Seed Propagation Protocol for *Spartina alterniflora* (Smooth Cordgrass)

Spartina alterniflora (hereafter referred to as *Spartina*) is the dominant salt marsh plant in coastal Georgia. Due to the vital role this species plays in salt marsh creation, maintenance, and ecosystem function, *Spartina* is often included as a biotic component in salt marsh restoration projects. To maximize success of nursery-produced transplants in marsh restoration, research suggests the use of local plants propagated from seed. This propagation protocol will provide methodology for the successful production of *Spartina* seedlings from seed collected in Georgia marshes.

1. Seed Collection

Spartina can be observed with inflorescences (flowering stalks) in the field as early as July or August (**Figure 1**). Inflorescences contain small, whitish flowers arranged as a spikelet and occupy the upper 20-35 cm of the stem (**Figure 2a**). Flowers are wind-pollinated and after pollination, light-brown seeds approximately 1 cm in length develop. In coastal Georgia, seed development occurs during September and October, and mature inflorescences containing seeds become more common in early to mid-October until early to mid-November (**Figure 1**). Mature spikelets can be determined in the field by examining the color of the floral stem (or peduncle) and the readiness of seed shed. Specifically, the peduncles of mature spikelets are brownish and seeds should easily shed with gentle disturbance (**Figure 2b**). Total number of seeds per spikelet varies, and previous research showed greater number of seeds per spikelet at marshes located further within the coastal system (i.e., further from ocean influence) and exposed to minimal anthropogenic disturbance. Additionally, both the number of seeds per spikelet and seed viability was greatest between mid-October to mid-November. To collect mature spikelets, a gallon-sized plastic bag should be placed over the spikelet to catch any seeds shed during collection, after which the spikelet should be removed from the stem. It is suggested that more spikelets than thought needed be collected, as many spikelets contain empty seeds with no embryos, and/or repeated visits are made to collect seeds, as not all inflorescences mature at the same time. Spikelets visibilly infected with fungus (e.g., ergot) and where birds have consumed seeds should be avoided.

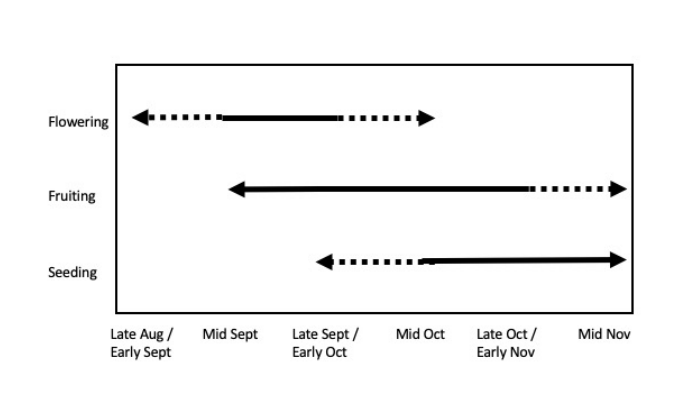

Figure 1: Reproductive status of *Spartina* in Chatham County, GA, over mutlipe sites and years. Solid lines represent greater than 10% of individuals in each reproductive state, and dottled lines represent less than 1% in each state.

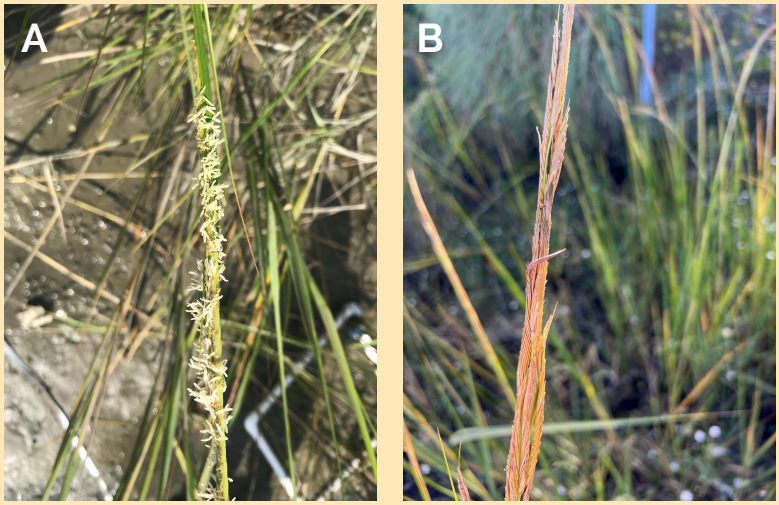

Figure 2: (A) *Spartina* inflorescence stalk with flowers. (B) Mature *Spartina* seed spikelet.

Collect seeds from mature spikelets using plastic bags between mid-October and mid-November at marshes with minimal marine influence and anthropogenic disturbance.

2. Seed Processing and Winter Storage

Once collected, seeds should be processed as soon as possible. Seeds can be easily removed from the spikelet by hand (**Figure 3**) and then placed in sealable plastic baggies half filled with freshwater. Spartina seeds are recalcitrant, which means that seeds cannot survive dry conditions. For best results, seeds should be stored at 10°C (50°F) for at least two months to break seed dormancy. Seeds are remain viable for approximately one year after collection.

Figure 3: Seeds can be easily removed from the spikelet by hand.

Figure 4: Filled and unfilled seeds as shown on a fluorescent light box.

3. Seed Germination

After at least two months of cold, wet storage, seeds should be removed from plastic bags and rinsed with freshwater water using a No. 16 or 18 sieve to avoid loss of seeds during rinsing. It has been reported that *Spartina* spikelets have a high occurrence of empty or sterile fruits (i.e., no embryo), so it is suggested that seeds be viewed on a fluorescent light box to determine the presence/absence of an embryo. Filled seeds, or those with embryos, will appear dark while unfilled seeds will appear lighter and almost translucent (**Figure 4**). Unfilled seeds can be discarded to minimize space and resources.

For germination, filled seeds should be place in glass culture dishes filled approximately halfway with freshwater. For best results, seeds should be placed under 12:12 day:night light conditions and daytime:nighttime temperatures of 24°C: 35°C (75°F: 95°F). Seeds should be checked regularly for germination, and freshwater should be added as needed to maintain water levels.

Seeds should be stored under cold conditions in sealable plastic baggies filled halfway with freshwater. After at least 2 months, seeds can be placed in glass culture dishes with freshwater for germination.

4. Seedling Growth and Maintenance

Once the shoot of germinated seeds is approximately 2.5 cm (1 inch) in length, seedlings should be planted in hydrated peat pellets (e.g., Jiffy-7 peat pellets) using forceps and placed in a heavy-duty plant tray. Seeds should maintained on a growth cart (or other growing area) under a 12:12 day:night light cycle for 1-2 weeks, after which seedlings can be maintained under greenhouse conditions. When seedlings reach heights of 15 cm (~6 inches), they should be transplanted into 15 cm (6-inch) diameter pots and a 2:1 topsoil:sand potting mixture. To maximize growth, salt marsh soil collected from natural marshes can be added to potting mixture. We recommend ~4 cups of salt marsh soil for a 15 cm diameter pot. Pots of seedlings should be placed in heavy duty plant trays and subirrigated with freshwater. Plants should be fertilized monthly.

Alternatively, *Spartina* seedlings can be maintained in an aboveground constructed wetland. Benefits of this alternative form of production include less labor, reduced water use, and growing conditions more similar to natural salt marshes, which may improve transplant success in restoration projects. Aboveground wetlands can be constructed with minimal cost and even be attached to a freshwater aquaculture system to provide an organic source of nutrients. Research has shown that seedlings maintained in an aboveground weltand designed to remediate freshwater aquaculture wastewater acheived equal growth to those maintained using traditional methods.

For questions, contact Dr. Heather Joesting at hjoesting@georgiasouthern.edu.

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