

potassium concentration in the outside medium increases, potassium absorption rate into root cells increases proportionately, but only to a certain point owing to saturation kinetics caused by the limited number of K⁺ binding sites for potassium carrier enzymes on the plant cell plasma membrane.

Another unlikely possibility is an allelopathic response of brome to wheat straw. Recommendations for growing brome as a forage crop include using wheat as a cover crop during seeding, precluding a negative reaction to wheat straw. Moreover, demonstrated allelopathic relationships tend to be between forbs and grasses (e.g., Hicks et al. 1989) rather than between different grasses.

Our results indicate a negative relationship between wheat straw application and early growth of brome. This may be due to potassium leached from straw or ammonium produced during initial decomposition, or possibly some other factor generated by wheat straw. Applying wheat straw to new grassland disturbances may provide some protection against brome invasion; however, more research is required to determine the mechanisms involved, the amount of straw needed, and whether the effect continues beyond the first year. Research into the tolerance limits to potassium and ammonium would be useful, as they could be applied directly as a controlling amendment if found effective. Brome has moved from farmland and roadsides into native grasslands in many parts of North America. Study into the effects of wheat straw, potassium, and ammonium on existing smooth brome stands and in new native prairie restoration sites could determine if these could aid in removing or controlling brome.

Acknowledgments

We thank Dr. Vern S. Baron and the editors for their review of the paper and their helpful comments. We acknowledge funding from Alberta Sustainable Resource Development, Husky Energy, and Canadian Natural Resources.

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Seed Propagation Protocol for Wigeongrass (*Ruppia maritima*) (Mississippi)

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Wigeongrass (*Ruppia maritima*), is a submerged aquatic vegetation (SAV) species that occurs in broad salinity zones from near freshwater (0.5 parts per thousand) to hypersaline (> 40 ppt) conditions (Kantrud 1991). Wigeongrass reproduces asexually and sexually; it produces enormous numbers of tiny seeds that are protected by sturdy coats. Its broad salinity tolerance and versatile growth strategies have made wigeongrass the most widely distributed cosmopolitan SAV species (Kantrud 1991, Green and Short 2003). In addition, this fast growing SAV can provide favorable conditions for growth of other SAV species that require stable habitat.

Often wigeongrass is the only SAV species that can be successfully established in transitional areas between freshwater SAV beds and the declining seagrass beds in many coastal wetlands (Cho et al. 2009). Methods to produce large numbers of wigeongrass transplanting units from seeds or vegetative segments have been developed in laboratories and suggested for potential use in SAV restoration (DeLeon et al. 1997, Ailstock and Shafer 2006). However, the practical use of this plant in restoration has been underappreciated owing to large stochastic variation in its coverage and the shallow root/rhizome structures. The conventional harvesting and transplanting methods using plugs, sprigs, and staples have had limited success in wigeongrass restoration. Moreover, growing a large number of transplanting units requires a large space, advanced facilities, and trained personnel to house and maintain large (500 L) water tanks with recirculating systems and

Table 1. Collection, storage, viability, and germination rates of wigeongrass (*Ruppia maritima*) based on accumulated data (2001–2009) on phenology, biomass, seed production, and effects of seed pretreatments (stratification). The probability reflects pure chance likelihood with nondiscriminating analysis. Viability was tested using the tetrazolium red method (Cho and Sanders 2009).

Propagation Step or Variable	Probability (%) or Quantity
Locating large wigeongrass beds, given known populations and six trips per growing season, April–August	60% per population per trip
Substantial seeds on wigeongrass plants for harvest, given repeat visits to the same bed	20% per trip
Amount of randomly collected seed-bearing plant materials	20–40 liters/person/h
Weight of 1 liter of wet wigeongrass materials	100–150 g
Percentage of reproductive shoots per liter of wet plant material	10%–40%
Number of inflorescences per reproductive shoot	10–20
Maximum number of seeds per inflorescence	8
Biotic potential of wigeongrass per season	20,000–40,000 seeds/kg of wet reproductive shoots
Intact, mature seeds at the time of a field collection	1%–10% of the biotic potential
Number of intact mature seeds per liter of wet sample	25–1000
Loss of seeds during transportation, handling, and seed harvesting	40%–50%
Viability of freshly harvested mature seeds	90%–95%
Viability after dry storage at room temperature (4–12 mo.)	30%–40%
Viability after cold storage (0–5°C, 3 mo.)	45%–55%
Germination rate of freshly harvested seeds	50%–95%
Germination rate after dry or cold storage (3–12 mo.)	20%–40%

controlled environmental conditions. However, seed collection, storage, and germination do not have such demanding requirements. Therefore, restoration practitioners with SAV nurseries can involve volunteers. The hands-on participation will improve public perception of the importance of SAV habitat and restoration.

Here, we report recent developments in restoration of SAV habitats using wigeongrass seeds from the coastal habitats of Mississippi and Louisiana in the northern Gulf of Mexico. The seeds require less labor and room to harvest, handle, transport, and store than whole plants. Also, collecting only reproductive shoots for seed harvest preserves most of the plant intact in the natural beds and causes less disturbance.

Based on our research, approximately 30% of the seeds can stay viable one year after maturation owing to inherent or induced dormancy (Cho and Sanders 2009). We present a simple procedure to collect, transport, sort, pretreat, and germinate wigeongrass seeds (Table 1). The users of the protocol need minimal preknowledge, skills, or special equipment. The information presented in Table 1 can be used to plan labor needs and number of seeds to be collected in order to produce a given number of seedling units.

Volunteers will initially need help from an expert and may require a permit to collect SAV, as well as a boat to locate and access natural wigeongrass beds. Wigeongrass beds are known to display significant seasonal and annual variations in size and location; the probability of locating substantial beds is 60%, even with expert assistance (Table 1). For example, the abundant wigeongrass beds in

our biennial SAV survey sites at the Grand Bay National Estuarine Research Reserve, Mississippi, can completely disappear in the late fall and winter months (Cho and May 2008). Some wigeongrass populations in tropical and subtropical habitats also display two growing seasons per year (Cho and Poirrier 2005). Therefore, knowing the phenology of local populations will improve the probabilities of seed collection.

Wigeongrass usually grows in shallow waters up to 1.2 m deep, so the plants can easily be collected by wading in the water. Seed production time is of limited duration and varies from location to location; further, seeds are easily detached from stems by strong waves and storms, so that only 20% of collection trips will find substantial amounts of seeds available for harvest.

The plant parts containing seeds should be hand-collected, leaving the stems and roots intact. Plastic containers with floats can be used to hold the collected plant parts (Table 1). The biotic potential, the maximum reproductive capacity under optimal conditions, of wigeongrass can be estimated as 20,000–40,000 seeds produced per kilogram of wet reproductive shoots per season (Table 1). In any given collection, only 1%–10% of the biotic potential may be intact mature seeds. Transportation, handling, and seed harvesting processes cause additional losses (Table 1), which can be reduced by sieving the water from the containers (mesh \leq 1 mm) to retrieve lost seeds.

The collected plants should be transported in the habitat water in plastic containers or insulated coolers with tops. Immediately after returning to the wet laboratory, the plants need to be aerated using an aquarium pump

to prevent decaying. If most seeds are mature (dark and hard), the seeds should be harvested within 12 hours after collection. When abundant immature seeds are attached, the plant material can be held in outdoor water tanks, such as inflatable swimming pools filled with tap water mixed with the habitat water, for a few days to a week. Then attached mature seeds can be hand picked and the tank water carefully sieved to retrieve the released seeds. Manual harvesting is the most labor intensive part of the procedure, but it also assures minimal loss of the collected seeds and is feasible without a complicated seed processing system (Orth and Marion 2007). One volunteer can harvest up to 250 seeds per hour.

Viability and germination rates of freshly harvested mature seeds are generally greater than 90% (Cho and Sanders 2009), and germination rate is the highest in freshwater. Germination does not require soil or additional nutrients, and it can be easily induced in a Petri dish or similar container with 1–2 cm of freshwater. Germination itself does not require light, but seedlings should be transferred to places that receive adequate natural sunlight (at least 12L/12D) or to a growth chamber. The fastest germination (within 2–3 days after germination induction) occurs when the mature seeds are air-dried for one week, then cold treated at around 5°C for one month, then placed in freshwater at temperatures above 18°C.

The seeds can be best stored for extended time periods (> 6 mo) when air-dried or kept in the habitat water at cold temperature (1–5°C), but the viability and germination rates after long-term storage are reduced (Table 1). We currently do not have any guidance on how long the seeds can be stored at cold temperature or dried for future uses in propagation.

Once the seedlings reach 1–2 cm long, they need to be planted, for instance on biodegradable mats, to promote root anchoring. Burlap fabric cut into 9 cm × 9 cm pieces can be used. Approximately a week later, the biodegradable mats with seedlings should be transplanted to 10 cm peat pots containing one part clean sand and one part topsoil for additional nutrients. Where appropriate, the salinity needs to be increased gradually (1 ppt per day) using commercial sea salts for saltwater aquaria sold at pet stores. The target salinity for coastal restoration should be 5–10 ppt, but this is dependent on the SAV nursery conditions or the restoration site conditions. Two to three weeks thereafter, the peat pots can be transported to research or commercial nurseries, such as the Gulf Coast Research Laboratory in Ocean Springs, Mississippi, or the U.S. Fish and Wildlife Service in Pensacola, Florida. Our protocol has proven effective along the northern Gulf Coast; other coastal locales and freshwater inland sites may experience different outcomes.

Acknowledgments

The research and restoration projects are supported by grants from the NOAA-ECSC (Grant No. NA17AE1626, Subcontract #27-0629-017 to Jackson State University), Mississippi Department of Marine Resources (Tidelands Project), Mississippi-Alabama Sea Grant Consortium (Grant Number USM-GR02639/OMNIBUS-R/CEH-29-PD). We thank Yvonne Sanders and many other undergraduate students at Jackson State University who participated in field and laboratory activities.

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