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Seed Age, Seed Treatment, and Growing Environment Effects on Two *Scirpus* Species

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ABSTRACT
A seed lot of woolgrass collected in 1992 and exposed to 43 months of cool-dry storage showed increased germination compared to another lot collected in 1995 that was stored in comparable conditions for just over five months. This would indicate that there appears to be some beneficial physiological changes that occur during storage. Four months of cold stratification increased germination of the older seed lot, but had no effect on the newly collected seed. Environmental conditions also affected woolgrass seed germination, with better germination shown on a flood bench where the growing media was constantly saturated compared to somewhat drier conditions on a normal greenhouse bench. Two lots of soft-stem bulrush seed collected in the same years and subjected to the same seed treatments and growing environments both responded to stratification. However, 45 months of cool-dry storage appeared to negatively impact germination of this species. There was a trend towards improved soft-stem bulrush germination on the flood bench compared to the drier conditions on the normal greenhouse bench.

INTRODUCTION
It has been reported that seed of wetland plants lose viability when allowed to dry after harvest and should be stored in water. Muenscher (1936) recommended storing wetland seed in water at 1-3°C, however, germination percentages for the three *Scirpus* species included in that test were very low. From a practical standpoint, it is difficult to store seed in water for a period of many years. Unless the storage containers are sealed to prevent moisture loss, water levels will require monitoring; however, such sealing would expose the seed to prolonged periods of anoxia. Some seeds also tend to germinate during water storage, even when stored in darkness. Harris and Marshall (1960) obtained about 50% germination from two *Scirpus* species stored outdoors for 7-9 months in spun glass bags under water of a natural wetland in northwestern Minnesota. Such storage would not be practical in the southern US.

Isley (1944) found that seed of many *Scirpus* species, collected mainly in New York, did not lose viability during dry storage, but were in a state of dormancy. Dry storage did not provide the appropriate environmental conditions for after-ripening to occur, but when dry stored seed was placed in low temperature storage in water or a moist medium (stratification), such as peat moss, sphagnum, or sand, this dormancy was overcome. Which stratification medium was used had little effect on germination of most species when the proper environmental conditions, including continuous light and a temperature of 30-32°C, were provided. Under these germination conditions, he also obtained a high germination

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percentage for some *Scirpus* species when seeds were stored in water at room temperature, which conflicts with the results reported by Muenscher (1936). He stated that many *Scirpus* species have a fairly long period of viability, and that those species with larger, heavy-walled achenes are likely to retain their viability longer than smaller-seeded ones. Garbish and McIninch (1992) reported from their personal experience that seed of various *Scirpus* species can be stored dry for periods greater than two years.

Wetland creation and restoration activities have resulted in a demand for wetland plants. More information is needed on propagation and seed storage requirements for wetland species adapted to the southeastern United States. A test was conducted at the Jamie L. Whitten Plant Materials Center, Coffeeville, Mississippi to determine 1) the effect of three to four years of cool-dry storage on Mississippi ecotypes of woolgrass, *Scirpus cyperinus* (L.) Kunth (Accession Number 9062741, released as Leaf River Source woolgrass), and soft-stem bulrush, *S. tabernaemontani* K.C. Gmel. (Synonym = *S. validus* Vahl) (Accession Number 9062740), 2) the effect of a four month seed stratification period on these species, and 3) the effect of two different growing environments, saturated conditions on a flood bench and drier conditions on a normal greenhouse bench, on germination of this seed.

MATERIALS AND METHODS

Seed lots used were collected in 1992 and 1995. Collection dates in 1992 were not recorded, but soft-stem bulrush was collected in June and woolgrass in August. In 1995, woolgrass seed was collected on September 29 and soft-stem bulrush on October 12. Seed was collected by hand pulling or cutting fruiting clusters from plants. These clusters were then placed in cloth bags and stored in a seed vault. Seed vault conditions were 55°F and 45% relative humidity, however, during the storage period there were short periods of time when the cooler malfunctioned and storage conditions varied from those listed above. The exact timing and duration of these periods was not recorded.

Although the seed of *Scirpus* species are actually contained in a dry, indehiscent fruit called an achene, in the discussion below, the entire structure will be referred to as a seed. The seed were freed from the fruit clusters using a brush machine (Westrup a/s, Slagelse, Denmark) and then hand screened to remove inert matter. On November 7, 1995, a sample of each seed lot was stratified by being placed on a brown paper towel which was moistened with tap water, placed in a self-sealing plastic bag with a sufficient quantity of additional water to ensure the paper would stay wet, and stored in a cooler without humidity control at 42°F. A paper towel was used as the stratification medium because it simplified later counting of seed samples for treatments, especially woolgrass, which has minute seed. Remaining quantities of seed were returned to dry storage conditions listed above. Therefore, total length of exposure to dry storage, in months, was 45, 41, 43, 39, 5, 1, 5+, and 1+ for 1992 non-stratified bulrush, 1992 stratified bulrush, 1992 non-stratified woolgrass, 1992 stratified woolgrass, 1995 non-stratified bulrush, 1995 stratified bulrush, 1995 non-stratified woolgrass, and 1995 stratified woolgrass, respectively. For ease of presentation, these lots will be referred to by their year of collection rather than months of dry storage in the discussion below.

Woolgrass seed treatments were sown on March 18 and 19, 1996 and soft-stem bulrush treatments on March 19 and 20, 1996. Lot size used was 100 seed per sample, except for the 1995 lot of soft-stem bulrush seed, where lot size was 50 seed used due to limited seed quantities. Experimental design was a factorial experiment in a randomized complete block with three replications. Stratified seed was surface dried to facilitate counting, but was not allowed to dry completely. Isley (1944) and Harris and Marshall (1960) found that germination of *Scirpus* seed was not greatly reduced when seed was allowed to dry for 14 days to one month following the stratification period. Appararently, these seed are not subject to secondary dormancy, so it is unlikely germination in this study would be affected by exposure to this short period of drying. Germination containers were 7" x 5-1/4" x 2-5/16" black plastic bedding plant liners. Growing media was a 3:1 mix of peat moss/sand amended with commercially recommended quantities of Osmocote 13-13-13 fertilizer, dolomitic lime, Micromax micronutrient fertilizer, and Aquagro wetting agent. The sand was not pasteurized before use, which did cause some minor weed problems during the experiment. Seed was spread as uniformly as possible on the surface of the germination medium and all containers were hand watered to ensure good seed contact with the medium. Germination conditions tested were a normal greenhouse bench where the seed flats were watered regularly to maintain moist conditions, and continuously saturated conditions on an ebb and flow greenhouse bench where water was maintained at ½ inch depths, except for short periods of time when the water was drained and the bench rinsed to remove algae. Seedling counts began when it was deemed that a sufficient number of seedlings were present, which for woolgrass was April 9, 1996 (22-23 days after sowing) and for soft-stem bulrush was April 16, 1996 (28-29 days after sowing). Two additional counts were made at three week intervals after the initial count. Some woolgrass seedlings, especially on the normal greenhouse bench, died prior to the initial count, but were counted and included in the germination percentage because germination had occurred. Later counts included only plants that were green and could be considered alive at the evaluation date. The data was subjected to an analysis of variance and appropriate mean separation was determined by using a Tukey's honestly significant difference test (HSD) at the 5 percent level of probability.

RESULTS AND DISCUSSION

Woolgrass: Woolgrass seed germination percentages for three evaluation dates are listed in Table 1. Because the seedling counts decreased markedly after the initial count, mean separation was performed only on that count.

Table 1. Mean germination percentage of two ages of woolgrass seed previously exposed to varying lengths of dry storage subjected to two seed treatments and two germination environments.

Seed Age	Treatment	Environment	First Count	Second Count	Third Count
			-----------------------------%-----------------------------------		
1992	Stratified	Normal Bench	49	1	1

		Flood Bench	87	53	11
	Non-Stratified	Normal Bench	22	3	2
		Flood Bench	70	63	7
1995	Stratified	Normal Bench	19	1	1
		Flood Bench	35	21	15
	Non-Stratified	Normal Bench	12	6	4
		Flood Bench	41	28	17
Mean			42	22	7

The fairly high germination percentages generally agree with the results of Isley (1944), who found that seed of this species germinated readily after exposure to a variety of storage conditions and pre-germination treatments. Germination percentages were highest for seed collected in 1992, even though both seed lots appeared to be of comparable seed quality, although this could not be determined objectively. This result conflicts with that of Isley (1994) who found that this species declined in viability after 24 to 36 months of storage. Seed in this test was stored dry for 39 months prior to initiation of the stratification period. However, Isley (1944) did find that seed that was stored for 18 months under various storage conditions showed greater germination than seed stored for only six months. Perhaps the 1995 seed lot, where time from seed collection to planting was five months, required a longer storage period to achieve maximum germination.

There was a significant seed treatment by age of seed interaction for initial germination of woolgrass seed (Table 2). Seed collected in 1992 responded positively to a four month stratification period whereas that collected in 1995 showed no response. It appears that the seed undergoes some physiological changes during storage that promote germination. These changes may not have occurred during the shortened storage period of the 1995 seed and this possibly could have prevented it from responding to the after-ripening treatment. There was also a significant interaction between germination environment and seed lot (Table 3). Initial seed germination was better for both seed lots on the flood bench with completely saturated growing media. Reduced germination shown for the 1995 seed on the normal bench compared to 1992 seed on the same bench was probably due to the overall lower germination of this seed lot. During this test, there was some problem in maintaining moisture levels on the normal greenhouse bench, especially over weekends when staff were not available to irrigate the containers. The majority of the seedling mortality discussed above was noted on the normal greenhouse bench. However, at later evaluation dates, the plants that survived on the normal bench were more vigorous than those on the flood bench, many of which were just barely alive by the final count. Michael Fournier (unpublished) reported that the treatments that provided best emergence of many wetland plants were not always the ones which provided best plant performance and that is most likely the case for woolgrass.

Table 2. Interaction between seed age and seed treatment on initial germination percentage of woolgrass seed.

Seed Age	Seed Treatment	% Germination
1992	Stratified	68a*

1992	Non-Stratified	46ab
1995	Stratified	27b
1995	Non-Stratified	27b

*Simple effect means in columns followed by the same letters are not significantly different according to Tukey's HSD at P<0.05.

Table 3. Interaction between seed age and growing conditions and on initial germination percentage of woolgrass seed.

Seed Age	Environment	% Germination
1992	Flood Bench	79a*
1992	Normal Bench	36bc
1995	Flood Bench	38b
1995	Normal Bench	16c

*Simple effect means in columns followed by the same letters are not significantly different according to Tukey's HSD at P<0.05.

Soft-stem bulrush: Table 4 contains the germination percentage data for soft-stem bulrush. Because germination counts declined for later evaluation dates, mean separation was performed only on the data from the initial germination count. Germination counts were fairly low for all seed treatments and were much lower that those for woolgrass. Isley (1944) determined that *Scirpus* species with smaller seed, such as woolgrass, are much easier to propagate from seed than larger seeded species like soft-stem bulrush. His soft-stem bulrush germination percentages generally were not much higher than those reported for the best treatments in this study, excluding the test where germination conditions were altered to provide continuous illumination and a temperature of 30-32°C. Ambient conditions in the greenhouse during this test may not have been conducive to germination of this species.

Table 4. Mean germination percentage of two ages of soft-stem bulrush seed previously exposed to varying lengths of dry storage subjected to two seed treatments and two germination environments.

Seed Age	Treatment	Environment	First Count	Second Count	Third Count
			------------------------------%------------------------ -----------		
1992	Stratified	Normal Bench	8	2	4
		Flood Bench	13	19	16
	Non-Stratified	Normal Bench	0	0	0
		Flood Bench	0	1	1
1995	Stratified	Normal Bench	26	11	13
		Flood Bench	21	10	7
	Non-Stratified	Normal Bench	0	1	1

	Flood Bench	7	5	3
Mean		9	6	6

There was a trend towards increased germination on the flood bench compared to the normal bench, but this increase was not significant. Table 5 shows the effect of length of dry storage on percent germination. Isley (1944) reported that germination of this species was negligible when seed was stored for less than six months and that germinability increased with storage of 18 to 24 months. In this test, the 1995 seed lot was stored for less than six months, so low germination counts could be expected, however, it germinated better than the 1992 lot which was stored for about 45 months. Possibly the 1992 seed lost viability during the prolonged dry storage period. Isley (1944) did find that soft-stem bulrush seed viability was not maintained during long-term storage as well as that of the larger fruited *S. americanus*. Another factor that may have affected these results was seed collection time; the 1992 seed lot was collected earlier in the year than the 1995 seed (June vs. October) which may have affected seed quality. Also, Isley used water as the germination medium for all tests and he found reduced germination when seed was germinated on peat moss. Using a peat moss based medium may have affected germination of this species, however, the length of time seedlings were observed in this study precluded the use of water as the germination medium.

Table 5. Percent germination of two lots of soft-stem bulrush seed exposed to varying lengths of dry storage.

Seed Age	% Germination
1992	5b*
1995	14a

*Main effect means followed by different letters are significantly different according to Tukey's HSD at P<0.05.

A four month period of stratification increased germination of soft-stem bulrush seed (Table 6). This basically agrees with the findings of Isley (1944), however, he found better germination resulted from longer stratification periods than those used in this test. For this species, he also found that the medium in which the seed was stratified did affect germination and that the best stratification medium was peat moss. In this study, the stratification medium used was a moist paper towel saturated with water. This may have reduced germination, but it would have been difficult to count seed samples for testing if the seed had been mixed in a peat moss stratification medium.

Table 6. Percent germination of soft-stem bulrush seed subjected to two seed treatments.

Seed Treatment	% Germination
Stratified	17a*
Non-stratified	2b

*Main effect means followed by different case letters are significantly different according to Tukey's HSD at P<0.05.

CONCLUSIONS

Prolonged exposure to dry storage conditions did not adversely effect germination of woolgrass, but did have some effect on germination of soft-stem bulrush. It is not necessary to store seed of either species in water, however, exposing the seed to a stratification period prior to planting will increase germination. It is critical to maintain adequate moisture in the growing medium during germination, however, later growth may be improved when the medium does not remain saturated. Woolgrass had higher germination percentages than soft-stem bulrush and would be the easier species to produce.

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