Jamie L. Whitten Plant Materials Center

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Seed Propagation Techniques for Wetland Plants

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ABSTRACT

An investigation of seed germination techniques for six species of wetland plants originally collected in Mississippi shows that production of seedlings in the greenhouse is possible for all accessions, except creeping burhead. Seed of the other five species could be stored dry, but all except woolgrass responded to a three to four month stratification period before planting. Germination rates of soft-stem bulrush, bulltongue, and powdery thalia were slightly better when scarified before stratification, however, in most cases, the improvement was not substantial enough to warrant this additional treatment. Two germination conditions were tested, saturated growing media on a flood bench and moist, but not saturated conditions on a normal greenhouse bench. Powdery thalia was the only species that germinated better on the normal bench; however, later seedling growth of woolgrass and soft-stem bulrush was better in that environment than in the saturated condition.

INTRODUCTION

Natural germination of most emergent wetland plants occurs during drawdown periods when water levels are severely reduced. In order to propagate these plants from seed, it is necessary to simulate the environmental conditions experienced during these drawdowns. According to Shipley and Parent (1991) these stimulating conditions include the following: 1) a period of cold, moist stratification followed by sowing the seed on the surface of a growing medium; 2) maintaining the medium in a wet, but not inundated state; 3) exposing the seed to light; and 4) a night/day temperature cycle of 20/30°C. Isley (1944) found that seed of *Scirpus* species required exposure to cool, moist stratification conditions for after-ripening to occur. He also found improved germination percentages when the seed was exposed to light and to high temperatures (20-25°C/30-32°C) during the germination period. Sharp (1939) stated that in a natural wetland, seed of *Sagittaria latifolia* (common arrowhead) germinates on the surface of shallow water where temperatures and radiation levels are the highest and then the seedlings settle to the bottom and root in the substrate.

Germination of species with thick seed coats or hard surrounding fruit structures may be improved if the seed coat is broken or scarified before planting (Crocker, 1907; Shipley and Parent, 1991). Andersen (1968), in his review of seed propagation techniques, stated that germination of common arrowhead can be improved by cutting the seed coat with scissors before planting. Crocker (1907) found that intact seeds of *Sagittaria variabilis* did not germinate, but high germination percentages resulted when the seed coat was ruptured. Isley

(1944) found only slight improvement in germination percentages for *Scirpus* species when concentrated sulfuric acid was used to reduce the thickness of the seed coat (acid scarification). He also tested various methods of physically penetrating the seed coat, but germination using these treatments was negligible.

Post-harvest seed storage conditions can also affect seed germination. Muenscher (1936) stated that seed of many wetland species must be stored in water at 1-3°C to retain viability. However, Garbisch and McIninch (1992), in a fairly extensive list of storage requirements for wetland plants, recommended moist or water storage for only a few species. Isley (1944) studied various storage methods for seed of *Scirpus* species. He found that germination of seed that was stored dry and then stratified had similar or, in some cases, better germination percentages than seed stored in water at 2-4°C. Germination of seed that was stored dry without stratification or in water at room temperature proved to be sporadic. Although maintaining seed in moist or water storage may be a viable option for holding seed from one season to the next, it would be somewhat more difficult to store seed in this manner for prolonged periods of time.

The Jamie L. Whitten Plant Materials Center (PMC) at Coffeeville, Mississippi collected wetland plants from various locations in Mississippi that showed potential for use in wetland mitigation and restoration plantings or for constructed wetlands for wastewater treatment. The species collected are listed in Table 1. Seed of all these species ripen above, or in the case of creeping burhead, both above and on the surface of the water, so they would be exposed to drying conditions before dispersal. A few of these species are not commonly marketed, so propagation methods have not been recorded. Limited propagation information is available on the others, but these southern ecotypes may respond differently than the northern ecotypes studied in the literature. Ecotypes of these species adapted to the climate of Mississippi would not be exposed to extended periods of cool, moist conditions appropriate for stratification. A three to four month stratification period would be the maximum to which local ecotypes would be subjected, depending upon the portion of the state in which the plants were located. Most of these species can be easily propagated by vegetative means, but production of seedlings may be more economical because they require less production space, and use of seedlings in wetland plantings would increase the genetic diversity of the planting population. Therefore, a study was initiated to determine the best seed storage conditions; pre-planting seed treatments, and germination environments for these accessions.

MATERIALS AND METHODS

Table 1. Species tested.

Common Name	Scientific Name	Accn. No.	Release Name
Woolgrass	*Scirpus cyperinus* (L.) Kunth	9062741	Leaf River Source
Soft-stem bulrush	*Scirpus tabernaemontani* K.C. Gmel. (Synonym = *S. validus* Vahl)	9062740	

Longbeak arrowhead	*Sagittaria australis* (J.G. Sm.) Small	9077062	
Bulltongue	*Sagittaria lancifolia* L.	9062745	
Powdery thalia	*Thalia dealbata* Fraser ex Roscoe	9059002	Indian Bayou Source
Creeping burhead	*Echinodorus cordifolius* (L.) Griseb.	9062853	Leflore Source

Seed of these accessions were harvested in late summer to fall of 1995 and 1996. Harvest dates varied between species and between years of collection. In each case, seed was collected when fully mature and before any significant seed shattering occurred. The term "seed" will be used to describe planting units, although most are actually indehiscent fruits. Woolgrass and soft-stem bulrush were harvested by hand pulling or cutting the fruit clusters from the stem. Longbeak arrowhead, bulltongue, and creeping burhead were harvested by manually shattering the fruit clusters and collecting the seed in a container as it fell. Powdery thalia was harvested by shaking the fruit clusters over a container which caught the falling seed. All necessary seed cleaning was performed before the seed was allowed to dry. Woolgrass and soft-stem bulrush seed was freed from the fruit clusters using a brush machine (Westrup a/s Slagelse, Denmark) and hand screened to remove inert matter. Powdery thalia seed was rubbed over a roughened surface to remove the papery fruit coverings. The other species required only hand screening to remove small amounts of trash.

Storage treatments used were dry storage in a cooler maintained at 55°F and 45% relative humidity, moist storage in a cooler at 42°F with no humidity control, and water storage in a cooler at 42°F with no humidity control. Storage and pre-plant treatments tested were based on published references or on previous work with that species at the PMC. Only those species where moist or water storage appeared to be beneficial were stored in that manner. All species were subjected to dry storage. Moist storage was tested on soft-stem bulrush, longbeak arrowhead, creeping burhead, powdery thalia, bulltongue, and for only the first year on woolgrass. Water storage was tested on soft-stem bulrush, longbeak arrowhead, bulltongue, and for the first year only, woolgrass. Woolgrass seed is so small that it is difficult to count samples for planting; during the first year of testing the moist and water storage treatments did not prove to be beneficial, so they were dropped from the second year of the test. Small quantities of seed of these accessions were divided from the main lot and placed in moist and water storage treatments immediately after seed cleaning. All seed in water storage was placed in a nylon-mesh container that was placed in a glass jar containing tap water. Water was not changed during the storage period for the first year of the test, however, it was changed monthly during the second year in an attempt to limit the algae growth seen during the first year. Isley (1944) found that, for the *Scirpus* species he tested, changing the water weekly during a six month storage period gave slightly better germination than storing seed in water that was not changed regularly. Moist stored seed was placed on a brown paper towel that was moistened and placed in a self-sealing plastic bag with sufficient additional water to maintain the moisture levels during the storage period. Placing the seed on the paper toweling facilitated later counting of seed lots for planting. Powdery thalia seed is too large to allow good seed contact with the paper towel, so it was stratified in moist sphagnum moss from which the seed was easily separated at planting. All remaining seed was dried thoroughly and placed in a self-sealing plastic bag for the dry storage treatments.

Pre-planting treatments used on dry-stored seed were a three to four month stratification period, scarification using mechanical means or sulfuric acid, and combinations of stratification and scarification. All species except longbeak arrowhead and bulltongue were stratified. These species were not given a separate stratification period because during the first test year for bulltongue and both test years for longbeak arrowhead, the seed collection date was so close to the date when the stratification treatment began, it was determined that there would be no effect of the dry storage period prior to stratification and the treatment would be indistinguishable from the moist storage treatment. All stratification treatments were placed in the cooler on November 7, 1995 and November 21, 1996. Stratification media and storage conditions were the same as those used for moist storage. The length of time that the seed was exposed to dry storage before commencement of the stratification period varied between species due to different harvest dates as discussed previously. Those species given the mechanical scarification and mechanical scarification plus stratification treatments were soft-stem bulrush, longbeak arrowhead, bulltongue, and powdery thalia. In 1995, mechanical scarification was done using a small mechanical scarifier (Fred Forsberg and Sons, Inc., Thief River Falls, MN), except for powdery thalia, which was scarified by placing the seed in a coffee can lined with sand paper and tumbling them with some gravel added to increase abrasion. There was a concern that the seed was not uniformly scarified using these methods so a different method was used for the second year of the test. In 1996, seed was scarified by placing it in a sand paper lined box and rubbing it underneath a sandpaper covered block, except powdery thalia where each seed was individually rubbed against the sand paper in the box. Soft-stem bulrush was the only species on which acid scarification and the combined treatment with stratification was tested. Seed was soaked in concentrated sulfuric acid for five minutes with periodic stirring, then rinsed in water to remove the acid. There was not enough seed collected in 1995 to attempt acid scarification because of the seed losses that occur during the rinsing process, but older seed collected in 1992 and seed collected in 1996 were both treated.

Seed from the storage and stratification treatments were removed from the cooler, seed samples counted and planted in the greenhouse at the same time as the dry storage treatments were planted. In 1996, this was done during the week of March 18-22 and in 1997 from March 20-27. Seed samples for some species were more time consuming to count than others, however, all treatments for a single species were planted within a two day period. All seed lots will be referred to by their seed collection year in the following discussion. Sample sizes consisted of 100 seed for all treatments except the following: 1995 soft-stem bulrush, 50 seed; 1995 powdery thalia, 20 seed; 1996 powdery thalia, 25 seed. Stratified, moist stored, and water stored seed were allowed to surface dry during planting, but were not allowed to dry completely. Isley (1944) and Harris and Marshall (1960) found that *Scirpus* seed stored in water at cool temperatures could be allowed to dry for a period of 14 days to one month without greatly affecting germination percentages. The effect of post-stratification drying on the other species is not known, but the planting operation would have been more difficult if the seed was maintained in a wet condition. Two additional treatments for the 1995 woolgrass seed lot were intact sections torn from the fruit cluster that were both stratified and planted dry. Because seed numbers in each treatment could not be determined, these were observational treatments only.

Experimental design was a factorial experiment in a randomized complete block with three replications. Germination containers were 7” x 5-1/4” x 2-5/16” black plastic bedding plant liners. Growing media used was a 3:1 mix of peat moss/sand amended with commercially recommended quantities of pelletized slow-release fertilizer, dolomitic lime, Micromax micronutrient fertilizer, and Aquagro wetting agent. The sand was not pasteurized before use in 1996 which caused some minor weed problems, so in 1997 it was placed in an electric soil sterilizer that was heated to 180°F for approximately 30 minutes. All species were planted on the surface of the growing media, except powdery thalia which was planted approximately ¼ inch deep. Seed was spread as uniformly as possible on the media.

Containers were placed in the greenhouse after planting and hand watered to ensure good seed contact with the growing medium. Although temperatures and light levels are critical for successful germination of most wetland species, instrumentation was not available to monitor these environmental conditions during the testing period. Two germination conditions were tested; one, where the containers were placed on a normal greenhouse bench and watered regularly to maintain a moist condition, and the other, continuously saturated conditions on an ebb and flow greenhouse bench where the water was maintained at ¼ to ½ inch depths except for short periods of time when the bench was drained and rinsed to remove algal growth. In the discussion below, these conditions will be referred to as the normal bench and the flood bench.

An initial seedling count was made when it was deemed that a sufficient number of seedlings were present to justify counting. These dates varied between species and between study years. Two additional counts were made at three week intervals following the initial count. A few seedlings of some species died prior to the initial count, especially on the normal greenhouse bench, when the surface of the growing medium tended to dry during the weekend when staff members were not available to irrigate the containers. Dead seedlings were counted and their numbers were included in the initial count because germination had occurred. Later counts included only those plants that were green and could be considered to be alive at the evaluation date. The study was analyzed with years of testing treated separately. When the data for all species was subjected to an analysis of variance (ANOVA), it was found that the germination percentages from the initial count were representative of treatment effects and mean separation was performed on those means using a Tukey’s honestly significant difference test (HSD) at the five percent level of probability.

RESULTS AND DISCUSSION

Woolgrass: Woolgrass was the earliest species to germinate. Data for the woolgrass treatments are presented in Table 2. Results for the 1995 and 1996 seed lots were similar for those treatments common to both years of testing. The 1995 seed showed a significant response to seed treatment. Dry stored woolgrass seed did germinate more quickly after the stratification treatment, but germination percentages were not significantly different from the dry storage treatment alone. These results conflict with those of Isley (1944) who found that woolgrass seed required stratification. His tests showed that a three month stratification period was not long enough to provide the required after-ripening for the ecotypes of this species he tested, and stratification for six months or longer was required to overcome dormancy. These results agree more closely with the observations of Garbisch and McIninch (1992) who recommended dry storage for woolgrass seed and related that there is no seed dormancy. Apparently, this ecotype of woolgrass does not have a dormancy mechanism that requires after-ripening. This plant was collected in the southern portion of Mississippi, where only short periods of chilling temperatures are common during the winter months. There was a trend towards improved germination of the 1995 seed stored in water compared to the other treatments, with the lowest germination percentages for moist stored seed.

Woolgrass showed a significant response to the growing environment in both years of testing; best germination was found to be in the saturated conditions on the flood bench. Seedling mortality on the normal bench was higher for this species than most of the other species tested, probably because of the small size of the seedlings, which made them highly susceptible to desiccation as the surface of the growing medium dried. This problem was more pronounced in 1996 than in 1997, probably due to the cooler temperatures and long periods of cloud cover experienced during the testing period in 1997. However, in both years later growth of the seedlings was more vigorous for surviving plants on the normal greenhouse bench. Many of the seedlings growing on the flood bench showed evidence of poor root growth due to a lack of aeration and there was extensive algal and slime mold growth of the media surface which may have been somewhat toxic to the seedlings. Most of the plants were barely alive by the third evaluation date. Personal observation of plants growing in the wild indicates that this species does require fairly wet conditions for germination, but plants become increasingly tolerant of drying substrates as they grow, with mature plants possessing a higher level of drought tolerance than would be expected of most wetland plants.

Table 2. Woolgrass mean initial germination percentages for seed exposed to three seed storage conditions, one pre-planting treatment, and two growing environments.

Storage/Treatment	Environment	1995	1996
Dry/Stratified	Normal Bench	19c*	17b
Dry/Stratified	Flood Bench	35ab	61a
Dry	Normal Bench	12c	9b
Dry	Flood Bench	41a	57a
Moist	Normal Bench	15c	-----
Moist	Flood Bench	32ab	-----
Water	Normal Bench	24bc	-----
Water	Flood Bench	44a	-----

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

There was a substantial amount of germination from the planted woolgrass infructescence pieces. It appeared that the stratified pieces showed greater levels of germination than the dry planted ones, but this could not be determined because seed numbers in each container were not known. This treatment was added to the study because there was a concern that the long perianth bristles attached to the achenes would limit the ability to successfully clean this seed from the surrounding fruit structures. This concern proved to be unfounded in this study because the amount of seed required for testing was not difficult to clean using the methods described above, however, there was a large quantity of seed that could not be removed using the available brush machine screen (mantle) size.

Soft-stem bulrush: Initial germination percentages for soft-stem bulrush are presented in Table 3. Germination percentages for this species are fairly low. These results basically agree with Isley (1944) who found that the germination rates of the lots of seed he tested were lower than for the smaller seeded *Scirpus* species and somewhat erratic. He found that germination rates of soft-stem bulrush increase with seed age and that germination of seed stored for less than six months was negligible. Time from collection to planting for the 1995 and 1996 lots would have been in the range of six to eight months. A longer period of storage may have improved germination rates of these two seed lots. Seed storage period for the 1992 seed was approximately four years, however, germination rates were generally lower for this lot than those of the younger seed. The oldest seed lot Isley (1944) tested was 24 months. Therefore, it seems likely that the 1992 seed could have lost viability during the four year storage period. Also, both Isley (1944) and Harris and Marshall (1960) found that this species responded positively to illumination and high temperatures during the germination period. Perhaps greenhouse light levels and temperatures were not high enough during the testing period to produce maximum germination.

The results for this species were not the same for all three seed lots. Both the 1992 and 1996 seed lots showed an interaction between the growing environments and the seed treatments, while the 1995 seed lot showed a significant response to seed treatment only. The reason for this difference is because, for the 1995 seed lot, seed treatments responded similarly to both growing conditions, while for the other two seed lots, the corresponding seed treatments on the flood bench germinated better than those on the normal bench. Although germination was better on the flood bench, observations made during the course of this study showed that later growth of the seedlings may have been better if the growing media had not remained constantly saturated. The plants that grew on the flood bench were taller and larger than those on the normal bench, but the plants became infested with aphids, which may have been due to the succulent growth or as a result of plant stress from the saturated media. Aphids were not attracted to the plants on the normal greenhouse bench. The best conditions for greenhouse production of this species may vary from those in which seedlings grow in nature. Harris and Marshall (1960) recommended sowing seed on exposed mud flats during either a spring or, more desirably, a fall drawdown. They recommended that the mud flats be kept very wet to slightly flooded. At the PMC, abundant germination of soft-stem bulrush seed from the seed bank resulted in one growing pond when the water levels were drawn down during the fall and early spring.

Dry stored seed responded positively to stratification for all three seed lots which confirms the findings of Isley (1944) and Garbisch and McIninch (1992) that seed of this species does have a dormancy mechanism that requires stratification. Isley (1944) did not find improved germination percentages until seed had been stratified for at least six months. It appears that this southern ecotype does not require as long a stratification period as northern ecotypes such as those used by Isley. He did find that the stratification medium used had an effect on germination of this species; stratification in water produced lower germination rates than stratification in peat moss. Although the seed was placed on a paper towel in this test, the conditions would more closely approximate those of water stratification. The response to the scarification treatments was variable. There was no response to either mechanical scarification or acid scarification alone. Acid scarification plus stratification increased germination of the 1992 lot, but not the 1996 lot. This erratic response to acid scarification agrees with the findings of Isley (1944). He obtained moderate improvements in germination percentages when seeds were treated with sulfuric acid, however, he found that it was difficult to treat the seeds for a sufficient length of time to remove the germination restricting pericarp (seed wall) without damaging the seed. It is entirely possible that the pericarp of the 1996 seed lot differed in thickness from that of the 1992 seed and therefore required a different duration of acid treatment to promote germination. Isley (1944) did not consider sulfuric acid scarification to be a practical seed treatment for this species, and these findings support that observation. Mechanical scarification plus stratification showed a moderately positive response for the 1995 and 1996 seed lots. These findings do not appear to agree with those of Isley (1944). He found that the various methods he employed of cutting through the seed coat generally had a negative effect on germination, rather than a positive one. He did not try a scarification treatment that treated the surface of the entire seed coat as used in this test. The variable response to mechanical scarification does not suggest that it is a viable treatment option. There was a positive response to moist storage for the 1995 and 1996 seed lots. This storage method could be recommended for short-term storage of this seed, with dry storage followed by stratification recommended for longer storage periods.

Table 3. Soft-stem bulrush mean initial germination percentages for seed exposed to three storage conditions, four pre-planting treatments, and two growing environments.

Storage/Treatment	Environment	1992	1995	1996
Dry/Stratified	Normal Bench	8bc*	26a	9bc
Dry/Stratified	Flood Bench	13b	21ab	5cde
Dry	Normal Bench	0d	0b	0e
Dry	Flood Bench	0d	7ab	0e
Dry/Mechanically Scarified + Stratified	Normal Bench	3cd	9ab	15ab
Dry/Mechanically Scarified + Stratified	Flood Bench	4cd	7ab	8bcd
Dry/Mechanically Scarified	Normal Bench	0d	0b	0e
Dry/Mechanically Scarified	Flood Bench	0d	0b	0e

Dry/Acid Scarified + Stratified	Normal Bench	11bc	-----	6cde
Dry/Acid Scarified + Stratified	Flood Bench	27a	-----	5cde
Dry/Acid Scarified	Normal Bench	0d	-----	1de
Dry/Acid Scarified	Flood Bench	0d	-----	0e
Moist	Normal Bench	-----	20ab	21a
Moist	Flood Bench	-----	17ab	14ab

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

Longbeak arrowhead: Table 4 lists the initial germination data for longbeak arrowhead. There was a significant seed treatment by growing environment interaction for both years of testing. For the 1995 seed, this interaction is explained by the positive response of all treatments except the dry stored seed to the conditions on the flood bench; the dry stored seed behaved the same on the normal and flood benches. There were no differences shown for the 1995 seed lot between the water storage, moist storage, and mechanically scarified plus stratified treatments on the flood bench. The responses for the 1996 seed were not the same as in the previous year; the interaction in this year is due to the significantly higher germination percentage of moist stored seed on the flood bench compared to all other treatments. In later counts (data not shown), the germination percentages were highest for water stored seed. However, even in the later counts, the germination of mechanically scarified plus stratified seed never equaled that of moist and water stored seed as it did for the 1995 seed. There was not a consistent response in initial germination percentages to the flood bench environment for the 1996 seed, however, when both years of testing are taken into account, the flood bench provided the best germination environment for this species. Growth rate of the seedlings on the flood bench was also better on the flood bench than on the normal bench.

Sharp (1939) recommended storing seed of common arrowhead on an outdoor platform, the conditions of which would closely approximate the moist storage conditions used in this test. Although he was working with a different *Sagittaria* species, the species included in this test is closely related and should behave in a similar manner. Muenscher (1936) found that no common arrowhead seed germinated without being stored in water, however, he admits that the seed lots he used were most likely immature because they were removed from the plant before the seed fell and may not behave in a similar manner as mature seed. Keddy and Constabel (1986) state that common arrowhead seed, because it is flat, would have a high surface area to volume ratio and should be sensitive to drying conditions, such as those found in coarse textured substrates. Garbisch and McIninch (1992) recommended dry storage for common arrowhead seed and report that there is no seed dormancy.

The data presented below shows that longbeak arrowhead did maintain viability best in moist or water storage, however, there was a response to mechanically scarifying and stratifying the seed. Since there was no dry storage plus stratification alone treatment, it is difficult to judge if the effect of this treatment was due to the scarification or the stratification treatment.

In an observational planting made in 1997, seed from the previous year's test was stored dry until the stratification date, when it was stratified along with the other treatments. Germination was evident, but germination counts were not made. This indicates that seed of this species did not lose viability during dry storage. Crocker (1907) states that for *Sagittaria* species, removal of the husk-like outer exocarp did not improve germination, but rupture of the inner, hardened endocarp did, as long as the embryo was not damaged in the process. It is doubtful that the scarification methods used in this test were sufficiently abrasive to rupture a high percentage of the endocarps of the seed treated. Therefore, when the results of this treatment and those of the moist and water treatment are examined, it does appear that this species has a physiological dormancy that is overcome by cool, moist storage.

Table 4. Longbeak arrowhead mean initial germination percentages for seed exposed to three storage conditions, two pre-planting treatments, and two growing environments.

Storage/Treatment	Environment	1995	1996
Dry	Normal Bench	0b*	1b
Dry	Flood Bench	0b	0b
Dry/Mechanically Scarified + Stratified	Normal Bench	0b	1b
Dry/Mechanically Scarified + Stratified	Flood Bench	12a	1b
Dry/Mechanically Scarified	Normal Bench	0b	7b
Dry/Mechanically Scarified	Flood Bench	0b	1b
Moist	Normal Bench	0b	6b
Moist	Flood Bench	13a	20a
Water	Normal Bench	0b	5b
Water	Flood Bench	15a	9b

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

Bulltongue: Bulltongue is another species of *Sagittaria*, but observation of its germination characteristics in the PMC growing ponds shows that it behaves quite differently than longbeak arrowhead. Few seedlings of longbeak arrowhead have become established in the production pond, however, in ponds where mature bulltongue plants have produced seed, copious amounts of seedlings have germinated throughout the spring and summer in years when the water levels were drawn down to a few inches in depth. The seed of this plant remains on the surface of the water for a long period of time after dispersal, and as Sharp (1939) found for common arrowhead, it may germinate on the surface of the water or possibly underneath the water.

The data for the greenhouse germination test is presented in Table 5. The ANOVA for the 1995 lot showed a significant response to growing environment, with better germination on the flood bench. Tukey's HSD did not separate the individual treatment means, probably due to the high magnitude of the error term. The least significant difference (LSD) value calculated at the five percent probability level was 17.57; using this value, the differences between the treatments become apparent. There was a significant response to both growing environment and pre-planting treatment for the 1996 seed lot. Although initial germination was better on the normal bench for this seed lot, later counts showed the germination rates on the flood bench equaled and then exceeded those on the normal bench (data not presented). Observation of seedling growth rates showed superior growth on the flood bench compared to the normal bench. From the results of this study, it appears that seedlings should be grown in wet growing media. Since the natural habit of this seed is to germinate either on the surface of or under shallow water, germination rates could possibly be increased by submerging the containers either before or after planting.

Table 5. Bulltongue mean initial germination percentages for seed exposed to three storage conditions, two pre-planting treatments, and two growing environments.

Storage/Treatment	Environment	1995	1996
Dry	Normal Bench	0a*	0b
Dry	Flood Bench	12a	0b
Dry/Mechanically Scarified + Stratified	Normal Bench	1a	9ab
Dry/Mechanically Scarified + Stratified	Flood Bench	21a	2b
Dry/Mechanically Scarified	Normal Bench	9a	2b
Dry/Mechanically Scarified	Flood Bench	21a	0b
Moist	Normal Bench	2a	10ab
Moist	Flood Bench	17a	3ab
Water	Normal Bench	1a	12a
Water	Flood Bench	23a	5ab

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

For the 1996 seed lot, initial germination was highest in the water storage treatment, but that treatment was not significantly different than the moist and the dry storage plus mechanically scarified and stratified treatments. The high initial germination rate of the water stored seed on the normal bench was probably an anomaly because, because by the final count, percent germination of this treatment on the flood bench was more than twice that on the normal bench (37% vs. 17%). There was a problem in this treatment year with a large number of seed germinating before removal from the water and moist storage treatment. This problem was not seen in the previous year, probably because the seed was collected approximately three months later and was therefore stored for a shorter period of time before planting. Seed that had not yet germinated was selected for the planting sample, but that seed might not have been the highest quality seed. By the final count, the germination percentage of the mechanically scarified plus stratified seed was not different than that of the mechanical scarification treatment alone, however, both of these treatments showed slightly better germination than the dry stored seed (data not shown). This potential scarification effect does not appear to support the general findings on *Sagittaria* seed presented above (Crocker, 1907). The endocarp of this species does not appear to be as hard as that of longbeak arrowhead, so possibly the scarification treatments employed were sufficient to rupture the endocarp and promote germination. Because no beneficial effect could be attributed to stratification alone, there does not appear to be an internal dormancy mechanism in this seed. This finding appears to confirm observations of immediate germination in the growing ponds. An observational study of the previous year's seed, similar to that of longbeak arrowhead, showed that seed that was stored dry for an extended period of time and then stratified did germinate, but non-stratified seed was not planted so its germination potential was not evaluated.

Powdery thalia: Powdery thalia seed is round to oval in shape and approximately ¼ inch in diameter, so it required different treatment methods than the previous species. Observations of the regeneration characteristics of this plant in the PMC growing ponds has shown that only a few seedlings have established naturally, mainly along the margins of the pond. Previous attempts at seedling production in the greenhouse have shown that germination is slow and somewhat erratic. Also, a few seedlings tend to die from no apparent cause.

The data on initial germination of powdery thalia is presented in Table 6. Initial germination rates were fairly low and seed continued germinating in small numbers throughout the test. For the 1995 seed lot, there was an interaction between growing environment and seed treatment. This is probably due to the low germination rates of the dry stored seed and the mechanically scarified seed on both growing environments. Other treatments germinated better on the normal bench, except for the mechanically scarified plus stratified treatment, which germinated better on the flood bench. This response of the mechanically scarified plus stratified treatment on the flood bench was probably an anomaly, because later counts (data not presented) showed that germination percentages for this treatment were not significantly different on the normal and the flood bench.

Table 6. Powdery thalia mean initial germination percentages for seed exposed to two storage conditions, three pre-planting treatments, and two growing environments.

Storage/Treatment	Environment	1995	1996
Dry/Stratified	Normal Bench	30a*	11ab
Dry/Stratified	Flood Bench	0b	0b
Dry	Normal Bench	2b	0b
Dry	Flood Bench	0b	1b
Dry/Mechanically Scarified + Stratified	Normal Bench	7b	17a
Dry/Mechanically Scarified + Stratified	Flood Bench	17ab	7ab
Dry/Mechanically Scarified	Normal Bench	2b	1b

Dry/Mechanically Scarified	Flood Bench	0b	0b
Moist	Normal Bench	12ab	8ab
Moist	Flood Bench	2b	0b

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

For the 1996 seed lot, there was a significant response in initial germination to seed treatment only. In this treatment year, the mechanically scarified plus stratified treatment was the best, followed by dry storage and moist storage. Although in this year, there was no significant response to growing environment, there was a trend towards improved germination on the normal bench. In both years, the plants on the flood bench were more susceptible to infestation by aphids than those on the normal bench, indicating that the plants were probably stressed. Overall, germination of this species was better on the normal bench, which seems to confirm the observations of seed germination in drier conditions along the margins of the growing ponds.

The response to stratification and moist storage indicates that this species has an internal dormancy that is overcome by the cool, moist treatment. Germination rates were better for the dry stored seed that was subsequently stratified than for the moist stored seed in both treatment years. Although not consistent, there also appears to be some beneficial effects from mechanical scarification when accompanied by stratification. The seed has a waxy coating which may prevent water infiltration. A previous attempt at acid scarification on a small quantity of seed showed that even a short duration treatment (less than 1 minute) was toxic. Because the germination rates of the mechanically scarified plus stratified seed were generally not different than those of the stratification treatment, it appears that stratification alone is probably sufficient to promote germination.

Creeping burhead: Initial germination percentages for creeping burhead (Table 7) were extremely low and little additional germination was noted at subsequent evaluation dates (data not presented). There were no significant differences between growing environments or pre-planting treatments for the 1995 seed. The ANOVA for the 1996 lot indicated that there was a significant effect due to the growing environment, but Tukey's HSD did not separate the individual treatment means. The LSD value calculated for these means was 2, so there was a slight response to moist and water storage on the flood bench. Although germination rates were slightly better on the flood bench, the differences were so minuscule that they will not support a recommendation. There was no detectable difference between pre-planting treatments in either year. The water storage treatment was added in the second testing year to see if germination rates could be improved using this storage method, however, it was not appreciably better than moist storage. There was a slight trend towards improved germination in these two storage treatments compared to dry storage. An attempt was made to mechanically scarify some seed from the 1996 lot, however, visual inspection of the seed did not indicate that the treatment had any effect on the seed coat, so this treatment was not added to the study. Seed germination has been noted in the PMC production ponds, mainly along the margins and in shallow water. However, this test obviously did not provide the appropriate conditions for germination of this species.

Table 7. Creeping burhead initial germination percentages for seed exposed to three storage conditions, one pre-planting treatment, and two growing environments.

Storage/Treatment	Environment	1995	1996
Dry/Stratified	Normal Bench	0*	1a**
Dry/Stratified	Flood Bench	1	0a
Dry	Normal Bench	0	0a
Dry	Flood Bench	0	0a
Moist	Normal Bench	0	0a
Moist	Flood Bench	2	2a
Water	Normal Bench	---	1a
Water	Flood Bench	---	2a

*ANOVA indicates there are no significant treatment effects at P<0.05.
**Treatment means in column followed by the same letters are not significantly different according to Tukey's HSD at P<0.05.

CONCLUSIONS

Woolgrass: Woolgrass germination rates in this test were fairly high. Isley (1944) also found that woolgrass seed placed in various storage conditions germinated readily. If equipment is not available to clean the seed from the surrounding fruit structures, a potential alternate planting method is to tear fruit clusters into smaller sections and plant them without further cleaning. This ecotype did not require stratification, although seed may germinate more rapidly if stratified. Although best germination rates were in the treatments with a saturated growing medium, later seedling growth may be improved if once the seedlings become established, the growing medium could be maintained in a moist, not wet condition.

Soft-stem bulrush: Soft-stem bulrush germination rates were fairly low. Isley (1944) found that germination of larger-seed *Scirpus* species with thicker walled seed was often less than satisfactory. However, both acid and mechanical scarification did not substantially improve germination. Moist storage or dry storage followed by a three to four month stratification, preferably using peat moss as the stratification medium, are recommended for this ecotype of soft-stem bulrush.

Longbeak arrowhead: Longbeak arrowhead germination rates were also fairly low, so seeding rates should be adjusted accordingly. Seed germinated best when stored in water or in a moistened state, but there was also some response of dry stored seed to stratification. Seedlings germinated and grew best in a saturated growing medium.
Bulltongue: Bulltongue seed germinates more readily than longbeak arrowhead, but the germination rates were lower than would be expected by the reseeding potential exhibited in the PMC growing ponds. The seed germinated better in a wet growing environment, but germination rates possibly could be improved by elevating the water level above the surface

of the growing medium. Seed germination rates were highest in the water storage treatment, but it appears that moist storage and probably even dry storage followed by a stratification treatment to rehydrate the seed may provide acceptable germination rates. Mechanically scarifying the seed using sand paper prior to stratification may also be beneficial.

Powdery thalia: Seed germinates slowly and sporadically over a long period of time. Dry storage is probably best for this seed but cold stratification for three to four months prior to planting is required for germination to occur. Germination may also be slightly improved if the seed is mechanically scarified before stratification. This species grew best when the growing medium was maintained in a moist, but not saturated condition.

Creeping burhead: Acceptable germination levels were not obtained with any combination of the seed treatments and growing environments tested. There was a slight trend towards improved germination with the saturated media conditions on the flood bench.

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