## THREE MISSISSIPPI ECOTYPES OF WETLAND PLANTS

## Janet M. Grabowski

Biologist, USDA-NRCS, Jamie L. Whitten Plant Materials Center, 2533 County Road 65, Coffeeville, MS, 38922-2652

Abstract--In 1996, the USDA-Natural Resources Conservation Service (NRCS) Jamie L. Whitten Plant Materials Center (PMC) released three locally collected, source identified wetland plants. Indian Bayou source powdery thalia (*Thalia dealbata* Fraser ex Roscoe) and Leflore source creeping burhead [*Echinodorus cordifolius* (L.) Griseb.] were collected in the Mississippi Delta and Leaf River source woolgrass [*Scirpus cyperinus* (L.) Kunth] from southeastern Mississippi. Both vegetative propagules and seedlings can be planted. PMC seed germination studies have shown that Indian Bayou and Leaf River germinate readily after exposure to cold, moist conditions (stratification). Indian Bayou germination and seedling growth was best in a moist, not saturated, growing medium, whereas best germination of Leaf River was in a saturated medium and seedling growth was better in moist medium. Germination of Leflore was very poor for all seed treatments in the test, even though seedlings have established in PMC plant production ponds.

## INTRODUCTION

Current interest in wetland restoration, enhancement, and mitigation has led to an increased need for sources of plants that are well adapted to local environmental and soil/water conditions. Most plant materials available commercially are from widely varying geographic regions. Plant materials originating from areas with vastly different physical and environmental characteristics are often not well suited to conditions on the planned planting site and use of these plants can lead to less than satisfactory performance. McNaughton (1966) found significant differences in growth habit, environmental tolerances, and dormancy patterns of several ecotypes of three Typha L. species collected from diverse sites in the United States. Growth patterns of each ecotype were matched for optimum survival and productivity to the environmental conditions common to their original source location. Although the ideal recommendation would be to use planting materials collected from local ecotypes, this is often impractical because availability may be lacking and costs prohibitive (U.S. Army Engineer Waterways Experiment Station 1978). Therefore, an alternate method of obtaining adapted plant materials is required.

Awareness of genetically controlled ecotypic differences between plant populations has led to development of a new type of plant release within the NRCS Plant Materials Program called a source identified release. A source identified release was collected from a natural plant population and has not undergone any testing or selection prior to its release.

Staff at the Jamie L. Whitten PMC, Coffeeville, Mississippi collected plants of several emergent wetland species within the state and released three of these for use in various types of wetland plantings. Although these ecotypes are best suited to areas within Mississippi, ecological differences within the state and among adjoining states are much smaller than in some other parts of the country. This fact along with the fairly broad natural geographic range of these species suggests that they may also be planted successfully in other areas in the southeastern United States with comparable climatic, hydrologic, topographic, and soil features. Plant material of these releases was provided to a limited number of local plant producers for increase and sale to the public. At present, vegetative propagules (clonal material) are being marketed; however, production of seedlings may be more desirable because genetic recombination could lead to greater diversity in the planting population and increased ability to withstand environmental stresses. This paper presents descriptive information on each of these releases as well as results of a study conducted to determine seed propagation methods for these plants.

# PLANT COLLECTIONS

## Indian Bayou

Thalia dealbata is a member of the Maranthaceae or arrowroot family. It is a rare inhabitant of shallow wetlands in the Mississippi Delta and other southeastern states. The Indian Bayou source of powdery thalia was collected by Travis Salley in 1989 from a homeowner's yard in Bolivar County, Mississippi. According to the homeowner, the plants were originally moved from Washington County, Mississippi. It is a herbaceous perennial arising from thick (up to 4-cm in diameter) rhizomes, with a bluish, glaucous coating on leaves, flower stalks, and flowers. The 1- to 1.2-m long leaves arise from the base of the plant and have a shape reminiscent of a Canna L., with a stout petiole and a large, ovate to lanceolate leaf blade. Flowers are produced from late May to September with fruit maturing throughout the summer. The attractive purple to bluish flower clusters are produced on scapes extending 0.6- to 1-m above the foliage. Fruit type is an urticle, consisting of a bladdery, membranous covering loosely surrounding a single seed. Seeds are approximately 6-mm in diameter, subglobose, dark brown speckled with tan or gray, with a conspicuous tan to brown hilum. There are approximately 3184 seeds per kilogram.

#### Leaf River

Woolgrass is a bulrush, in the Cyperaceae or sedge family that is commonly found in shallow wetlands throughout the state of Mississippi. Its native range covers most of the eastern United States and Canada, extending westward into eastern Texas (USDA-Soil Conservation Service No Date). Leaf River source was collected by B.B. Billinglsey, Jr., Harvey Huffstatler, and Jeff Tillman from a site near the Leaf River in Jones County, Mississippi. It is a clump-forming perennial with short rhizomes. The grass-like basal leaves are up to 1.5-m in length and arch outwards from the base of the plant like a fountain. The flowering culms are 1.2- to 1.8-m tall, leafy, somewhat coarse, and obtusely triangular. The dense inflorescence contains numerous pale green spikelets that become brown and wooly as the seeds mature. The tiny achenes are light tan, with six long, twisting perianth bristles. Flowering begins in June and seeds mature by September. The achenes were too small to be accurately counted by PMC equipment, but it was estimated that there are upwards of 18 million per kilogram.

#### Leflore

Creeping burhead can be found in swamps, marshes, and ditches in the Southeast and lower Midwest, westward into Oklahoma and Texas (Godfrey and Wooten 1979). It is a member of the Alismataceae or water-plantain family. The Leflore source was collected by B.B. Billingsley, Jr., Joe Snider, Joel Douglas, and Janet Grabowski from a flooded lowland in Leflore County, Mississippi. Leflore is an annual or short-lived perennial that often spreads or creeps by rooting scapes. Basal leaves are broadly ovate, cordate at the base, 5- to 18-cm long and almost as wide. The principal veins are conspicuous and impressed on the upper surface of the leaf blade. Petioles are 10- to 50-cm long, enlarged and spongy towards the base. The leafy scapes are upright when young becoming prostrate, up to 1-m or more in length, producing new plantlets at the tip and nodes. Numerous whorls of 12- to 20-mm wide flowers, with three white petals and rounded, greenish centers, are produced at nodes along the scape. Flowering begins in June and continues until frost. The fruiting heads are round, bur-like clusters of small brown, flattened achenes, which have a long beak on one end. There are approximately 5 million achenes per kilogram.

#### VEEGETATIVE PROPAGATION

All of these releases can be vegetatively propagated by division of the parent plant. The large size of the shoots and rhizomes of Indian Bayou dictate that a fairly large planting piece is required. A section about 15to 24-cm long with a few shoots is ideal. Plantings made by the PMC on a Wetland Reserve Program (WRP) site have indicated that Indian Bayou propagules are capable of withstanding fairly adverse environmental conditions after planting (data not presented); these results can probably be attributed to the large amount of food reserves stored in the rhizomes.

Leaf River clumps can be dug and individual shoots divided somewhat easily. Older clumps tend to die back in the center, so actively growing shoots around the margins of the plant should be selected. It is also a good idea to discard shoots containing an old flowering culm, because these either will not regenerate or, if they do, the shoot produced will be very weak. Care should be taken in handling Leaf River plants because the edges of the leaves are very sharp and can cut deeply into flesh.

Established Leflore plants can be dug and divided into a small number of propagules. These plants do not have a very deep root system, which makes them fairly easy to dig. Small plants produced on the flowering scape can also be removed and planted.

## SEED GERMINATION STUDY Methods and Materials

Seeds were harvested in late summer to fall of 1995 and 1996 (harvest dates varied between species and between year of collection). Seeds were cleaned immediately after collection without drying. Leaf River seeds were loosened from fruit clusters using a brush machine (Westrup a/s Slagelse, Denmark) and then hand screened to remove inert matter. Indian Bayou seeds were rubbed over a roughened surface to remove the papery fruit coverings. Leflore seeds required only hand screening to remove small amounts of trash.

Three storage treatments were tested: 1) dry storage in a cooler at 13 degrees C and 45 percent relative humidity; 2) moist storage in a cooler at 5.5 degrees C; and 3) water storage in a cooler at 5.5 degrees C. Dry storage was tested on all species. Moist storage was used for all seed lots, except the 1996 Leaf River lot. Water storage was tested on the 1995 seed lot of Leaf River and 1996 Leflore only. Small quantities of seeds of each species were divided from the main lot and placed in moist and water storage immediately after cleaning. All seeds in water storage were put in a nylonmesh bag and then submerged in tap water in a glass jar. 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 algae growth. Moist stored seeds of Leaf River and Leflore were placed on a moistened brown paper towel and placed in a self-sealing plastic bag with sufficient additional water to maintain high moisture levels during the storage period. Indian Bayou seeds are too large to allow good seed contact with paper toweling, so seeds were stratified in moist sphagnum moss from

which they were easily separated at planting. All remaining seeds were dried thoroughly and placed in a self-sealing plastic bag for the dry storage treatments.

Pre-planting treatments applied to dry stored seeds were a 3- to 4-mo stratification period (Strat.), and for Indian Bayou seed only, scarification (Scar.) using mechanical means, and combinations of stratification and scarification. In 1995, seeds were scarified by placing them in a coffee can lined with sand paper and tumbling with some gravel added to increase abrasion. A concern that seeds were not uniformly scarified using this method required use of an alternate method in the second year, where seeds were individually rubbed against sandpaper. All stratification treatments were placed in the cooler during November 1995 and 1996.

Treatments were planted in the greenhouse within a one-week period in March 1996 and 1997. Stratified, moist stored, and water stored Leaf River and Leflore seeds were allowed to surface dry during the planting process to facilitate sample counting. Sample sizes used were 100 seeds of Leaf River and Leflore for each treatment and 20 seeds of the 1995 lot and 25 seeds of the 1996 Indian Bayou lot. Germination containers were 17.8-cm x 13.3-cm x 5.9-cm black plastic bedding plant liners. Growing medium used was a 3:1 peat moss/sand growing medium amended with commercially recommended quantities of pelletized slow-release fertilizer, dolomitic lime, Micromax micronutrient fertilizer, and Aquagro wetting agent. Seeds were sown on the surface of the growing medium, except Indian Bayou seeds, which were planted approximately 6-mm deep. Seeds were spread as uniformly as possible on the medium.

Two germination conditions were tested: 1) moist conditions, where containers were placed on a normal greenhouse bench and watered regularly (normal bench); and 2) continuously saturated conditions on an ebb and flow greenhouse bench (flood bench). Water on the ebb and flow bench was maintained at a depth of 6- to 12-mm except for short periods of time when the bench was drained and rinsed to remove alga growth. Experimental design was a factorial experiment in a randomized complete block with three replications arranged as a split plot, with germination condition as the main plot and seed treatment (storage condition and pre-planting seed treatment) as the split plot.

An initial seedling count was made when it was deemed that a sufficient number of seedlings were present to justify counting. Two additional counts were made at three-week intervals following the initial count. A few seedlings of some species died before the initial count, especially on the normal greenhouse bench, where the surface of the growing medium dried for short periods of time. Dead seedlings were counted and their numbers were included in the initial count because germination had occurred. Later counts included only those plants that had some green tissue. Total germination could not be determined, so results presented are from the count with highest overall number of seedlings. The study was analyzed with years of testing treated separately. Data was subjected to an analysis of variance and appropriate mean separation was performed using a least significant difference test (LSD) at P = 0.05. All data on seed lots is referenced by year of seed collection (1995 and 1996) not year of testing.

### Results and Discussion Indian Bayou germination

Indian Bayou appears to have complex germination and seedling growth requirements. Only a few seedlings have established naturally in PMC ponds,

mainly along the margin of the pond. Previous attempts at seedling production in the greenhouse indicated that germination was erratic and slow.

Initial germination percentages in this study were fairly low and seeds continued to germinate in small numbers throughout the test. For the 1995 seed lot, there was a significant response to seed treatment only (Table 1). Overall germination was better on the normal bench than on the flood bench. In this environment, seeds responded positively to moist storage and both stratification treatments. The high germination percentage for scarified plus stratified seeds compared to stratified seeds indicate that this seed lot responded to scarification.

For the 1996 seed lot, there was a significant interaction between seed treatment and germination condition. This interaction was probably due to poor germination of all treatments on the flood bench. Also, germination of dry stored seeds was poor on both benches. Germination of scarified seeds on the normal bench was significantly higher for this seed lot than any other treatment, which is curious because of the low percentage for this treatment in the previous year. The scarification method used for this seed lot was more vigorous and may have increased response to this treatment. However, this cannot explain the response of scarified plus stratified seeds, where germination was not different than stratified seeds.

In both years, seedlings on the flood bench were more susceptible to infestation by aphids than those on the normal bench, indicating that the plants were probably stressed. This improved germination and growth on the normal bench appears to corroborate observations of seedling establishment in drier conditions along the margins of PMC growing ponds. The response to stratification and moist storage indicates that Indian Bayou has an internal dormancy that is overcome by cold, moist treatment. Germination percentages were slightly higher for dry stored seeds that were subsequently stratified than for moist stored seeds regardless of treatment year, which suggests capability for long-term seed storage. Mechanical scarification, especially when accompanied by stratification also appears to be beneficial, perhaps because seeds have a waxy coating that may prevent water infiltration. A previous attempt at sulfuric acid scarification on a small quantity of seeds showed that even a short duration (< 1-min) treatment was toxic (Janet Grabowski, unpublished data). However, because mechanical scarification did not consistently improve germination, it appears that stratification alone is probably sufficient to promote germination.

### Leaf River germination

Results for the 1995 and 1996 seed lots were similar for those treatments common to both years of testing (Table 2). The 1995 lot showed a significant response to seed treatment. Dry stored Leaf River seeds appeared to germinate more quickly after stratification, but germination percentages were not significantly different from the dry storage treatment alone, which conflicts with Isley (1944) who found that woolgrass seeds require stratification for 6-mo or longer to overcome dormancy. This southern Mississippi ecotype is adapted to areas with only short periods of chilling winter temperatures and apparently does not have a dormancy mechanism that requires stratification. Garbisch and McIninch (1992) also reported a lack of seed dormancy. There was a trend towards improved germination of the 1995 seeds stored in water but the difference was significantly higher than only the dry stored seeds on the normal bench and the moist stored seeds on the flood bench. Leaf River seeds showed a significant response to germination conditions in both years of testing. Highest germination was on the flood bench with saturated conditions. Seedling mortality on the normal bench was higher for this species, 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, probably due to cooler temperatures and longer periods of cloud cover in 1997. However, later seedling growth in both years was more vigorous for surviving plants on the normal bench. Seedlings growing on the flood bench showed evidence of poor root growth due to a lack of aeration and there was extensive algae and slime mold growth on the growing medium surface, which may have been toxic to the seedlings. Most of the plants on the flood bench were barely alive by the third evaluation date. Observations made of plants growing in the wild indicate that this species requires 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 anticipated for a wetland plant.

#### Leflore germination

Mean germination percentages for all treatments were extremely low (< 5 percent) in both years (data not presented). For 1995 and 1996 seed lots, there was a significant response to germination condition, with best germination on the flood bench. However, these differences were too minuscule to support a recommendation on proper germination and seedling growth environments. Storage treatments yielded the same results in both years. Although water storage was added in the second year to see if germination could be improved over that of moist storage, no improvement was observed. Water and moist stored seeds showed a slight trend towards improved germination compared to dry storage. An attempt was made to mechanically scarify some seeds from the 1996 lot, but visual inspection after treatment did not indicate that this treatment had any appreciable effect on seed coverings. Seed germination in the PMC production ponds has been noted, mainly along the margins and in shallow water; however, this test apparently did not provide suitable conditions for germination.

## SUMMARY

These three source identified releases can potentially be used in wetland plantings in Mississippi and perhaps other areas in the Southeast. Because of their specific germination requirements, direct seeding is probably not advisable. However, greenhouse seedling production is practical for Indian Bayou and Leaf River, with stratification recommended for Indian Bayou seeds. Additional research would be necessary to determine methods to successfully propagate Leflore from seed.

#### REFERENCES

Garbisch E.W. and McIninch S. 1992. Seed information for wetland plant species of the northeast United States. Restoration and Management Notes 10:85-86.

Godfrey, R.K. and Wooten, J.W. 1979. Aquatic and wetland plants of southeastern United States monocotyledons. Athens (GA): The University of Georgia Press. 712 p.

Isley, D. 1944. A study of conditions that affect the germination of *Scirpus* seeds. Ithaca (NY): Cornell University Agricultural Experiment Station. Memoir 257. 28 p.

McNaughton, S.J. 1966. Ecotype function in the *Typha* community-type. Ecological Monographs. 36: 297-325.

U.S. Army Engineer Waterways Experiment Station. 1978. Wetland habitat development with dredged material: engineering and plant propagation. Tech. Rep. DS-78-16. Vicksburg, MS. 158 p.

USDA-Soil Conservation Service. No Date. Midwestern wetland flora field office guide to plant species. Lincoln, NE. 615 p.

Table 1.--Indian Bayou mean germination percentages for seed exposed to two storage conditions, three pre-planting treatments, and two germination conditions.

Storage/Pre-treatment	Germination Condition	1995	1996		
			%		
Dry/Strat.	Normal Bench	35	17		
Dry		8	1		
Dry/Scar. + Strat.		25	17		
Dry/Scar.		5	40		
Moist		27	15		
Dry/Strat.	Flood Bench	0	3		
Dry		0	3		
Dry/Scar. + Strat.		15	8		
Dry/Scar.		0	9		
Moist		3	1		
LSD (P 0.05)		17	13		

Table 2.--Leaf River mean germination percentages for seed exposed to various seed storage conditions, one pre-planting treatment, and two germination conditions.

Storage/Pre-treatment	Germination Condition	1995	1996	
		%		
Dry/Strat.	Normal Bench	19	17	
Dry		12	9	
Moist		15		
Water		24		
Dry/Strat.	Flood Bench	35	61	
Dry		41	57	
Moist		32		
Water		44		
LSD (P 0.05)		8	23	