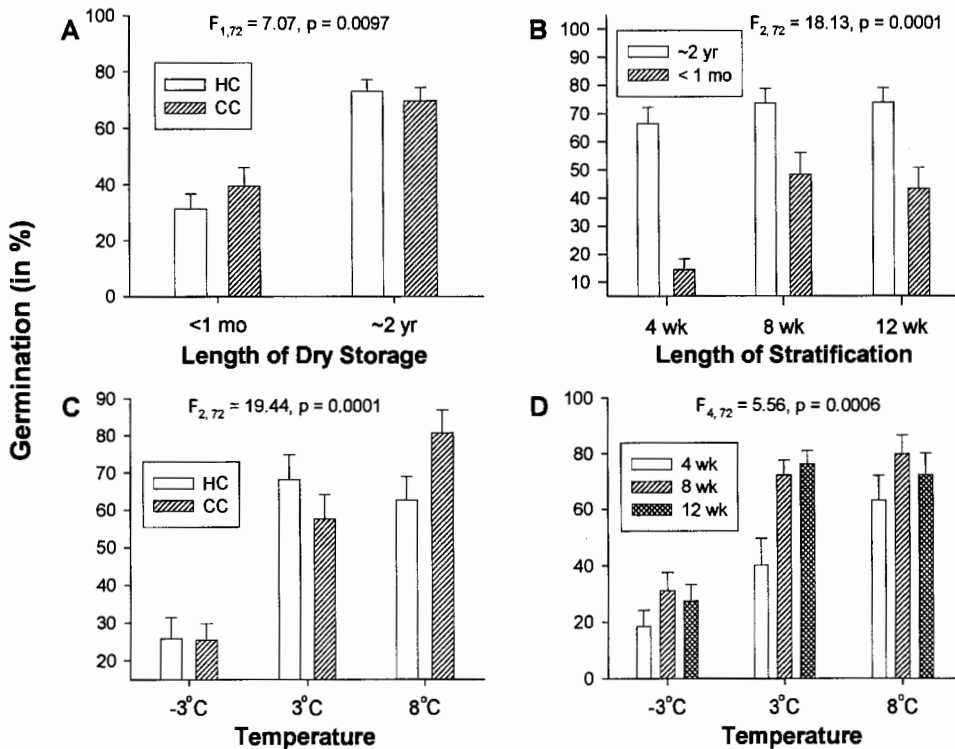


Figure 2. Significant two-way interactions (average percentage germination \pm SE): (A) site \times length of dry storage; (B) length of dry storage \times length of stratification; (C) site \times temperature; (D) length of stratification \times temperature. HC = Huntingdon County, CC = Centre County.

fied for eight or 12 weeks was consistently high over all combinations of site and length of dry storage (Figure 3A). Seeds from HC germinated best after a stratification period of eight or 12 weeks at 3°C, while seeds from CC germinated best after a stratification period of eight or 12 weeks at 8°C (Figure 3B). Dry storage had a significant effect on germination of seeds that were not subjected to cold stratification ($F_{1,8} = 36.68, p = 0.000$). Seeds dry-stored < one month had a much lower percentage germination than seeds dry-stored for two years ($1.7 \pm 1.05\%$ and $26.1 \pm 6.29\%$, respectively, mean \pm SE).

Experiment 2 – Effect of gibberellic acid, length of dry storage and site

Germination of seeds from HC ($56.7 \pm 5.30\%$) was significantly higher than that of seeds from CC ($40.59 \pm 5.66\%$, $F_{1,16} = 7.29, p = 0.016$). Germination of seeds stored dry for > two years ($56.92 \pm 6.51\%$) was significantly higher than that of seeds stored dry for four months ($40.38 \pm 4.14\%$, $F_{1,16} = 6.99, p = 0.018$). Addition of gibberellic acid (GA) had no overall effect on germination, yet the interaction of length of dry storage and addition of GA on germination was significant. Germination of seeds dry-stored for

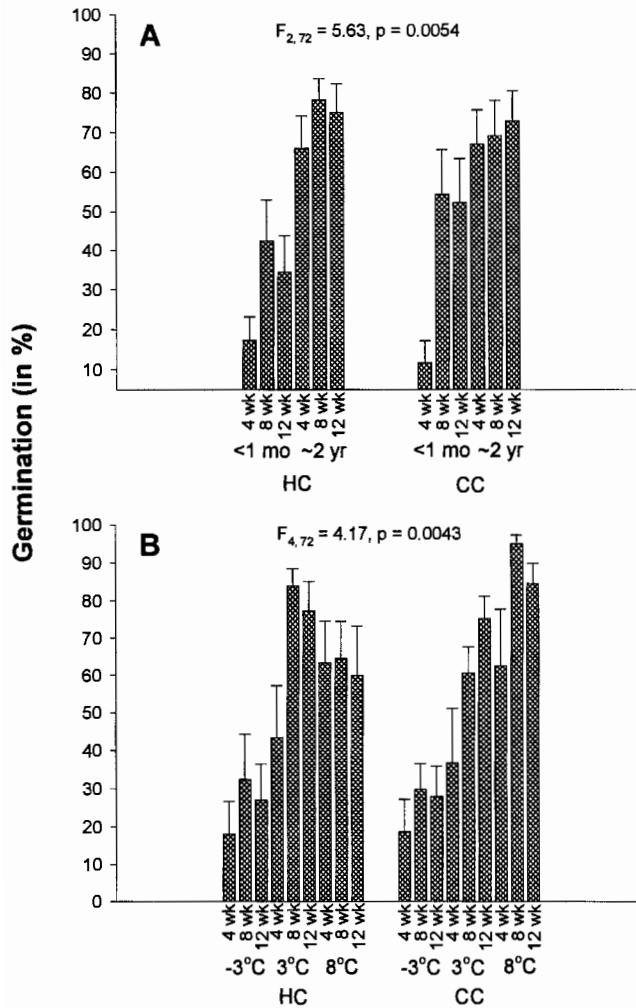


Figure 3. Significant three-way interactions (average percentage germination \pm SE): (A) site \times temperature \times length of stratification; (B) site \times length of dry storage \times length of stratification. HC = Huntingdon County, CC = Centre County.

> two years was unaffected by the addition of GA, while germination of seeds dry-stored for four months increased with the addition of GA (Figure 4A). The interaction of site and length of dry storage was also significant. Germination of seeds collected from HC and dry-stored for four months was lower compared to seeds dry-stored for > two years while germination of seeds collected from CC from both dry storage lengths was similar (Figure 4B).

Discussion

In this study, seeds subjected to dry storage for two years had a higher percent germina-

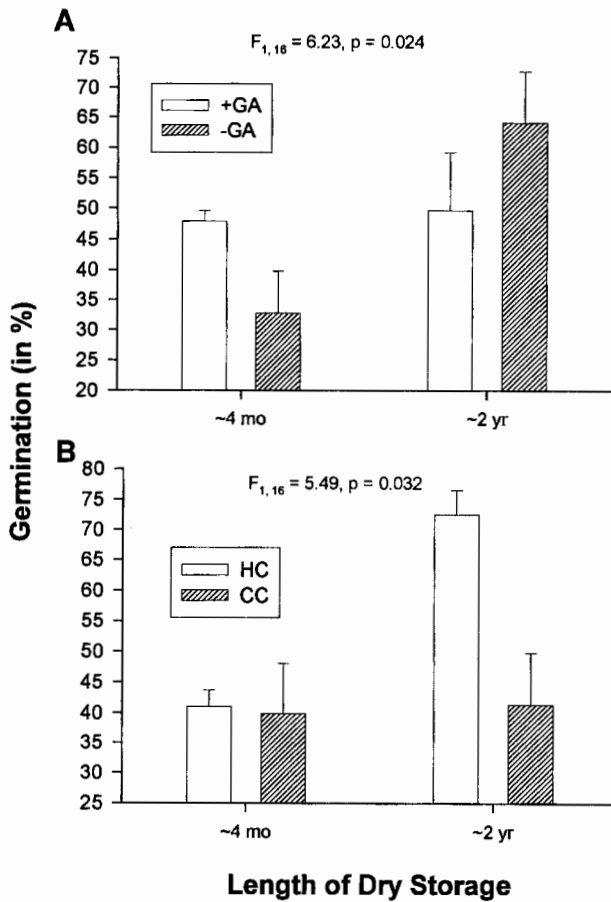


Figure 4. Significant two-way interactions for gibberellic acid (GA) experiment (average percentage germination \pm SE): (A) addition of GA \times length of dry storage; (B) site \times length of dry storage. HC = Huntingdon County, CC = Centre County.

tion than seeds subjected to dry storage for one to four months. In fact, seeds stored dry for two years were able to germinate (26%) without any cold stratification. A period of dry storage thus is an important controlling factor of germination in *S. ancistrochaetus*, which may seem surprising for a wetland species. However, previous studies have found that a period of seed drying may be an important factor in enhancing germination of certain wetland plant species (Larson and Stearns, 1990; Baskin and Baskin, 1982; Dietert and Shontz, 1978; Maguire and Heuterman, 1978). Ponds that support *S. ancistrochaetus* are usually temporary and are subjected to periods of drawdown; therefore, seed drying under natural circumstances is a possibility. Many interactions between factors were also important and must be taken into consideration when attempting to recommend the best germination procedure. Seeds stratified at 3 or 8°C for eight or 12 weeks had the highest germination, taking the factors of length of dry storage and site into account. The interactions involving seed origin, length of dry storage and

length of stratification were also significant. Seeds stratified for eight or 12 weeks had the highest germination over all combinations of site and length of dry storage.

Addition of GA had an effect on germination only through the interaction with length of dry storage. GA addition enhanced germination in seeds dry-stored for four months, while percentage germination was unaffected by GA addition in seeds dry-stored > two years. Germination studies using excised *Avena fatua* embryos produced a similar result; although GA did not affect germination of embryos from seeds after-ripened by drying, it did enhance germination of dormant embryos (Myers, Foley and Nichols, 1997). Our results with *S. ancistrochaetus* provide further evidence that GA can enhance the germination of dormant seeds (Simpson, 1990; Mayer and Poljakoff-Mayber, 1989).

Results concerning ecotypic differences in germination cues between seeds from the two sites used in this study are equivocal. While no overall difference in seed response between sites was observed in the first study, some differences in seed germination between sites were evident in the second study. Furthermore, in both studies, site was a significant factor in interaction with other factors, suggesting that sites do respond differently to different conditions to enhance germination. Since the effects of age/developmental stage of seed and seed origin could not be separated in this study, making any conclusions about ecotypic differences is difficult. The two sites from where seeds were collected were most likely subjected to different climatic conditions due to the distance between them, and therefore seeds from each site may have been at different developmental stages when collected. Plants from the southern site (HC) did flower and set seed earlier than plants from the northern site (CC), further suggesting that the maturity of seeds may have been different when gathered (Lentz, personal observation). Attempts were made to correct for this difference by collecting seeds from the two sites a month apart in 1996, but no examination of the embryo or seed to determine age was made. Clearly, more controlled studies must be conducted in the future to separate possible ecotypic differences in germination requirements from differences in germination requirements due to seed maturity.

The interaction of length of dry storage and site was significant for both experiments. However, the results are contradictory (Figure 2A vs. Figure 4B). The low percent germination for seeds collected from CC and subjected to > two years of dry storage (Figure 4B) may be due to the three month difference between the first and second experiment. It is possible that the extension of dry storage time from the first to the second experiment may have been detrimental to seed from CC only, as prolonged dry storage can reduce seed viability (Deno, 1993; Larson and Stearns, 1990). This possible reduction in viability of seeds dry-stored > 2 years from CC, without a similar reduction in similar seeds from HC, can also explain the differences in results from the first to second experiment concerning the main effect of site.

By combining results from both studies, we recommend that, for the highest germination in the shortest amount of time, freshly collected seeds with addition of GA or air-dried seeds should be used, followed by a cold stratification period of eight weeks at 3 or 8°C. A stratification period of 12 weeks would give similar results, but the time re-

quirement is obviously longer. Future studies are needed to fully explain factors that control germination success in the field and further define the conditions required for maximal germination success. However, we have shown that high germination success of this species can be attained under laboratory conditions using very simple techniques. Germination under optimal conditions averaged approximately 80%, which greatly improves the percentages previously attained in this species and significantly contributes to efforts towards its conservation.

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