

**REPRODUCTIVE BIOLOGY OF SMOOTH CORDGRASS**  
**(*SPARTINA ALTERNIFLORA*)**

A Thesis

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## ABSTRACT

Smooth cordgrass (*S. alterniflora*) is a perennial grass that dominates the salt marsh in tidal wetlands along the Atlantic and Gulf Coast of North America and has been used for preventing soil erosion and restoring wetlands. Accessions collected from south Louisiana were studied to investigate flowering phenology, pollen viability, crossability, and seed production. *S. alterniflora* exhibited protogynous flowering where stigmas exerted 2 to 5 days from the floret prior to anthesis. Pollen shed primarily between 8:00 and 10:00 AM. Pollens were viable with average germination of 69% and stigma was receptive after exertion. Pollen germinated in 15 minutes and pollen tubes reached micropyle in 55 to 75 minutes after contacting stigma. Protogyny could be used to produce controlled hybrid without emasculation and it could reduce tedious labor required for making crosses.

*S. alterniflora* was cross-pollinated with 52% seed set for cross-pollination and 26% for self-pollination. Flowering started in early July and ended by the middle of October with a flowering peak between early September and early October. During the flowering peak, seed set, kernel weight, and seed viability were positively correlated to flowering date while unfilled and total seeds/panicle were negatively correlated with flowering date. Kernel weight, flowering date, seed weight/panicle, and panicle height were positively correlated with seed set. Plant flowering during the peak period might produce better seed set and seed weight. Field investigation showed an average seed set of 47% with range from 0 to 94% for *S. alterniflora*, which provided large opportunity for selection. Several lines with improved characteristics were selected and would be valuable for recurrent selection program with an objective of developing improved *S.*

*alterniflora* populations. However, short term breeding objective should be focused on selection of plants from native collection that have high percentage of seed set, germination, and broad adaptability.

## CHAPTER 1. LITERATURE REVIEW

### 1.1 Introduction

*Spartina alterniflora*, commonly known as smooth cordgrass, saltwater cordgrass, or oyster grass, is a perennial grass native to the Atlantic and Gulf Coast of North America and dominates the salt marsh community in tidal wetlands along the Atlantic seaboard and Gulf Coast. *S. alterniflora* is the most important species in seaside habitats, covering vast areas to the exclusion, or nearly so, of other species. It is also found on the Pacific Coast of the United States, where it is considered an invasive species. Duncan and Duncan (1987) reported that *S. alterniflora* plants have soft stems and hairy sheath margins with leaves up to 40 cm long and 1.25 cm wide. Leaf blades are flat, not scabrous on the margins. *S. alterniflora* has smooth flowers, lacking hairs, and panicles are erect to arching. The panicle is usually 10 to 30 cm long and composed of five to thirty spikes alternately arranged and appressed to main axis with ten to fifty sessile spikelets along one side of the axis of each spike. It has narrow terminal inflorescence. Plants grow in the high salt marshes, especially at edges of saltpans. Plants may be only 40 cm tall including the inflorescence in some areas. *S. alterniflora* spreads in mid to high tide levels in salt and brackish marshes and is of major ecological importance as a habitat for fish, birds, mammals, and invertebrates, and as primary producer of organic matter for coastal food chains.

Due to vigorous spreading ability and strong underground rhizome growth, *S. alterniflora* has been used for preventing soil erosion and restoring wetlands along coastal areas. The species colonizes primarily by vegetation when pieces of its roots, or of whole plants, float into a suitable area and become established. It can also spread by

seeds that float into a suitable area and germinate. Once established, *S. alterniflora* spreads vegetatively and forms ring-shaped clumps of individual clones. These clones are tall and conspicuous in open mudflats. New stems grow along the outer edge, gradually increasing ring diameter with each growing season. As plants age, dying vegetation can often be found in the middle of these rings. As clones spread they grow into each other and form a dense single-species meadow. The success of *S. alterniflora* can be attributed to its high rate of spread, tall and dense canopy that can shade out other plants, and its ability to colonize low intertidal regions.

Callaway and Josselyn (1992) compared vegetative and reproductive characteristics between *S. alterniflora* and the native grass, *S. foliosa*, in South San Francisco Bay. The characters such as intertidal distribution, phenology, aboveground and belowground biomass, growth rates, seed production, and germination rates were investigated. They concluded that *S. alterniflora* has a wider intertidal distribution than *S. foliosa* and out produces the native species in all aspects that were studied. *S. alterniflora* has a much better chance of becoming established in new areas than the native species, and once established, it spreads more rapidly vegetatively than the native species.

*Spartina* species are among the few salt marsh plants that have been introduced outside their native range for erosion control due to their abilities to colonize open areas, stabilize eroding shorelines, and reclaim land. They have been intentionally or accidentally introduced into countries such as the United Kingdom, France, The Netherlands, New Zealand, and China. Many of these introductions have been regarded as successful for the purposes intended. *S. alterniflora* was accidentally introduced into

the United Kingdom in the mid 1800's, possibly with shipping ballast (Doody, 1982). In 1950's the New Zealand Department of Agriculture introduced *S. alterniflora* from Florida to replace less successful *S. anglica* in the north island and it successfully acclimatized in most of the northern harbors (Bascand, 1970). *S. alterniflora* was introduced into China in 1979 because of its potential ability for producing higher biomass. Five years later, successful plantings amounted to 260 ha (Zhou and Xu, 1985). The introduction was made to stabilize coastlines; accelerate land reclamation; produce green manure, animal fodder, fish feed and fuel; increase invertebrate production; and partially control waterway siltation and pollution (Chung, 1993). Its value as green manure for rice culture, goat and fish feed and raw material for making paper has also been demonstrated.

Unfortunately, there are growing concerns about the negative impacts of *S. alterniflora* in regions where it has been introduced and it is now considered an invasive species. Aberle (1993) outlined negative effects of *Spartina* invasion as degradation of wildlife habitat, threat to aquaculture, loss of native plant communities, sedimentation, loss of property values, wrack deposition, and effects on insect populations. *S. alterniflora* has become an aggressive invasive species after it was introduced into areas along the Pacific Coast of United States. *S. alterniflora* was placed on the noxious weed list of Washington and Oregon State in 1989 and 1990 respectively. California does not recommend it for further introductions or for use in saltmarsh restoration projects. However, in the Atlantic and Gulf coasts, *S. alterniflora* is still the most important species for soil erosion control and wetland restoration.

## **1.2 Wetland Losses and Restoration**

### **1.2.1 Wetlands in the United States**

Wetlands are areas where water covers the soil, or is present at or near the surface of the soil, all year or for varying periods of time during the year, including during the growing season. Wetlands include marshes, swamps, bogs, and similar areas found in generally flat vegetation areas, between dry land and water along the edges of streams, rivers, lakes, and coastlines.

Wetlands are defined by the U.S. Army Corps of Engineers (COE) and the U.S. Environmental Protection Agency (EPA) as “areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and under normal circumstances do support, a prevalence of vegetation typically adapted to life in saturated soils”. The COE cites the following situations as strong evidence of wetlands occurrence: 1) Area lies in a floodplain or contains low spots on or above the soil surface for more than seven consecutive days during the growing season. 2) Plant communities are present in areas having standing water for part of the growing season. 3) Area has soils known as peats, mucks or heavy clays. 4) Area is periodically flooded by tides including strong wind-driven or springtides. Wetlands help regulate water levels within watersheds, improve water quality, reduce flood and storm damage, provide important fish and wildlife habitat, and support hunting, fishing, and other recreational activities.

Today, in the 48 mainland states of the United States, there are approximately 4,168,410 sq. km. of wetlands, reduced more than 53% from the 8,943,870 sq. km. estimated during the colonial times (LSU Agricultural Center, 1995). Wetlands have

been tremendously reduced due to expanding population and growing need for food, fiber and housing.

### **1.2.2 Coastal Wetlands Losses**

Coastal wetlands are among those wetlands not specifically associated with agricultural activities. They have ecological value in primary production, nutrient cycling, as habitat for fish, birds and other wildlife and in stabilizing shorelines.

Louisiana has 14,164.5 sq. km. of coastal wetlands, currently accounting for more than 40% of the salt marsh in the contiguous United States. These coastal wetlands are more productive than many intensely used agricultural lands. However, coastal wetlands have been experiencing catastrophic losses due to development activities and soil erosion.

Louisiana accounts for 80% of the nation's coastal land loss at rates ranging between 64.75 to 90.65 sq. m. per year. At this rate, Louisiana could loose another 2132.769 sq. km. of coastal wetlands by the year 2050 (<http://www.savelawtlands.org/olesite/newaffects.html>).

Louisiana's coastal wetlands provide habitat for fisheries, waterfowl, neotropical birds and fur bearers; protection for oil and gas exploration and production, and waterborne commerce; amenities for recreation and tourism; flood protection; and the context for the unique Cajun culture. Continued losses of Louisiana's coastal wetlands will deprive Louisiana, the Gulf Coast Basin, and the nation as a whole, of vitally important fish, wildlife, and other wetland-related economic and environmental benefits. The economic value of the wetlands is enormous, and its benefits go well beyond the local and state levels by providing a positive economic impact for the entire nation.

### 1.2.3 Restoration

Recovery of marshes after natural or human disturbance is often slow under natural conditions. Banking the shorelines is an effective way to minimize soil erosion and water loss and results in significant improvements immediately. However, it is costly and requires continuous maintenance after the banks have been built. Replanting vegetation can accelerate marsh restoration, greatly reduce expense, and be maintained longer. Along the east coast of the U.S., *S. alterniflora* is the dominant angiosperm in regularly flooded marshes and is the principal species used in marsh re-establishment projects (Woodhouse et al., 1974, 1976; Garbish et al., 1975). It has also been used effectively to stabilize shorelines along the Gulf Coasts (Dodd and Webb, 1975).

Successful marsh restoration by vegetation depends on site selection and plant management. Broome et al. (1988) pointed out some factors influencing marsh restoration: (1) Marsh slope: Within limits, the more gentle the slope, the more area on which marsh can be established. Slopes must be sufficient for good surface drainage to prevent ponding and subsequent increases in salinity due to evaporation. Slopes of 1-3% are preferable. (2) Salinity variation of estuaries: Substrate salinities > 45 ppt (parts per thousand) may prevent establishment of *S. alterniflora* at mid-latitudes (35 °N) along the eastern coast of U.S., whereas transplants of *Puccinellia maritime* (Huds.) Parl. may survive.

### 1.3 Biological Study

Knowledge of taxonomy, genetics, reproductive biology, and physiology is important for understanding development of *S. alterniflora* within a region and may assist in germplasm collection and plant improvement. Since *S. alterniflora* is a strong invasive

species, studies have been conducted on reproductive mechanism to understand how this species hybridizes with the native species and occupies new areas. This knowledge could also be used for genetic improvement of this species.

### 1.3.1 Genus and Chromosome Number

The genus *Spartina* belongs to the *Poaceae* (grass) family and includes 17 species worldwide (Mobberley, 1956; Partridge, 1987) (Table 1.1). *Spartina* species are perennial rhizomatous wetland grasses that occur primarily in estuarine systems, but are also found in inland freshwater habitats. The natural distribution of these species centers on the Atlantic coast of North and South America with smaller populations occurring on the west coast of North America, Europe, and Africa (Partridge, 1987).

*S. alterniflora* has two major growth forms, generally recognized as “short form” (less than 45 cm in irregularly flooded high marsh) and “tall form” (higher than 45 cm in regularly flooded low marsh), found in North America (Valiela et al., 1978).

Controversy exists as to whether the height forms differ genetically or simply reflect differences in the environment to which they are exposed. Church (1940) reported existence of two levels of polyploidy within *S. alterniflora* and indicated that they were morphologically and ecologically distinct. The short form, *S. alterniflora* var. *glabra* (Muhl.) Fern, was characterized as being octoploid ( $n=7$ ) with 56 chromosomes and the tall form, *S. alterniflora*, var. *pilosa* (Merr.), was characterized by being more robust decaploid ( $n=7$ ) with 70 chromosomes. However, Mobberley (1956) concluded that it was impossible to correlate taxonomic differences with chromosome ploidy level. Marchant (1970) conducted a cytological survey of *S. alterniflora* populations in Canada and north-eastern U.S.A. and excluded the basic number of 10 for *S. alterniflora* and

stated that both the tall and short growth forms have the same chromosome number of 62, which is anomalous with respect to the basic number of  $x = 10$  generally found in the genus. It is not very clear where the two additional chromosomes in the complement of *S. alterniflora* originated, but by inference from basic number in all other species, it may still be assumed that they represent a tetrasomic condition.

**Table 1.1. Species of the Genus *Spartina* (Mobberley, 1956).**

<i>S. arundinacea</i>	<i>S. alterniflora</i>	<i>S. bakeri</i>	<i>S. ciliata</i>	<i>S. foliosa</i>
<i>S. X caespitosa</i>	<i>S. spartinae</i>	<i>S. longispica</i>	<i>S. cynosuroides</i>	<i>S. maritima</i>
<i>S. densiflora</i>	<i>S. neyrautii</i>	<i>S. gracilis</i>	<i>S. townsendii</i>	<i>S. anglica</i>
<i>S. patens</i>	<i>S. pectinata</i>			

*S. alterniflora* shows a range of morphological and physiological variation over wide geographic areas as well as within marsh areas. Seneca (1974) conducted a two year study on morphological and physiological differences among separate geographic populations of *S. alterniflora* and concluded that four broadly distributed populations exist along the Atlantic and Gulf coasts of the United States (New England, the Mid-Atlantic, the South Atlantic and the Gulf coasts). O'Brien et al. (1999) employed randomly amplified polymorphic DNA (RAPD) to assess the genetic variability in tall form *S. alterniflora* collected from the Atlantic and Gulf coasts and found that the plants could be classified into three geographic areas (New England, South Atlantic, and Gulf coast), closely approximating the three types defined by Seneca (1974).

Genetic diversity exists in *S. alterniflora*. Gallagher et al. (1988) investigated within-marsh growth forms of *S. alterniflora* in a 9-year study and indicated that these growth forms have a genetic basis, either in the basic coding information or regulatory mechanisms, that becomes permanently set at an early development stage. Anderson and Treshow (1980) pointed out that plant height in *S. alterniflora* might be determined by a

combination of environmental and genetic factors. Genetic differences within *S. alterniflora* among locations may provide enhanced opportunity for plant improvement.

Daehler et al. (1994) investigated viable reproductive output among clones of *S. alterniflora* in San Francisco Bay and found that inflorescences of self-pollinated plants generally had lower seed set than controls, and none of the self-pollinated seeds were viable. They concluded that spikelet viability is not a function of clone size and there is some genetic control of spikelet viability in *S. alterniflora* as shown by the uniformity of seed set within large clones.

### **1.3.2 Physiological Study**

#### **1) Photoperiod and Temperature**

*S. alterniflora* responds to a regime of shortening day lengths for plant growth and flowering. Seneca and Broome (1972) conducted a controlled-environment study by exposing seedlings to a series of thermoperiods under both short- and long-day photoperiods and the results indicated that *S. alterniflora* seedlings grown under short-day conditions (9-hr high intensity light period) were shorter, had a lower biomass, and produced more culms and rhizomes than those under long-day conditions (9-hr high intensity light period plus a 3-hr low intensity interrupted dark period) at the same temperatures. Flowering occurred earlier under short-day than under long-day conditions in the 22-26 °C and 26-30 °C temperature regimes. In another controlled-environment and field study, Seneca (1974) found there were considerable morphological and physiological variations among various populations of *S. alterniflora* from the Atlantic and Gulf coasts. Southern populations flowered later, exhibited a longer growing period, and were less sensitive to changes in photoperiod than northern populations.

## 2) Salinity

*S. alterniflora* is defined as a facultative halophyte. It can tolerate a broad range of salinities from near fresh water to ocean concentrations. Salt crystals can often be seen on the leaves during the growing season. Germination tests by Mooring et al. (1971) indicated that *S. alterniflora* seeds germinate best in fresh water, and that a gradual decline in germination occurs as salinity is increased.

Study in South Carolina Department of Natural Resources showed that *S. alterniflora* growth was negatively correlated with soil salinity. The highest growth rate occurred at salinities of 20 ppt or less, with the upper limit for salt tolerance being 60 ppt. Marshes with soil salinities above 75 ppt did not tend to have stands of *S. alterniflora*. Height of *S. alterniflora* was also inversely related to soil salinity levels. Along the creek and riverbanks where soil salinity level is lowest, *S. alterniflora* reached a maximum height of three meters. As the soil salinity increased with distance from the riverbank, the average height of the species decreased from one meter on the levee to less than 50 cm tall at the upper reaches of salt marsh where the soil salinity level was highest (<http://www.csc.noaa.gov>).

## 3) Seed Dormancy

*S. alterniflora* sets seed in the fall and seeds are subsequently dispersed but remain dormant until early the next spring (Mooring et al., 1971). Seed propagation is an important method by which *S. alterniflora* moves into new area. The initial establishment of seedlings is very important in determining which species will dominate at a new location. Seedling establishment rates are determined by seed production, seed germination, and seedling survival.

Dormancy can help seeds survive through cold winter. Plyer and Carrick (1993) studied seed dormancy in *S. alterniflora* with regard to the specific site of the dormancy mechanism and the influence of three growth-regulating substances: gibberellic acid (GA3), fusicoccin (FC), and abscisic acid (ABA). The site of the mechanism was determined by assessing the germinability of surgically altered dormant seeds. It was found that dormancy could be broken surgically by altering the scutellum or chemically by applying fusicoccin. Dormancy could be restored by abscisic acid in the former case but not the latter. The results showed that the dormancy mechanism is located in the scutellum and consists of at least two sequential steps, and also involves a leachable chemical inhibitor.

#### **4) Fertilization**

Nutrient supplies in regularly flooded salt marshes are usually adequate for growth of *S. alterniflora*. However, freshly deposited or exposed sandy substrates are usually deficient in nutrients, particularly nitrogen and phosphorus (Woodhouse and Knutson, 1982). As with many terrestrial plants, *S. alterniflora* requires a small amount of fertilizer at the time of planting. Practice, in Marine Program of Cornell Cooperative Extension of Suffolk County Marine Program (<http://www.seagrant.sunysb.edu/Pages/FactSheets-PDF/AmericanBeachgrass.pdf>), recommended the use of slow release fertilizer (approximately 30 gm per plant) that will provide nutrients throughout the first growing season and minimize nutrient release to the environment. A balanced fertilizer with a minimum 3-4 month release period is recommended for all plantings. Materne (2001) recommended high nitrogen slow-release fertilizer tablets, between 15 and 25

grams and having a nitrogen content of not less than 15% or more than 30%, be used in saturated and/or anaerobic soils.

However, in some studies, plant growth in fertilized plots was not significantly different from unfertilized plots. Webb et al. (1989) studied *S. alterniflora* response to fertilizer, planting dates, and elevation in Galveston Bay, Texas. The results showed that elevation was the dominant factor affecting parameters measured rather than fertilizer. The use of fertilizers did not appear necessary and produced no significant differences in height or density.

### **1.3.3 Reproductive Biology**

Although *S. alterniflora* salt marshes are a valuable natural resource, protection and preservation of these wetlands has been ignored for a long time. In the past, many acres of *S. alterniflora* have been destroyed by man's activities. Consequently, creating new salt marshes has become a desirable objective (Woodhouse et al., 1972).

Understanding reproductive biology of *S. alterniflora* may help to improve this species and contribute to marsh restoration.

#### **1) Flowering Phenology**

*S. alterniflora* flowers annually with variable flowering dates through out its geographic distribution. Mobberley (1956) noted that flowering is from June to October in North America, from December to June in South America, and from July to November in Europe. Eleuterius et al. (1984) observed *S. alterniflora* flowers between September and November in Mississippi. However, the length of flowering period for individual plant species varied from year to year, affected by change of temperatures in the spring. In South San Francisco Bay, *S. alterniflora* begins to flower in late July / early August

and sets seed in late September / early October (Callaway and Josselyn, 1992). Seneca (1974) associated earlier flowering with short photoperiods and with more northerly seed sources.

## **2) Pollination**

Protogynous flowering is a common phenomena among grass species in which stigmas exert prior to anther dehiscence. This process allows anthers from the early flowers to dehisce pollens as neighboring stigmas are becoming receptive and increases the possibility for cross-pollination.

In *S. alterniflora*, soon after the inflorescence emerges from the sheath of the uppermost leaves, white stigmas exert from the flowers, starting at the tip of the inflorescence and progressing toward the base. In most cases, however, anthers appear on each spikelet shortly after the stigmas of the same flower have turned brown and presumably are no longer functional. This phenology of anthesis suggests that inflorescence might be largely open pollinated, but that there might be some self-fertilization of late-appearing stigmas by anthers of the same inflorescence. Somers and Grant (1981) observed that inflorescences emerge over a period of about 3 to 4 weeks and provide abundant opportunities for cross-pollination among different inflorescences with little self-pollination of spikelets on any inflorescence. They found only 3.6% of the spikelets from inflorescence enclosed in plastic tubes contained seed and 8.5% of those enclosed in paper bags contained seeds while on control plants 62.8% of the spikelets contained seed. Their results indicated that cross-pollination predominates in *S. alterniflora* flowering.

*S. alterniflora* produces abundant pollen of high fertility. In a study on the threat to native *S. foliosa* by invasion and hybridization with *S. alterniflora* in San Francisco Bay, Anttila et al. (1998) observed that *S. alterniflora* exceeded *S. foliosa* in all components of male fitness and yielded about 21-fold greater pollen production per square meter. Pollen of *S. alterniflora* also had a higher germination rate than *S. foliosa*. As an invasive species, *S. alterniflora* poses a hybridization threat proportional to its advantage over natives in gene flow. In the environment where it grows as a native species, *S. alterniflora* may have a better chance to mix with other clones and adapt itself to specific environments.

### **3) Seed Set**

Reproduction from either rhizomes or seed is necessary for rapid colonization of bare substrates. Seed is an effective way to spread *S. alterniflora* into a new place. In both native and introduced *Spartina* populations, seed output has been found to be variable (Broome et al., 1974; Bertness et al., 1987; Sayce, 1988; Callway and Josselyn, 1992) and *S. alterniflora* marshes have been noted for their unpredictable seed production across years (Hubbard, 1970; Broome et al., 1974). In general, seed viability is typically low and seeds only remain viable for about one year (Sayce, 1988; Mooring et al., 1971). There are reports in the literature that *S. alterniflora* produces very few viable seeds (Chapman, 1960; Larimer, 1968) and that seeds are not as important as rhizomes in spreading this grass.

Daehler and Strong (1994) collected seed samples from more than 200 clones and observed germination rates ranging from 0% to 59%, indicating substantial variation in reproductive output among clones. Pollination manipulations showed that *S. alterniflora*

is outcrossing, but pollen supplements did not increase seed germination rates. Seed viability was not related to clone size; however, clones located lower in the intertidal region or far up a drainage slough averaged fewer germinations per spikelet, suggesting clones in areas with lower genetic density may have lower spikelet viabilities.

Inflorescences in the self-pollinated treatment generally had lower seed set than controls, and none of the self-pollinated seeds were viable.

#### **4) Seed Propagation**

Transplants are more vigorous and survive well on exposed sites and at lower elevations, but transplanting is labor intensive compared to seeding. Seeding is an economical and effective method of establishing *S. alterniflora* in salt marshes.

**Seed Harvesting and Storage:** Selecting the proper location is important in harvesting a good seed supply. The quality and quantity of seed produced varies greatly from one stand of *S. alterniflora* to another. Broome et al. (1974) pointed out that the most vigorous plants set the most seeds and these are generally found in areas recently colonized by *S. alterniflora*. There is little flowering and seed production in the short height zone areas of older marshes. Taylor (1938) made a similar observation on Long Island, New York, and reported that thick stands of *S. alterniflora* did not flower.

Broome et al. (1974) noted that excellent germination occurred when seeds were harvested as near the shattering stage as possible, stored in burlap sheets at 2 to 3 °C for one month, and submerged in estuaries or sea water at 2 to 3 °C until seeding the following spring. Seed stored longer than one year was of little use. For handling large seed quantities, Broome et al. (1988) further developed the technique and suggested that seed should be harvested as near as possible to maturity or just prior to shattering and

transferred to large burlap sacks which are then transported to an area for temporary storage or threshing. Seed can be easily threshed in a thresher designed for small grain following storage at 1 to 4 ° C for ~ 1 month. After threshing, seed must be stored in covered containers filled with estuarine water, sea water or artificial sea water of ~ 35 ppt salinity at 2 to 4 ° C.

**Artificial Seeds:** Biotechnology can play an active role in marsh restoration. Techniques to solve the low seed viability problem in *S. alterniflora* and speed up the seeding process are being developed. Utomo et al. (2000) developed a tissue culture protocol for clonal propagation of *S. alterniflora* in which a half-gram of plant callus tissue could be induced to regenerate into 25 or more plantlets. The encapsulated micro-plantlets can have 86% germination rate and can be stored at 1 to 5 ° C inside closed containers under a low light intensity for 6 weeks without loss of viability. This technique can produce individual plantlets encapsulated within a protective gel to form the equivalent of a plant-produced seed. The artificial seeds have the potential to be used as a substitute when plants fail to produce seeds naturally, or when they produce only a small number of fertile seeds. This technique might overcome poor seed production in *S. alterniflora* and might make large-scale plantings possible. However, it will take a long time to put into practice in marsh restoration.

#### **1.4 Plant Improvement**

A wide range of morphological and physiological variation exists in *S. alterniflora* and these inherent genetic differences (ecotypes) have resulted from prolonged growth of the various populations in their natural locales. This extensive variation may provide us the basis for plant improvement. However, there is little

information concerning how to genetically improve *S. alterniflora*. Since it is a cross-pollinated perennial grass, establishing a breeding program by learning from successful experiences with other grasses could be tried.

Breeding self-pollinated crops involves exploiting the homozygous nature of the individual plants, while breeding of cross-pollinated species is based on the heterozygous nature of the individual plants. Therefore, in cross-pollinated crops, the breeders' focus is on population improvement rather than individual plants, and more emphasis is given to quantitative inheritance in breeding systems than in self-pollinated crops (Poehlman and Sleper, 1995).

There are a few unique reproductive and breeding characteristics for cross-pollinated perennial grasses. Vogel and Pederson (1993) summarized in a review on cross-pollinated grass breeding as follows: cross-pollinated by wind in nature and largely self-incompatible so it has not been feasible to develop and maintain inbred lines; small floral parts, making hand emasculation tedious and difficult; many of the grasses are polyploids, which complicates inheritance of traits, and most traits are controlled by numerous genes while few genes have been determined or mapped due to complex inheritance and the inability to self-pollinate plants; vegetatively propagated by stolons, rhizomes, tillers, or buds on culms; highly heterozygous and substantial additive genetic variation exists in most grasses for most agronomic traits; and due to use in thickly seeded stands as forages or turf grasses, individual plant selection is not possible under these conditions and evaluation and selection is usually done in space-planted nurseries. Breeding for cross-pollinated grasses aims to maximize the utilization of additive genetic variation.

## **1) Ecotype Selection**

At the initial stage of breeding a new species, it is necessary to collect, assemble, and evaluate germplasm for the specific region. Ecotype selection can lead to rapid development and release of excellent cultivars (Vogel and Pedersen, 1993). Ecotype selection was not developed by any single individual but rather evolved over time. Collecting and bulking seed from many plants at a site is preferable to collecting and maintaining seed collections from individual plants for cross-pollinated grasses since the objective is to obtain a representative sample of the genes at a site. Space-planted evaluation plots give the breeder an opportunity to observe the relative amount of phenotypic variation within accessions and to make within accession selection from the original evaluation trials. Data from the evaluation trials are used to select the best ecotypes or accessions.

## **2) Recurrent Selection**

Recurrent selection attempts to increase the frequency of desirable alleles for particular quantitatively inherited characters by repeated cycles of selection. Ecotype selection can be used to assemble, evaluate, select, and intermate germplasm to produce the necessary base populations for recurrent selection. Burton (1974, 1982, 1992) developed a more efficient and effective form of recurrent selection known as restricted recurrent phenotypic selection (RRPS) in perennial forage grass breeding. The method stratified the selection nurseries into smaller selection units and improved realized gains from selection.

There are two known *S. alterniflora* cultivars, 'Vermilion' and 'Bayshore', both released by the Natural Resources Conservation Service Plant Material Program

(<http://plants.usda.gov>). ‘Vermilion’ was released in 1989 for use in the Gulf of Mexico northern basin and it has been used in Louisiana wetland restoration projects. ‘Bayshore’ was released in 1992 for use on the Atlantic Coast. The original clone of ‘Bayshore’ was collected from a shore along Chesapeake Bay and was increased vegetatively for testing. ‘Bayshore’ was selected for release because of its consistently good rhizome spread and production and its ability to survive while submerged under water for long periods during the establishment year. Although ‘Bayshore’ is capable of seed production, all commercial propagation is vegetative (Hamer et al., 1994).

### **1.5 Objectives**

Rapid vegetative spread by rhizomes, associated with tolerance to a wide range of salinity, makes *S. alterniflora* an ideal plant for coastal wetlands stabilization. However, the high cost of vegetative establishment of this species has limited its potential for rapid stabilization of disturbed wetland areas. The project “ Biological approaches to coastal wetlands restoration” undertaken by LSU Agricultural Center and USDA-NRCS aims at improvement and utilization of *S. alterniflora* to help build the coastal marshland in Louisiana. The project involves collection and characterization of native populations of *S. alterniflora* from south Louisiana, investigation of reproductive biology, and development of a breeding program to produce lines with improved seed set and vegetative characteristics.

As a part of the project, this investigation was carried out to understand in detail the reproductive biology of *S. alterniflora*, which will be helpful in establishing a breeding program in the future. Therefore, the objectives for this thesis research were: (i) to investigate the flowering habit in *S. alterniflora*; (ii) to ascertain the possibility for

hybridization; (iii) to examine flowering phenology of *S. alterniflora* in south Louisiana; (v) to investigate relationships between flowering date and seed production and provide information on seed production for further plant selection.

## **CHAPTER 2. MODE OF REPRODUCTION, POLLEN VIABILITY AND CROSSABILITY IN *SPARTINA ALTERNIFLORA***

### **2.1 Introduction**

Plant improvement programs involve creation of genetic variation followed by selection of genotypes with desirable attributes. Knowledge of the mode of reproduction is essential to determine the appropriate breeding methodology for improving a particular plant species. Since gene recombination is the most common mechanism utilized to create new genotypes in plant improvement programs, it is necessary to understand the details of pollination, fertilization, and seed development for the species.

Self- and cross-pollination are the main reproduction methods in sexually reproducing plant species. Breeding self-pollinated crops is based on exploiting the homozygous nature of individual plants. The techniques for breeding self-pollinated crops are well developed and include assembly of germplasm, hybridization, pedigree selection, mass selection, recurrent selection, and hybrid development (Poehlman and Sleper, 1995). Breeding methods for cross-pollinated species differ from those for self-pollinated species because the breeders focus on population improvement as well as line development in cross-pollinated crops. Recurrent selection is largely used and several modified recurrent selection procedures have been developed (Burton, 1992).

Even though mating system differences exist, hybridization is the common method to obtain gene recombination between genetically different parents. Hybridization is affected by species, flowering nature, and environment. Emasculation is the process of removing anthers in bisexual flowers to prevent self-pollination. It allows hybridization of selected individuals to produce genetically superior progeny with a combination of desirable traits from both parents. Although emasculation is time-

consuming and tedious, it is necessary in many crops to make hybridization possible. However, in some grass species, it is impossible to emasculate the florets because the small florets are likely to be damaged during emasculation and because of the large number of florets on an inflorescence.

Protogyny occurs in species such as walnuts, pearl millet, avocados, and many perennial grass species. In protogynous flowering, stigmas exert before anther dehiscence. Protogynous species have stigma receptivity prior to pollen shed therefore promoting cross-pollination. Protogynous flowering offers a significant advantage in making crosses since it eliminates the tedious emasculation process (Shafer et al., 2000).

*S. alterniflora* dominates the Atlantic and Gulf coasts of North America and has great ecological value. It is propagated by seed or vegetatively and exhibits protogynous flowering. *S. alterniflora* has complete florets, however, the floret palea are tightly closed and are easily broken when emasculating the florets.

Since controlled crossing is an important step in plant improvement, it is necessary to understand the flowering behavior of *S. alterniflora* and the limitations to get viable seeds from cross-pollination. Information about stigma receptivity, pollen viability, timing of pollination, and time needed for the pollen tube to reach the ovary and micropyle is critical for successful hybridization.

There are several steps that lead to successful hybridization. After pollen lands on the stigma, the pollen interacts with the pistil in a series of sequential events: pollen germination, pollen tube penetration into stigma, pollen tube growth through style, pollen tube entry into ovule and embryo sac, and double fertilization. Failure to accomplish any of these steps will prevent successful seed setting.

Pollen studies have been conducted to determine limitations to successful hybridization prior to double fertilization. Pollen viability is an important parameter since pollen must be viable and capable of germination at the time of pollination in order to have successful seed set. Heslop-Harrison et al. (1984) defined viability as “ the competence of individuals of a given pollen population to deliver male gametes to the embryo sac”. Since aniline blue fluorechrome (ABF) has affinity for callose (a carbohydrate in pollen tubes), the aniline blue fluorescence (ABF) method has been an important technique for studying the progressive phases of pollen tube growth, enabling visualization of callose in pollen tubes (Dumas and Knox, 1983). ABF has been used in dallisgrass (Burson, 1987), blue panicgrass, and kleingrass (Burson et al., 1983) to study self- and cross-incompatibility during the hybridization process.

Buffelgrass is predominantly cross-pollinated and some accessions exhibit protogynous flowering behavior where the stigmas are extruded from the florets prior to anther exertion. Shafer et al. (2000) observed that buffelgrass exhibited protogynous flowering with the protogynous intervals ranging from 1 to 4 days. Across all accessions, pollens germinated within 15 min of contacting the stigma, and pollen tubes reached to the micropyle within 2 to 6 hours, depending on the accession and the pollen source. Mean seed set ranged from 11 to 76% and from 22 to 80% among accessions following self- and cross-pollination, respectively. Their investigation revealed that variation exists for protogynous interval within buffelgrass, and the stigmas are receptive when exerted from the floret and remain receptive throughout the protogynous interval. This finding demonstrated that protogyny could be used to produce controlled hybrids in sexual buffelgrass without emasculation.

Although considerable studies on growth and development of *S. alterniflora* have been carried out, lack of information on reproductive biology is a major handicap for initiating a systematic breeding program. Determining the primary mode of reproduction is an important task when initiating a plant improvement program. This is because it can help to indicate the amount of genetic variability that can be expected from plant introductions or among cultivars of a species. More importantly, it can help to determine how the existing variability can be most readily exploited. Therefore, objectives of this part were (i) to determine the duration of the protogynous intervals within accessions, (ii) to ascertain pollen viability and receptivity of stigmas in self, direct, and reciprocal crosses, and (iii) to determine seed set under both self- and cross-pollination.

## **2.2 Materials and Methods**

### **2.2.1 Materials**

One hundred and twenty six *S. alterniflora* accessions were collected in the fall of 1998 (Table 2.1). This collection included 10 elite lines, from an earlier collection by the Plant Material Center of NRCS (including 3 accessions from Texas), and 116 native accessions from 11 parishes across southern Louisiana. A native accession was identified as a group of clones with a similar phenotype within a small geographic area. About 100 panicles for each accession were harvested and kept in plastic bags. The collections were stored in a cold storage room (5° C) at the Ben Hur Farm, LSU Agricultural Center, Baton Rouge, LA. In January 1999, seeds were threshed by hand from the panicles for each accession. Seeds were kept in plastic containers (500 ml) with salt solution (0.4% NaCl and 0.05% Vitavax 200) and stored in a refrigerator at 5° C. Vitavax 200, a fungicide manufactured by Gustafson, contains 17% carboxin and 17% thiram and its

application for grain seed treatment is 2.06-2.74 g/kg (www.agsco-agdepot.com/infosheets).

**Table 2.1. Geographical distribution of *S. alterniflora* accessions collected in south Louisiana during the fall of 1998.**

Parish	No. of accessions	Latitude	Longitude
Calcasieu	14	30°3'17.79"N~30°13'9.31"N	93°14'36.11"W~93°22'6.22"W
Cameron	37	29°37'49.29"N~30°0'21.43"N	92°45'50.01"W~93°53'46.34"W
Iberia	2	29°35'41.63"N~29°35'47.47"N	92°0'25.24"W~92°0'32.03"W
Jefferson	11	29°11'22.79"N~29°38'39.51"N	89°58'34.21"W~90°5'36.30"W
Lafourche	13	29°4'3.79"N~29°30'20.32"N	89°57'33.06"W~90°23'48.41"W
Orleans	2	30°1'26.89"N~30°2'41.24"N	89°44'10.01"W~89°49'25.23"W
Plaquemines	4	29°18'53.43"N~29°34'40.19"N	89°40'13.70"W~89°54'20.82"W
St. Bernard	6	29°44'23.48"N~29°59'20.73"N	89°28'20.41"W~89°55'35.97"W
St. Tammany	2	30°9'45.10"N~30°10'23.55"N	89°37'43.37"W~89°40'25.23"W
Terrebonne	15	29°3'12.19"N~29°18'12.98"N	90°28'43.90"W~91°0'44.81"W
Vermilion	10	29°33'55.34"N~29°40'31.53"N	92°2'23.56"W~92°32'1.01"W
Other	10	Elite lines from earlier collection by the Plant Material Center of NRCS, USDA, including 3 accessions from Texas.	

### 2.2.2 Protogynous Intervals

Germination tests were performed on all accessions after seeds were hand threshed from the panicle. Twenty accessions were selected based on seed germination test and eighty plants (four plants/accession) were transplanted into individual pots (20 cm diameter and 18 cm high) in the greenhouse. The pots were filled with a mixture of Jeffy-Mix Plus (Jiffy Products of America), soil, and sand (1:1:2). The pots were maintained in fiberglass pans (376 cm x 150 cm x 7.5 cm) filled with fresh tap water in the greenhouse. Insecticides, 'Orthene' and 'Pounce', were applied to the plants as needed.

The protogynous interval of flowering accessions was checked and verified under the greenhouse conditions. Data collection included dates for panicle emergence, stigma exertion, and anthesis. The date of first stigma exertion in a panicle was recorded on a tag. The florets on each tagged inflorescence were examined daily to determine the first date of anthesis. The interval between stigma exertion and anthesis is the protogynous interval. All flowering panicles for each accession were recorded and dates were averaged by accession.

### **2.2.3 Pollen Viability, Stigma Receptivity and Seed Set**

Plant 98NR30BG1 was identified as good pollen producer during 1999 flowering season. To investigate crossability of *S. alterniflora*, it was used as the common male parent for crosses with nine female plants. In order to make sufficient crosses, plants that flowered around the same time as 98NR30BG1 were identified and clonally increased. The pots were kept in fiberglass pans, filled with fresh tap water, in the greenhouse. Bamboo sticks (180 cm long) were used to support the panicle after a cross was made. The greenhouse was maintained at approximately 28 °C under natural light conditions and photoperiod. The greenhouse cooling system was automatically managed by setting the thermostat at 28° C. Once 28° C was reached, the exhaust fans come on pulling air through a cooling pad. The cooling pad was made of thin, paper like wood fiber that had water circulating through it.

#### **1) Pollination Techniques**

Hand pollinations were made to investigate stigma receptivity, pollen tube growth, and crossability. Panicles of the female parent were bagged with dialysis tubing (Spectra/Por®4 Membranes, tubing was cut into 40 cm pieces for use) prior to stigma

exsertion. Dialysis tubing was used to prevent pollen contamination and avoid excessive moisture buildup around the inflorescences. Before making a cross, the tubing was removed from the panicle and the florets were checked for stigma exsertion. Female panicles were prepared by cutting off all florets, about  $\frac{1}{4}$  of the panicle, which had already exserted stigmas. The florets at the lower  $\frac{1}{3}$  of the panicle were also cut to avoid self-pollination. Stigma exsertion on the remaining panicle was fairly uniform resulting in increased cross-pollination with reduced risk of self-pollination.

Pollen shed was at its maximum between 8:00 and 10:00 AM. A piece of watch glass was used to collect pollen from the male parent during this morning hours. Immediately after pollen collection, a fine camel-hair-brush (# 9) was used to transfer pollen onto the female parents by softly touching the stigma. After hand pollination, the panicle was re-bagged with dialysis tubing, tied to a bamboo stake and left until seed harvest.

## **2) Stigma Receptivity and Pollen Viability**

Crosses, reciprocal crosses and self-pollination, were made from two accessions (98NR30BG3 and 98NR7BG3) to study pollen tube growth and stigma receptivity. Crosses were made as described previously. To determine stigma receptivity and pollen viability, floret samples were taken at pre-determined intervals following pollination. The first sample was taken 15 minutes after initial pollination and subsequent samples were collected at 20 minutes intervals up to 115 minutes after pollination. Samples were fixed and stored in FAA (63% ethyl alcohol, 5% formaldehyde, and 5% acetic acid). Ten pistils were randomly dissected from the floret sample and prepared for examination under fluorescent microscopy by a modification of Kho and Baër's (1968) technique.

Pistils were placed in 1 N NaOH for 15 minutes, transferred into 0.1% (w/v) aniline blue solution for at least 30 minutes, and examined with a Olympus BH-2 microscope equipped with a reflected light fluorescence attachment (BH2-RFL) (Burson, 1987).

Pollen germination was determined by counting the number of germinated and non-germinated pollen grains on each stigma. Three viewing sections under the microscope were counted. At each sampling interval, pollen tube growth was recorded as the maximum distance the tubes had grown into each pistil. The number of pollen tubes at each developmental stage (to styles, to ovary, and to micropyle) was recorded. Pollen germination and stigma receptivity were determined under both self- and cross-pollination (direct crosses and reciprocal crosses).

### **3) Seed Set**

The common male parent, NR9830BG1, was crossed with nine plants from seven accessions to investigate crossability in *S. alterniflora*. Crosses and reciprocal crosses were made following above-mentioned technique. Self-pollination was made by bagging the panicle in dialysis tubing before pollen shed. All crosses remained bagged with dialysis tubing until harvest. The panicles were harvested individually in December 2000 and kept moist in plastic bags and refrigerated at 5° C. Panicles were threshed by hand and total seeds and filled seeds were counted for each panicle. A fluorescent light box (Model 1012) was used to help distinguish unfilled seeds from filled ones. Most unfilled seeds were transparent when placed under the fluorescent light. Any seed that was not distinguishable by light was manually manipulated to determine its status. Seed set was calculated by dividing filled seeds with total seeds.

#### **2.2.4 Self vs. Open Pollination**

In order to compare the effect of self- vs. open- pollination on seed set, plants that flowered around the same time were used. Plant materials for this study were obtained from LSU Rice Research Station, Crowley, LA. In July 1999, 10 accessions at flowering were taken from the Crowley field nursery and transplanted into a greenhouse at Baton Rouge. The accessions were designated from 'A' to 'J'. After one year's growth in the greenhouse, three accessions ('D', 'G', and 'J') with similar flowering date and plant height were selected. In 2000, each accession was vegetatively repotted into 12 pots resulting in a total of 36 pots (20 cm x 18 cm).

A complete randomized block design (RBD) was used with self and open pollination as treatments. The experiment was repeated in three locations (Ben Hur Farm, Gourrier, and East Campus Greenhouse). At each location, four plastic pools (150 cm x 30 cm) were filled with fresh water to contain pots. One pool, designated DGJ, contained one pot of each of the three accessions for open-pollination. The remaining three pools, designated DDD, GGG, and JJJ, contained 3 pots of the same accession for self-pollination. Within each location, individual pools were set at least 15 meters apart to avoid cross-pollination among accessions. Self- and open-pollinations were made naturally. After determination of flowering and start of seed ripening, polyester bags (45 cm x 40 cm) were used to wrap the panicles and capture mature seeds upon shattering in late November. Bamboo sticks (180 cm long) were used to support the plants and the polyester bags were tied to the bamboo sticks. Three panicles from each pot were harvested individually and stored in a plastic bag with a wet paper towel. The following data were collected; total seeds, filled seeds, unfilled empty seeds per panicle, seed

weight per panicle, and germination % on filled seeds. Seed determination was done under a florescent light box as described previously. Percentage of filled seeds over total seeds was calculated.

### **2.2.5 Data Analysis**

Data were analyzed by SAS (SAS Institute, 1999). Standard analysis of variance (ANOVA) procedures were used as appropriate. Least significant difference (LSD) was used for mean separation differences at 0.01 or 0.05 probability levels.

## **2.3 Results and Discussion**

### **2.3.1 Protogynous Intervals and Flowering Habits**

There was considerable variation in flowering time among accessions. Among 20 accessions planted in the greenhouse in 1999, plants within accession showed different flowering status. All plants in two accessions (98NR27 and 98NR71) failed to flower. Some accessions (98NR7 and 98NR26) had good flowering in some plants but did not flower in other plants. This was probably caused by genetic difference among plants within accession. Poor flowering in some accessions might be also caused by insufficient vegetative growth due to late June transplanting in 1999. Accession 98NR7, 98NR30, and 98NR107 demonstrated good flowering ability with long panicles and abundant pollens. 98NR30BG1 was identified as a good pollen provider and was used in the subsequent crossing tests.

Replicated observations on inflorescences for eight accessions showed that the protogynous interval, the period between stigma exertion and anthesis, averaged to 3.4 days with the range from 2.3 to 5 days (Table 2.2). There were significant differences among accessions. Plants within accession also showed variation. This interval is

**Table 2.2. Protogynous flowering interval of *S. alterniflora* accessions collected in south Louisiana and grown in the greenhouse at Baton Rouge, LA during 1999.**

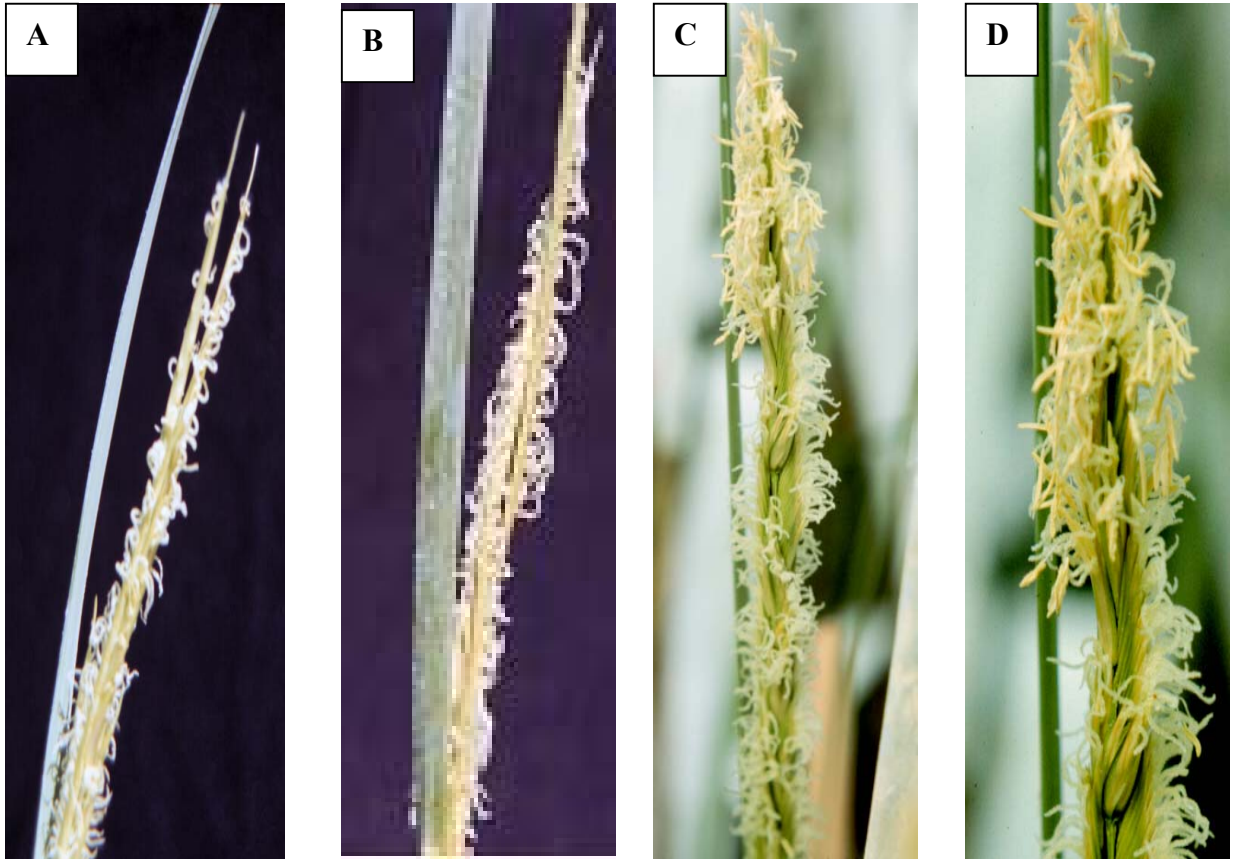
Accession	Number of panicles	Protogynous interval (day $\pm$ SE)
98NR19	3	2.3 $\pm$ 0.44
98NR30	2	3.5 $\pm$ 0.55
98NR38	3	4.0 $\pm$ 0.44
98NR47	3	4.0 $\pm$ 0.44
98NR60	5	3.0 $\pm$ 0.35
98NR61	2	2.5 $\pm$ 0.55
98NR107	2	5.0 $\pm$ 0.55
98NR109	2	3.0 $\pm$ 0.55
Mean		3.41
LSD <sub>0.05</sub>		1.49

sufficient to allow foreign pollens to fertilize with the female in its own florets. This result for protogynous interval did not agree with Somers and Grant (1981) who observed that the peak for anther emergence was about 2 weeks later than that for stigma emergence in one *S. alterniflora* population. Protogynous flowering suggests that abundant opportunities exist for cross-pollination to occur among *S. alterniflora* plants.

After emergence from the sheath of the flag leaf, panicle started to exert stigmas. Stigma exertion started at the upper 1/3 part of the panicle and proceed simultaneously to the top and bottom of the panicle. Anther exertion and pollen shedding followed the same pattern as stigma exertion (Fig. 2.1).

The blooming of *S. alterniflora* was normally occurred between 8:00 to 10:00 AM after early morning dew in plant was gone. Maximum pollen shedding occurred during this period and is was the best time for collecting pollen and making crosses. The phenology of anthesis suggested that the inflorescence might be largely cross-pollinated by foreign pollen. However, late exerting stigmas could be self-pollinated by pollens

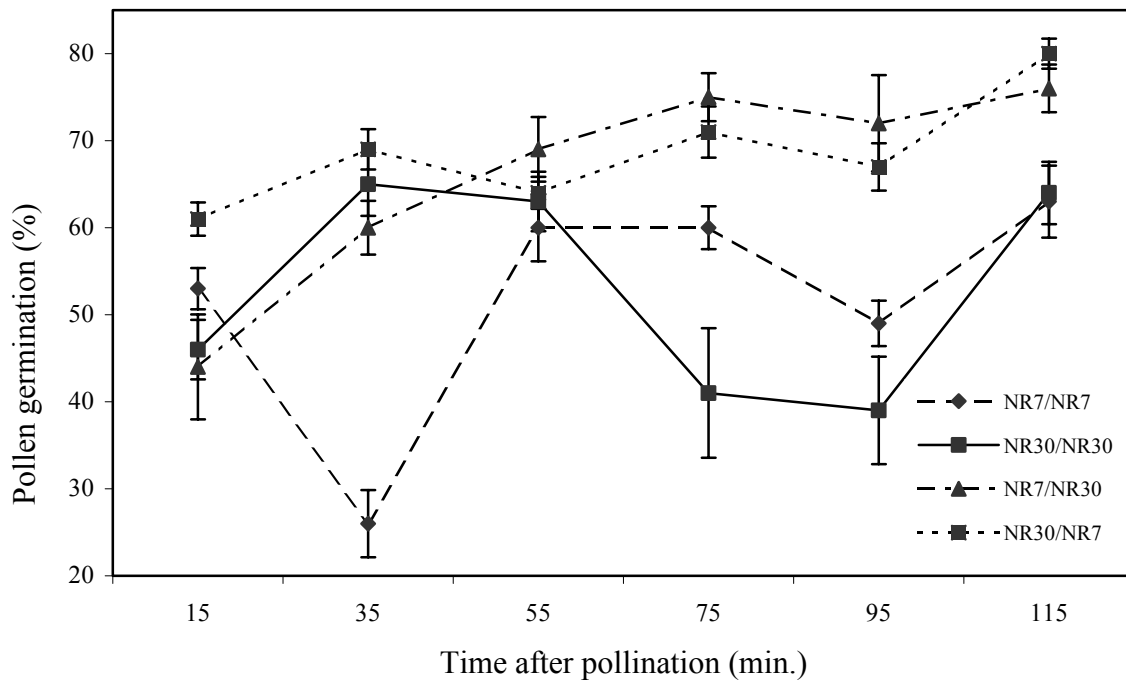
released from the top anthers in the same inflorescence. Information, such as the whole time required for the panicle to complete flowering, is called for in order to better understand the flowering process.



**Fig. 2.1. Protogynous flowering and sequence of flowering events in *S. alterniflora*. A and B. Stigma exertion: starting at the upper 1/3 part of the panicle and progressing simultaneously toward the top and bottom of the panicle. C and D. Anther exertion and pollen shedding: following the same pattern as stigma exertion (C and D: Courtesy of S. Harrison).**

### 2.3.2 Pollen Viability and Stigma Receptivity

In order to investigate pollen viability and stigma receptivity in *S. alterniflora*, 98NR7BG3 (NR7) and 98NR30BG3 (NR30) were used as parents to make crosses, reciprocal crosses, and selfs in the greenhouse. Florets were sampled after pollination and pollen germination and pollen tube growth was recorded.



**Fig. 2.2. Pollen germination on stigma for self- and cross-pollination of two *S. alterniflora* accessions grown in the greenhouse during 2000.**

Pollens started germination immediately after contact with the stigma. Pollen tubes were observed within 15 min after pollen landed on stigmas in both self- and cross-pollination (Table 2.3). Average pollen germination under self-pollination was 52% for NR7 and 53% for NR30. Average pollen germination under cross-pollination for cross NR7/NR30 and its reciprocal cross NR30/NR7 was 66% and 69% respectively. Pollen germination of accessions within self- or cross-pollination was not different. However, cross-pollination had higher germination than self-pollination (Fig. 2.2). Even though significant differences exist, both self- and cross-pollination produced high pollen germination. The overall mean of germination was 69% in this study, which was still less than pollen germination rate observed by Daehler and Strong (1994) who counted about 90% viability of pollen in four clones in California. There was sufficient viable

pollen growth to provide the basis for successful fertilization. These results suggest pollen viability is not a limitation for seed development in *S. alterniflora*.

**Table 2.3. Pollen germination on stigma and pollen tube growth for self- and cross-pollination in two *S. alterniflora* accessions grown in the greenhouse during 2000.**

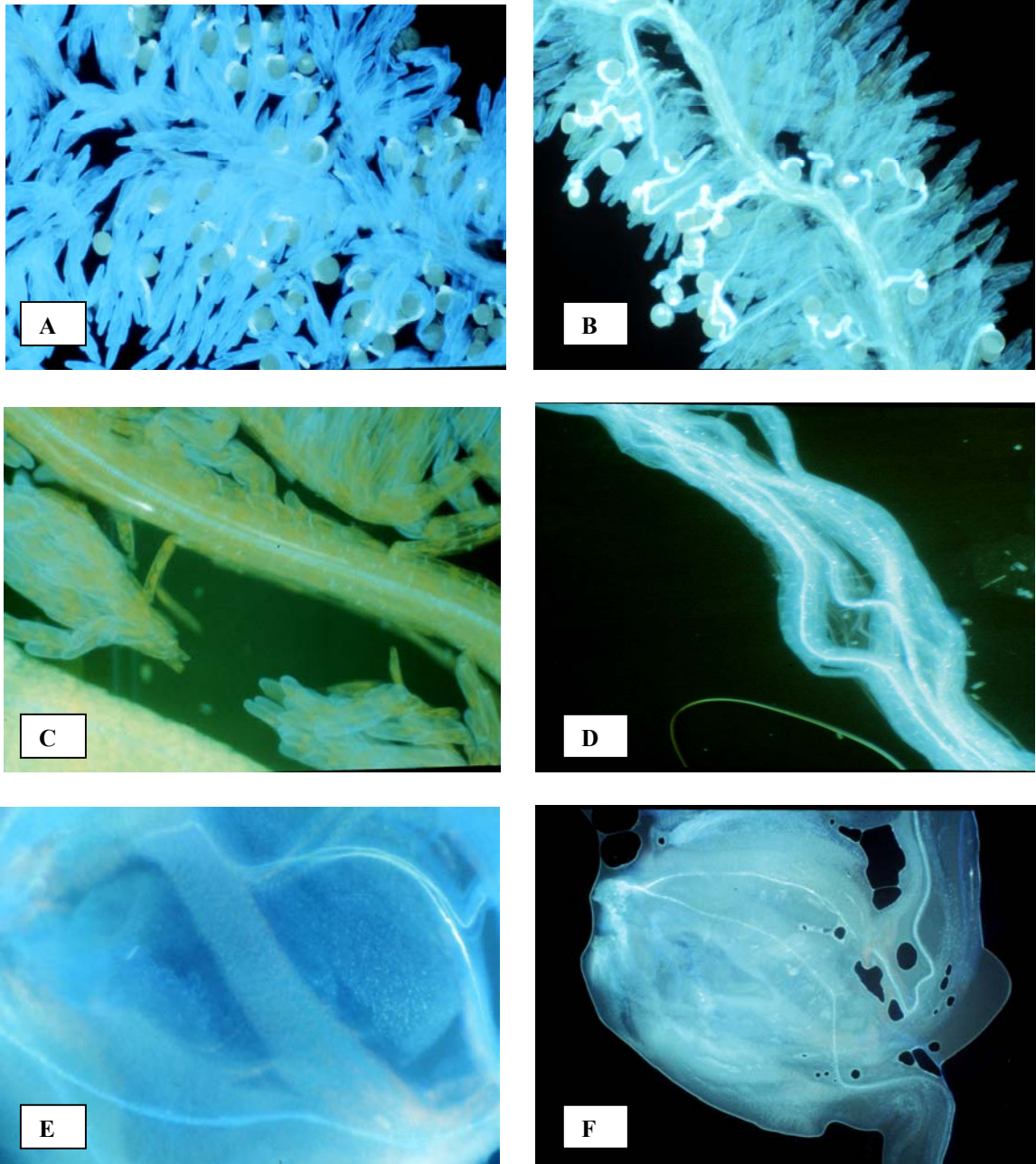
Cross	Time after pollination min	Pistils observed	Pollen grains observed no	Pollen germination %	Tubes to:		
					Style	Ovary	Micropyle
98NR7BG3/98NR7BG3 (NR7/NR7, Self)	15	10	2263	53	5	1	0
	35	10	1309	26	11	2	0
	55	10	1881	60	19	15	10
	75	10	2822	60	16	15	11
	95	10	1776	49	20	11	8
	115	10	1112	63	27	17	15
Mean				52 <sup>b†</sup>			
98NR30BG3/98NR30BG3 (NR30/NR30, Self)	15	10	2084	46	3	0	0
	35	10	2467	65	8	1	0
	55	10	2772	63	11	5	2
	75	10	1148	41	15	5	4
	95	10	732	39	23	12	8
	115	10	1375	64	6	4	1
Mean				53 <sup>b</sup>			
98NR7BG3/98NR30BG3 (NR7/NR30, Cross)	15	10	1877	44	0	0	0
	35	10	3000	60	10	2	0
	55	10	2311	69	18	10	3
	75	10	2084	75	24	16	9
	95	10	1444	72	28	16	10
	115	10	3704	76	25	14	8
Mean				66 <sup>a</sup>			
98NR30BG3/98NR7BG3 (NR30/NR7, Reciprocal)	15	10	3322	61	0	0	0
	35	10	3289	69	4	0	0
	55	10	3174	64	10	4	0
	75	10	3756	71	15	11	2
	95	10	3101	67	19	13	5
	115	10	2585	80	36	20	10
Mean				69 <sup>a</sup>			

† Means with the same letter are not significantly different at  $LSD_{0.05}=4.3$ .

After germination in the stigma, pollen tubes penetrated the stigma papilla, elongated and grew through the stigma branches and into the central axis of the stigma (Fig. 2.3). The whole process from pollen placement on stigma to pollen tubes reaching the micropyle ranged from 55 minutes to 75 minutes, depending upon accessions and types of pollination. NR7 had faster pollen tube growth than NR30, regardless of self- or cross-pollination. In NR7, pollen germination and tube growth into the ovary occurred within 15 minutes after pollen contacted the stigma and it took 55 minutes for pollen tube growth to the micropyle in both self- and cross-pollination. For self-pollination in NR30, pollen reached the style 15 minutes after contacting stigma, the ovary in 35 minutes, and to the micropyle in 55 minutes. Under cross-pollination, pollen tube growth in case of NR30 was similar to self-pollination but 20 minutes late for each stage. These results indicated that pollen tube reached the micropyle within 55 to 75 minutes after contacting the stigma. *S. alterniflora* was readily fertilized by cross- and self-pollination. Pollen germination occurs once pollen contacts the stigma. Pollen germination and viability were more than adequate for pollination. Observation on pollen tube growth showed that tubes proceeded through the micropyle into the female gametophytes and fertilization occurred. Stigmas were receptive after exertion and hybridization should be a routinely successful procedure.

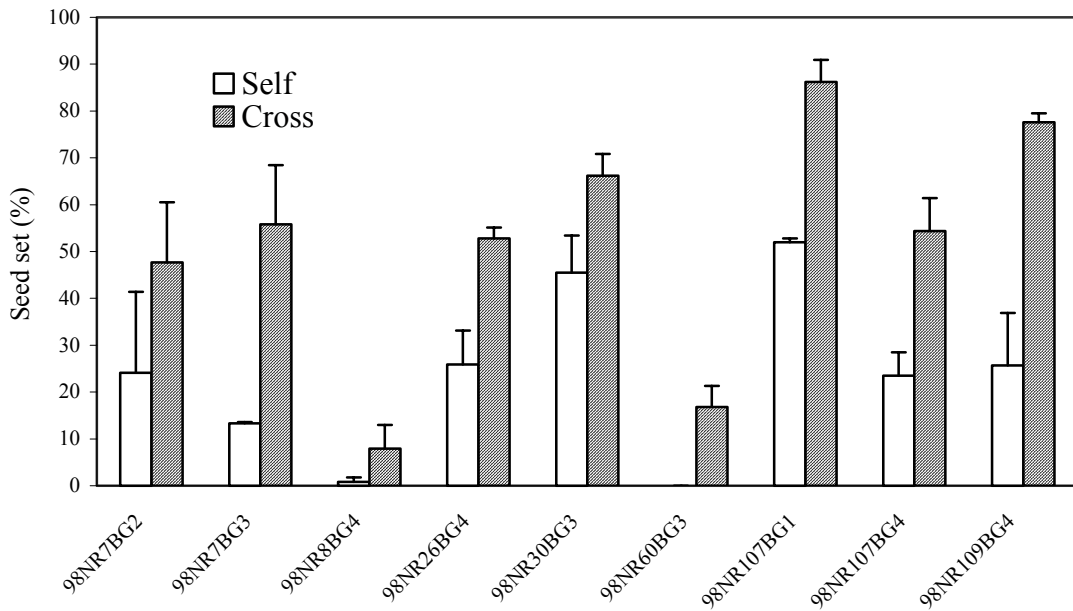
### **2.3.3 Seed Set**

*S. alterniflora* seed set for cross-pollination was significantly higher than that for self-pollination (Table 2.4). Average seed set for cross-pollination was 52% with a range of 8% to 86% while average seed set for self-pollination was 23% with a range of 0 to 52%. Plants appeared to have consistent trend for seed setting in both self- and cross-



**Fig. 2.3. Fluorescent micrographs showing various developmental stages of pollen grain germination and pollen tube growth following pollination in *S. alterniflora*. A. Germination of pollen on stigma papillae 15 minutes after pollination (x100); B. Penetration of pollen tube into the stigma and growth along the style (x100); C and D. Elongation of pollen tubes along the style and toward the ovary in 20 minutes after germination (x200); E and F. Pollen tubes reaching the micropyle in 55~75 minutes after pollination (x200).**

pollinations. Plants with low seed set for self-pollination also had low seed set for cross-pollination (Figure 2.4). 98NR8BG4 and 98NR60BG3 had low seed set (0.8% and 0%, respectively) when selfed and they had low seed set when crossed with the common male parent (7.9% and 16.8% respectively). Likewise, those plants that set more seeds when selfed also had better seed set when crossed.



**Fig. 2.4. Seed set of selfs and crosses with the common male for nine *S. alterniflora* plants grown in the greenhouse during 2000.**

Crosses and their reciprocal crosses were made in the greenhouse to compare seed set (Table 2.5). Results showed there was no significant difference for seed setting between cross and reciprocal cross. Average seed sets for cross and reciprocal were 53.9% and 65.9% respectively. 98NR107BG4 and 98NR109BG4 showed the largest difference for seed set (42.1%) between cross and reciprocal cross. 98NR7BG3 and 98NR30BG3 showed the lowest difference (1.5%) between cross and its reciprocal. It indicated that maternal effects for seed setting were not significantly different in *S. alterniflora*.

**Table 2.4. Seed set of selfs and crosses with the common male parent for nine *S. alterniflora* plants grown in the greenhouse during 2000.**

Cross	Panicles	Total seeds	Filled seeds	Seed set
		/panicle	/panicle	set
		no		%
<u>Self</u>				
98NR7BG2/98NR7BG2	2	626	151	24
98NR7BG3/98NR7BG3	2	1173	157	13
98NR8BG4/98NR8BG4	2	379	3	1
98NR26BG4/98NR26BG4	2	748	194	26
98NR30BG3/98NR30BG3	2	767	349	46
98NR60BG3/98NR60BG3	2	717	0	0
98NR107BG1/98NR107BG1	2	281	146	52
98NR107BG4/98NR107BG4	2	431	101	24
98NR109BG4/98NR109BG4	2	280	72	26
Mean				23
<u>Cross with common male parent</u>				
98NR7BG2/98NR30BG1	3	713	340	48
98NR7BG3/98NR30BG1	3	634	354	56
98NR8BG4/98NR30BG1	2	315	25	8
98NR26BG4/98NR30BG1	2	927	489	53
98NR30BG3/98NR30BG1	2	252	167	66
98NR60BG3/98NR30BG1	4	439	74	17
98NR107BG1/98NR30BG1	4	312	269	86
98NR107BG4/98NR30BG1	5	264	143	54
98NR109BG4/98NR30BG1	2	343	266	78
Mean				52
Mean (diff)				28**
SE (diff)†				5

\*\* Significant difference at the 0.01 probability level.

† Standard error for the difference between means of cross and self.

Due to the protogynous flowering nature of *S. alterniflora*, cross-pollination is likely to predominate during the flowering period (Daehler and Strong, 1994). We observed that *S. alterniflora* produced less seed in self-pollination than that in cross-pollination. This result shows a similar reducing trend for self-pollination as observed by Somers and Grant (1981), who found a 20-fold reduction in seed set from self-pollinated inflorescences in a Delaware population.

**Table 2.5. Seed set for crosses and reciprocal crosses in *S. alterniflora* grown in the greenhouse during 2000.**

Cross	Panicles	Total seeds	Filled seeds	Seed Set
		/panicle	/panicle	Set
		no		%
98NR7BG2/98NR107BG4	1	346	128	37
98NR107BG4/98NR7BG2	3	188	113	60
98NR7BG3/98NR30BG3	1	770	578	75
98NR30BG3/98NR7BG3	1	636	487	77
98NR107BG1/98NR109BG4	3	173	119	69
98NR109BG4/98NR107BG1	4	214	107	50
98NR107BG4/98NR109BG4	3	392	136	35
98NR109BG4/98NR107BG4	2	320	246	77
Mean (cross) <sup>†</sup>				54
Mean (reciprocal) <sup>‡</sup>				66
Mean (difference) <sup>§</sup>				12 <sup>†NS</sup>
SE (difference) <sup>¶</sup>				13

<sup>†NS</sup> Not significant at the 0.05 probability level.

<sup>†</sup> Average for seed sets of four direct crosses.

<sup>‡</sup> Average for seed sets of four reciprocal crosses.

<sup>§</sup> Difference of means for direct and reciprocal crosses.

<sup>¶</sup> Standard error for difference between direct and reciprocal crosses.

This investigation revealed that *S. alterniflora* varied in protogynous intervals from 2 to 5 days. Stigmas were receptive upon exertion from the florets, and remained receptive until anthesis. These findings demonstrated that the protogynous nature of *S. alterniflora* could be used to produce hybrids in sexual genotypes without resorting to tedious, time-consuming hand emasculations. Hand pollination can increase seed production and the species can produce adequate seeds to support a plant improvement program. However, it is necessary to develop a safe method for preventing self-pollination while making hybridization. Morphological markers and molecular markers could be used to determine if hybridization is successful. It has been observed that *S.*

*alterniflora* stem colors, green or purple, might be a useful genetic trait for identifying crosses. Molecular markers can identify crosses efficiently and may be a better choice.

### 2.3.4 Self vs. Open Pollination

Results from self vs. open pollination study showed that there were significant differences for seed set and germination of filled seeds between the accessions. However, pollination type (self or open) did not affect seed weight, seed set, or germination (Table 2.6). Among accessions used in this study, there were no significant differences for seed set between accession ‘G’ (50%) and ‘D’ (42%) but both ‘G’ and ‘D’ were significantly higher than accession ‘J’ (12%). For germination of filled seeds, ‘G’ and ‘J’ were not significantly different, with average germination rates of 91% and 88% respectively; both were significantly higher than ‘D’ (73%).

**Table 2.6. Mean squares from the analysis of variance for effects of self- and open-pollination on seed weight per panicle, seed set, and germination on filled seeds for *S. alterniflora* grown at Baton Rouge, LA during 2000.**

Source	Degrees of freedom	Seed weight /panicle	Seed set	Germination
Replications	2	0.11	579.06	119.21
Accessions	2	0.41	2386.12*	534.60*
Pollination types	1	0.08	904.54	120.64
Accession x pollination type	2	0.01	466.09	47.56
Error	10	0.17	318.56	94.56

\* Significant at the 0.05 probability level.

Since *S. alterniflora* is protogynous flowering species, long time exposure of stigmas before pollen shedding might encourage cross-pollination among plants. However, there were no significant differences for seed set between self and open pollination under natural condition in this study. Poor seed set might be due to lack of pollen during flowering, resulting in insufficient pollination. Since each pool used for

open-pollination (DGJ) contained only one plant from each accession, wind, rain washing or other factors at flowering might have resulted in insufficient pollen from neighboring plants for successful pollination. Since much more pollens would be available for successful open pollination in field conditions, further investigation is required for a meaningful conclusion.

## CHAPTER 3. FLOWERING PHENOLOGY AND SEED PRODUCTION IN *SPARTINA ALTERNIFLORA*

### 3.1 Introduction

Plant has long been recognized as an excellent adjunct to the erection of physical structures in erosion control. Due to its vigorous vegetative growth and strong colonizing and stabilizing ability, *S. alterniflora* becomes one of the most used species for erosion control in the Atlantic and the Gulf Coastal marshes. The Coastal Wetlands Planning, Protection and Restoration project (CWPPRA), initiated in Louisiana in 1990, placed emphasis on revegetation of areas damaged by both man-made and natural forces. Plant has been used to help slow wave action and bind soil together (<http://www.lacoast.gov/cwppra/index.htm>). *S. alterniflora* is a dominant species in the coastal marshes of Louisiana and is frequently used for restoration and stabilization in these marshes. The demand for plants of this species is growing for both eroded and newly constructed marsh areas.

To meet the demands for revegetation projects in wetland restoration, the Natural Resources Conservation Service (NRCS) Plant Materials Program of USDA has initiated collection and selection of native *S. alterniflora*. Two cultivars, ‘Vermilion’ and ‘Bayshore’, have been released for planting in the wetland areas (<http://plants.usda.gov>). ‘Vermilion’ was released in 1989 for use in the Gulf of Mexico northern basin. It was selected from a Louisiana ecotype from Vermilion parish with superior growth performance. ‘Bayshore’ was released in 1992 for use in the Atlantic Coast and has superior rhizomatous growth, stem density, foliage abundance and overall vigor. It is extremely salt tolerant and possesses the desired characteristics for revegetation and

stabilization of tidal stream banks (Hamer et al., 1994). However, both cultivars are poor seed producers and propagated by vegetative means.

Typically, *S. alterniflora* exhibits poor seed production and vegetative multiplication followed by potting is required for transplanting in marshes, which is expensive and labor intensive. Croughan et al. (1998) reported that twenty-five labor hours were required for planting 4047 square meters of *S. alterniflora*. Broome et al. (1988) estimated a requirement of 10 person-hours for broadcasting the seed and cultivation of one ha. *S. alterniflora* and about 150 person-hours for transplanting sprigs or potted plants over one ha on a 0.6 meter spacing using a mechanical auger to drill holes. The intensive labor costs for transplanting sprigs or potted plants has limited the progress of marsh revegetation projects.

There is a need for identification of uniquely fertile plants that produce viable seeds in large numbers for marsh plantings. Selection and improvement for better seed production would help to speed the marsh restoration and stabilization by providing an abundant supply of viable seeds. In response to latitude gradients on the coasts, *S. alterniflora* has developed different ecotypes and this has allowed it to attain a worldwide distribution, inhabiting salt marshes over various environments (Anderson and Treshow, 1980). The natural variations existing within this species provide opportunities to select desirable traits, including reproductive traits from the collection. Evaluation on native ecotypes may help us to develop genotypes with prolific seed-producing ability and make propagation from seeds feasible.

Yield components for cereal crops include number of reproductive units per unit area, number of grains per reproductive unit, and average weight per grain. Selection

among *S. alterniflora* accessions for these same yield components should result in higher seed set and improved seed production and viability. Advanced biotechnology applications such as tissue culture, genetic engineering, and artificial seeding may also be helpful in improving propagation and genetic quality of this important coastal wetland plant.

Much work on *S. alterniflora* has been emphasized on plant establishment. There were little information concerning on seed production. Currently released cultivars are still poorly setting seeds and intensive labors are required for pot transplanting. One aspect of the project “ Biological approaches to coastal wetlands restoration”, undertaken by LSU Agricultural Center and USDA-NRCS, is aiming at development of a breeding program to produce lines with improved seed set and vegetative characteristics. As a part of the project, this research attempted to investigate seed production in the field and determine the relationship among seed reproductive traits. The objectives of this study were (i) to determine the flowering phenology of *S. alterniflora* in south Louisiana, (ii) to investigate components of seed production and relationships between flowering date and seed production, and (iii) to evaluate reproductive characteristics of the selected plants in the current breeding program and provide information concerning seed production for further selection.

## **3.2 Materials and Methods**

### **3.2.1 Materials**

One hundred and twenty six *S. alterniflora* accessions were collected in the fall of 1998. This collection included 10 elite lines, from an earlier collection by the Plant Material Center of NRCS (including 3 accessions from Texas), and 116 native accessions

from 11 parishes across southern Louisiana. A native accession was identified as a group of clones with a similar phenotype within a small geographic area. Accession collection and seed processing are described in Chapter 2.

### 3.2.2 Field Design and Management

Seed germination tests were undertaken for all accessions in the spring of 1999. Four hundred seeds were randomly chosen from each accession and placed on 4 germination papers (100 seeds each). Each paper was placed in a plastic bag, kept moist and maintained under ambient laboratory conditions. Based on seed germination and

**Table 3.1. *S. alterniflora* accessions chosen for field study in 1999 and plants selected at Baton Rouge, LA during 2000.**

Accession evaluated in 1999	Origin (Parish)	Plants selected in 2000
98NR7	Lafourche	98NR7-8, -12, -14, -15 -16
98NR8	Lafourche	98NR8-4, -5, -12, -13, -17
98NR19	Jefferson	
98NR25	Orleans	
98NR26	Lafourche	98NR26-3, -4, -10, -13, -18
98NR27	Terrebonne	98NR27-2, -3, -13, -19, -20
98NR30	Lafourche	
98NR38	Vermilion	98NR38-4, -7, -9, -16
98NR47	Cameron	98NR47-4, -5, -14, -16, -18
98NR60	Iberia	
98NR61	Calcasieu	
98NR70	Calcasieu	
98NR71	Calcasieu	
98NR72	Calcasieu	
98NR82	Cameron	98NR82-3, -6, -10, -19, -20
98NR86	Cameron	
98NR98	Terrebonne	
98NR99	Jefferson	98NR99-1, -2, -3, -4, -18
98NR107	Jefferson	
98NR109	Terrebonne	98NR109-11

seedling growth, twenty accessions were selected for field evaluation (Table 3.1).

Twenty seedlings for each of the 20 selected accessions were transplanted to pots in the greenhouse and then planted into the field.

A Randomized Complete Block Design (RCBD) was used in the field. The 400 plants were planted in four replications with each replication containing a complete set of five plants from each selected accession (100 plants per replication). A 61 m x 61 m pond with 60 cm high levees was built to carry out the experiment. Plants were transplanted into the pond at a 2.4 m x 2.4 m spacing in the summer of 1999. The field was irrigated every other week and kept under water unless for field investigation. Herbicide was used to control weeds. In the summer of 2000, based on plant growth, spread, disease resistance, and growth vigor over one growing season, 40 plants were selected for further evaluation.

### **3.2.3 Data Collection**

#### **1) Flowering Phenology**

The date of the first panicle emergence from the sheath is referred as the flowering date. Like most cereal grasses, *S. alterniflora* has a prolonged flowering process since many tillers develop from each plant and flowering for the whole clone is not concentrated in a short time period. Starting from June 2000, flowering data were recorded twice a week until flowering ended in October. At the peak of flowering, data were collected every other day.

#### **2) Seed Production**

Seed production data was collected on the 40 selected plants (lines). *S. alterniflora* has very strong vegetative spreading ability and can develop many

reproductive shoots in a short time period. During the flowering season, fifteen shoots for each line were randomly chosen and tagged with the date of panicle emergence for each shoot. Before seeds matured, dialysis tubes (4.5 cm x 40 cm) were used to bag the panicle and catch any shattered seed.

In December 2000, plant height of flowering shoots, from the base of the culms to the tip of the apex of the inflorescence, was measured for each tagged shoots. Ten panicles from the 15 tagged shoots for each line were harvested after measuring the plant height. The panicles were kept in moist plastic bags in a refrigerator at 5° C for two weeks until threshed. Panicle length, from the point of insertion of the lowest spike to the tip of the uppermost spike, was measured in the laboratory and then the spikelets were removed from each inflorescence by hand. A fluorescent light box (Model 1012) was used to help distinguish unfilled seed from filled. Most unfilled seeds were transparent when placed under the fluorescent light. Any seed that was not distinguishable by light was manually examined to determine its status. Seed set was calculated as the percentage of filled seed relative to total seed.

Filled seeds were kept in plastic containers of salt solution (0.4% NaCl and 0.05% Vitavax 200) and stored in a refrigerator at 5° C. Vitavax 200 (manufactured by Gustafson) contains 17% carboxin and 17% thiram and its application rate to seed treatment on grains is 2.06-2.74 g/kg ([www.agsco-agdepot.com/infosheets](http://www.agsco-agdepot.com/infosheets)). Seed germination tests were performed on filled seeds for each individual panicle. Seeds were placed on germination paper in petri dishes. Germination was carried out under ambient temperature and light. When a panicle had sufficient filled seeds, the germination test

was replicated 3 times with 100 seeds in each petri dish. When there were less seed, seed number for each dish was reduced proportionately.

### **3.2.4 Data Analysis**

Data were analyzed by SAS (SAS Institute, 1999). Standard analysis of variance (ANOVA) procedures were used as appropriate. Least significant difference (LSD) was used for mean separation at 0.01 or 0.05 probability levels. Proc REG and CORR were used to analyze trait relationships.

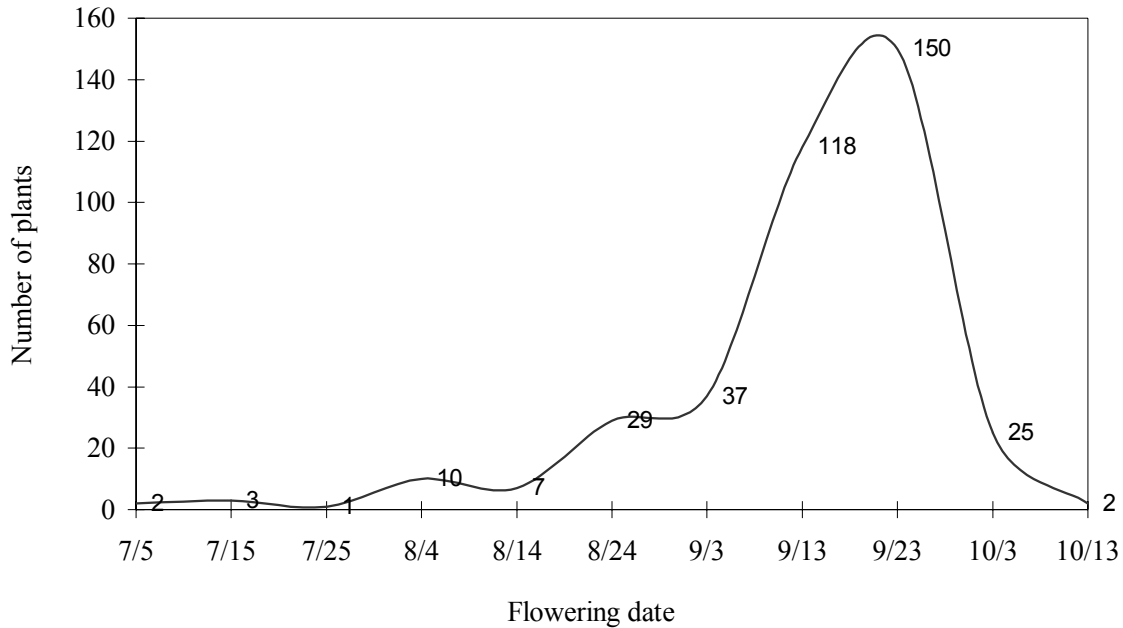
## **3.3 Results and Discussion**

### **3.3.1 Flowering Phenology**

It is difficult to determine the flowering date by the percentage of the flowering plants, as commonly used in cereal crops since *S. alterniflora* has strong tillering ability and an indeterminate flowering habit. Therefore, date of first panicle emergence was used to indicate flowering time in this study.

Observations on individual plants for all accessions showed that *S. alterniflora* initiated flowering in early July and terminated flowering by the middle of October, resulting in a 3.5 months flowering period. Due to vigorous spreading ability, the plants produced numerous tillers. However, it was observed that late flowering shoots grew shorter panicles and produced less viable seeds than early flowering shoots. The earliest flowering plant was found in accession 98NR70 (collected from Calcasieu parish) with a flowering date of July 10th. The latest flowering plant was found in accession 98NR102 (collected from Jefferson parish) with a flowering date of October 10th.

The flowering peak in *S. alterniflora* was between early September and early October (Fig. 3.1) with 85% of all plants flowered during this period. *S. alterniflora*



**Fig. 3.1. Distribution of flowering date (first panicle emergence) for 400 individual plants from 20 *S. alterniflora* accessions grown at Baton Rouge, LA during 2000.**

exhibited protogynous flowering and stigmas exerted from the florets 2 to 5 days earlier than pollen shed in the same floret. Stigmas were white and receptive upon exertion from the floret. Anthers started exerting and shedding pollen around 8:00 AM after morning dew was dried up in the plant. Maximum pollen shed lasted about two hours. Stigmas changed color from white to brown and became shriveled within a few days after the pollination.

There were significant differences among accessions for average flowering date (Table 3.2). The effect of replication and replication x accession interaction was non-significant for the flowering date. The average flowering date for accession ranged from September 4 (accession 98NR70, collected from Calcasieu parish) to September 23 (accession 98NR38, collected from Vermilion parish) (Fig. 3.2).

By comparison of flowering date, accessions could be divided into two groups: early (those flowered before September 14) and late (those flowered after September 14).

**Table 3.2. Analysis of variance of flowering dates for 20 *S. alterniflora* accessions grown at Baton Rouge, LA, during 2000.**

Source	DF	MS	F	Pr > F
Replication	3	140	0.64	0.5877
Accession	19	563	2.58	0.0004**
Replication x Accession	57	122	0.56	0.9955
Error	305	218		
Total	384			

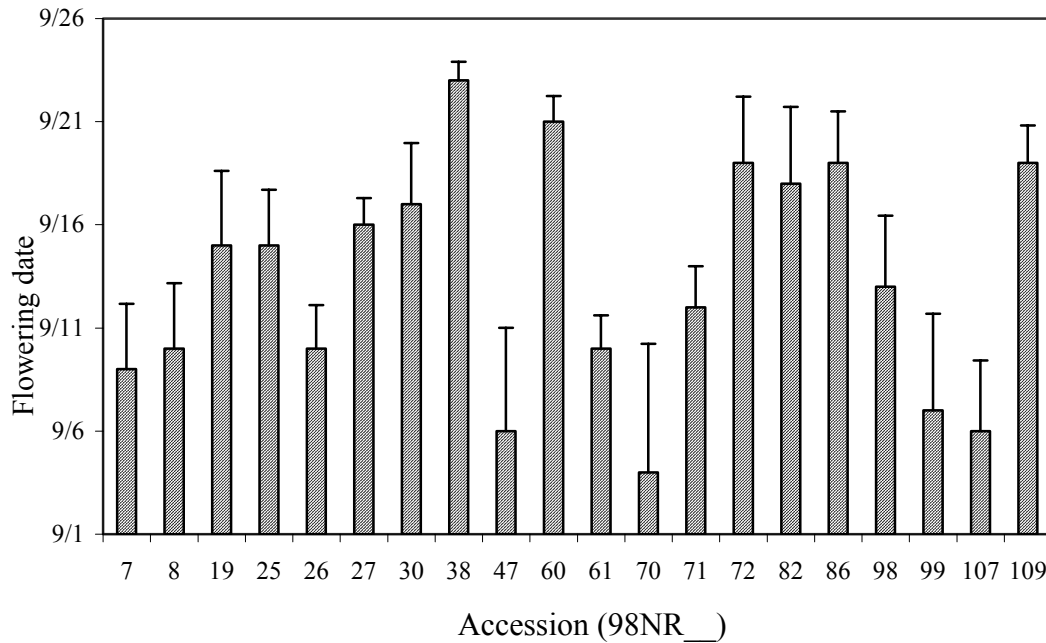
\*\* Significant at the 0.01 probability level.

The early group included nine accessions from three parishes, located in latitudes from 29°4'3.79"N to 30°8'53.19"N and longitudes from 90°3'24.32W to 93°19'52.33"W.

The late group included 11 accessions from 8 parishes located within latitudes from 29°3'28.24"N to 30°3'17.79"N and longitude from 89°49'25.23"W to 93°18'27.26"W.

Large variation for flowering date was observed within accessions during 2000 (Table 3.3). The early flowered accessions generally had high standard deviations. Accession 98NR70, the earliest accession, had the largest standard deviation (26.46) with the earliest plant flowering on July 10th and the last plant flowering on October 8th. Accession 98NR38, the flowered latest, had the smallest standard deviation (3.98). Plants within this accession flowered from September 18 to October 1. Of all accessions grown in 2000, three accessions had standard deviations higher than 20, showing larger variation in flowering dates; 10 accessions had a standard deviation between 10 and 20; and 7 accessions had standard deviations less than 10%.

*S. alterniflora* is a perennial species and flowers annually with variable flowering dates throughout its geographic distribution (Mobberley, 1956; Eleuterius et al., 1984; Callaway and Josselyn, 1992). Seneca (1974) associated earlier flowering with short photoperiods and with more northerly seed sources. However, there was no obvious



**Fig. 3.2. Mean of flowering dates for 20 *S. alterniflora* accessions grown at Baton Rouge, LA during 2000.**

evidence to identify a relationship between flowering date and geographic location of the collection in this study. This is probably because the collection was conducted across south Louisiana that is a relatively narrow range of latitudes.

Information on flowering can aid in the collection and improvement of plant species. Somers and Grant (1981) found no measurable difference in time of emergence or progression of flower development associated with different soil types or salinity of the water used for growing the plants. They concluded that the inherent genetic differences resulted from prolonged growth of the various populations in their natural locales. Variation in flowering provides opportunity to select desirable lines from different ecotypes. Data indicated that the flowering peak is between early September and early October in the south Louisiana and it would be the best time for taking floral observation or making crosses for plant improvement.

**Table 3.3. Variation, range, and comparison of flowering dates for 20 *S. alterniflora* accessions grown at Baton Rouge, LA, during 2000.**

Accession	Location	Latitude	Longitude	Mean (m/d)	Std dev	Min	Max	Comparison†
98NR38	Vermilion	29°39'13.05"N	92°31'20.96"W	9/23	3.98	9/18	10/1	A
98NR60	Iberia	29°35'41.63"N	92°0'25.24"W	9/21	5.16	9/13	10/3	AB
98NR72	Calcasieu	30°3'17.79"N	93°18'27.26"W	9/19	14.04	8/14	10/5	ABC
98NR86	Cameron	29°42'43.92"N	92°45'56.45"W	9/19	10.91	8/21	9/28	ABC
98NR109	Terrebonne	29°3'28.24"N	90°42'22.27"W	9/19	7.45	9/4	10/3	ABCD
98NR82	Cameron	29°37'41.21"N	92°45'54.12"W	9/18	16.62	8/7	10/9	ABCD
98NR30	Lafourche	29°19'24.37"N	90°14'24.81"W	9/17	13.26	8/28	10/10	ABCD
98NR27	Terrebonne	29°3'55.37"N	90°28'43.90"W	9/16	5.83	9/6	9/25	ABCDE
98NR19	Jefferson	29°32'21.41"N	90°3'11.27"W	9/15	16.20	7/18	9/26	ABCDEF
98NR25	Orleans	30°2'41.24"N	89°49'25.23"W	9/15	11.73	8/21	10/8	ABCDEF
98NR98	Terrebonne	29°16'39.43"N	90°55'53.58"W	9/13	14.98	8/7	10/5	BCDEFG
98NR71	Calcasieu	30°8'53.19"N	93°19'52.33"W	9/12	8.69	8/21	9/25	BCDEFG
98NR8	Lafourche	29°26'56.94"N	90°16'3.23"W	9/10	14.11	8/17	9/28	CDEFG
98NR26	Lafourche	29°4'3.79"N	90°19'35.50"W	9/10	9.44	8/21	10/1	CDEFG
98NR61	Calcasieu	30°3'58.87"N	93°19'47.52"W	9/10	7.07	8/28	9/25	DEFG
98NR7	Lafourche	29°26'56.94"N	90°16'3.23"W	9/9	14.22	8/7	10/3	DEFG
98NR99	Jefferson	29°34'33.90"N	90°3'24.32"W	9/7	20.98	7/10	9/26	EFG
98NR47	Cameron	29°41'1.94"N	92°47'43.36"W	9/6	22.42	7/18	10/1	FG
98NR107	Jefferson	29°37'32.88"N	90°5'21.36"W	9/6	15.34	7/31	9/25	FG
98NR70	Calcasieu	30°5'32.86"N	93°17'54.22"W	9/4	26.46	7/10	10/8	G

† Means with the same letter are not significantly different at  $LSD_{0.05}=9.38$ .

### 3.3.2 Seed Production and Flowering

#### 1) Seed Production Traits in *S. alterniflora*

Plant selection was made in the field during 2000 growing season. Forty plants were selected across accessions based on plant height, spreading ability, and disease resistance. To investigate seed production traits, ten panicles from each selected plant were randomly chosen and tagged at their first panicle emergence date. Plant height showed small variation among seed reproductive traits, ranging from 101 cm to 234 cm, with an average plant height of 172 cm (Table 3.4). *S. alterniflora* produces long

panicles with average panicle length of  $25 \pm 3.8$  cm and ranged from 13 cm to 37 cm.

The average total seed number per panicle was  $333 \pm 7.39$  cm and ranged from 70 to 984 cm. A plant from accession 98NR8 was found to have the most seeds per panicle (437 seeds/panicle) while a plant from accession 98NR47 had the least seeds (191seeds/panicle).

**Table 3.4. Mean, standard error (SE), range, and coefficient of variation (CV) of reproductive traits for *S. alterniflora* grown at Baton Rouge, LA during 2000.**

Trait	Average	SE	Minimum	Maximum	CV (%)
Flowering date (m/d) †	9/16	0.5	8/21	10/1	34
Panicle height (cm) ‡	172	1	101	234	14
Panicle length (cm)	25	0.2	13	37	16
Seed weight/panicle (g)	0.88	0.02	0.17	2.61	47
Kernel weight (g/1000 kernel)	2.76	0.05	1.00	5.05	36
Total seeds/panicle (no)	333	7	70	984	44
Filled seeds/panicle (no)	149	6	0	665	80
Unfilled seeds/panicle (no)	184	8	12	764	84
Seed set (%)	47	2	0	94	65
Germination (%)§	60	2	0	100	61
Viability (%)¶	28	1	0	83	86

† Flowering date was recorded as date of the first panicle emergence in a plant.

‡ Panicle height was measured from ground to the top of the panicle for the flowering shoot from which the panicle was randomly chosen for seed production study

§ Germination was made on filled seeds for each panicle.

¶ Viability was estimated by multiplying seed set with germination rate.

Seed set showed large variation among selected plants across accessions (Table 3.4). The average panicle seed set across plants was 47%, ranging from 0 to 94% with CV (Coefficient of Variation) of 64.76%. Accession 98NR27 had the highest mean seed set of 69% and accession 98NR99 had the lowest mean seed set of 16%. Among selected plants, the highest seed set was found in 98NR27-13 (87%) and the lowest was in 98NR99-4 (<1%) (Table 3.9). This large variation should provide excellent opportunity to improve seed set by selection.

## **2) Relationship between Flowering Date and Seed Production**

Effects of temporal variation on flowering date and subsequent seed traits were examined to determine relationship between flowering date and seed production (Table 3.5). There was a close relationship between flowering date and presence of filled seeds within panicles (Fig. 3.3). During this initial stage of flowering (August and early September), seed set ranged from 0 to 29%. When plants entered the flowering peak (from early September to early October), seed set increased. Except for plants flowering on September 12<sup>th</sup>, with seed set of only 2%, subsequent panicles set more seeds with seed set ranging from 46% to 71%. Seed set showed steady increasing trends until the end of the flowering peak period.

Seed production data were sorted by flowering date. Simple linear regression analysis on seed traits with flowering date indicated that total seeds per panicle and unfilled seeds per panicle were negatively related with flowering date; plant height, kernel weight, filled seed per panicle, germination and viability were positively related with flowering date; and panicle length and seed weight per panicle were not significantly related with flowering date (Fig. 3.4 and Table 3.6). Late flowering within peak flowering period would help to increase seed quality and quantity. R squares for three regression models (seed set, kernel weight, and unfilled seeds/panicle) ranged from 0.597 to 0.786, indicating that flowering date had strong effects on these traits. Conversely, R squares for panicle length and seed weight/panicle were very low (0.063 and 0.04, respectively), indicating less effect of flowering date on these two traits.

**Table 3.5. Mean of reproductive traits over flowering dates for *S. alterniflora* grown at Baton Rouge, LA during 2000.**

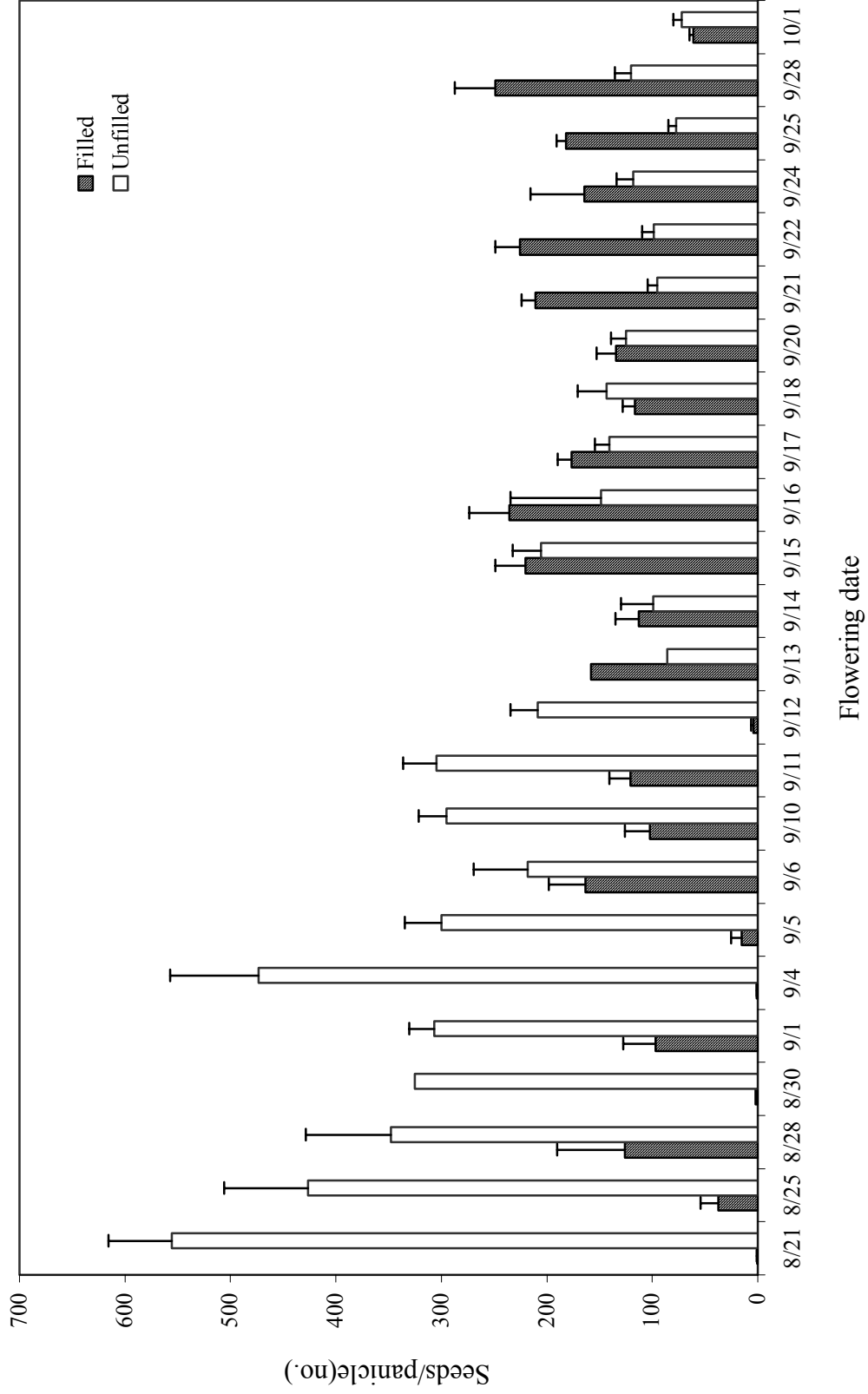
Flowering date	Panicle height†	Panicle length	g		Total seeds /panicle	no		Seed set‡	%		Seed viability¶
			Seed wt /panicle	Kernel wt /1000 kernel		Filled seeds /panicle	Unfilled seeds /panicle		Germination§	Seed viability¶	
8/21	139	25	0.88	1.56	556	0	555	0	0	0	
8/25	170	27	0.61	1.40	464	37	426	8	41	5	
8/28	176	25	1.15	2.52	474	126	314	29	30	23	
8/30	132	26	0.54	1.64	327	2	325	1	0	0	
9/1	166	25	0.66	1.65	404	97	307	18	9	13	
9/4	168	26	0.69	1.58	474	1	473	0	0	0	
9/5	140	22	0.57	1.84	315	15	300	6	11	1	
9/6	183	27	1.00	2.73	381	163	198	48	21	37	
9/10	169	27	0.76	1.93	398	102	295	25	8	17	
9/11	162	24	0.83	2.08	426	121	305	30	47	12	
9/12	128	21	0.35	1.64	213	4	209	2	19	1	
9/13	153	25	0.71	2.91	244	158	86	65	33	21	
9/14	186	28	0.76	3.70	212	113	99	56	72	40	
9/15	178	26	1.22	2.98	426	220	209	51	52	30	
9/16	170	31	1.01	2.87	385	236	149	71	6	4	
9/17	195	26	1.07	3.39	317	176	148	56	65	37	
9/18	157	27	0.59	2.27	260	116	143	46	55	20	
9/20	178	24	0.71	2.62	259	134	130	49	45	25	
9/21	179	22	1.00	3.35	306	211	109	68	38	30	
9/22	174	24	1.10	3.35	324	225	94	67	69	46	
9/24	171	21	0.95	3.33	282	164	118	55	51	38	
9/25	171	24	0.90	3.47	259	182	93	71	72	52	
9/28	185	26	1.33	3.64	369	249	120	64	57	49	
10/1	186	22	0.40	3.01	133	61	72	46	65	30	

† Panicle height was measured from ground to the top of the panicle for the flowering shoot from which the panicle was randomly chosen for seed production study.

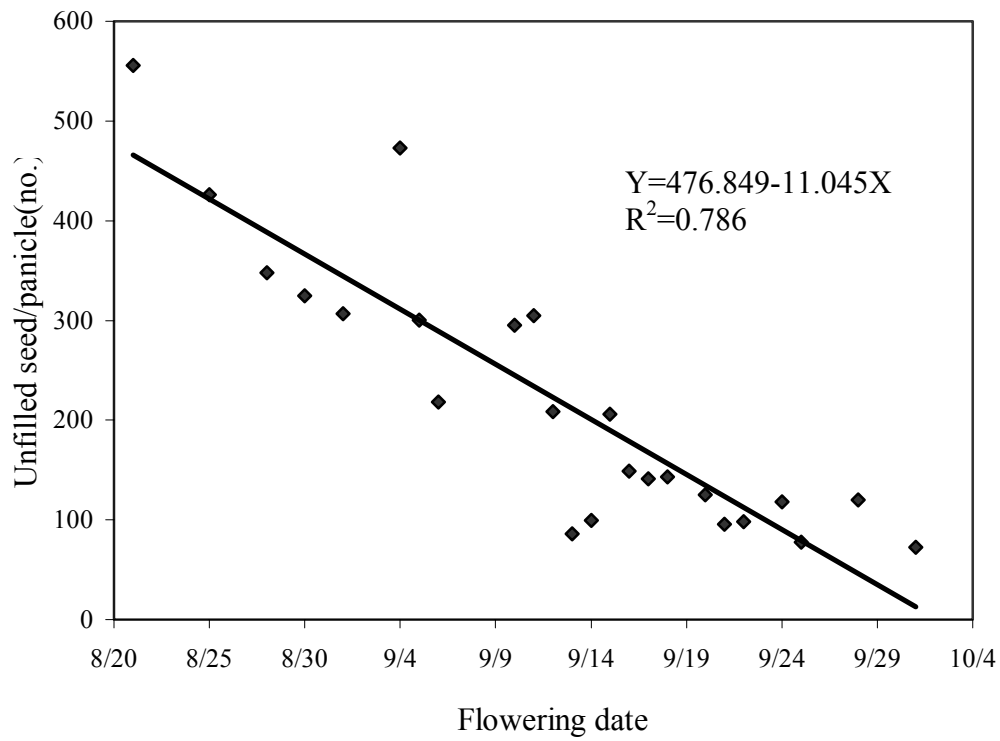
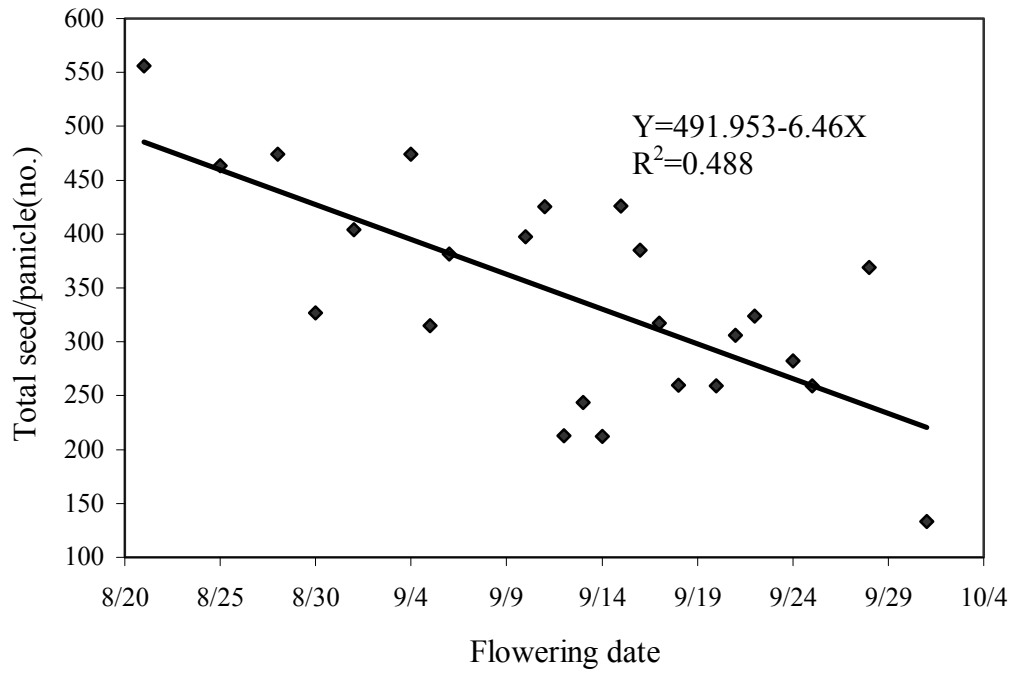
‡ Seed set was the average seed set for all panicles flowered at the same day.

§ Germination was undertaken on filled seeds for each panicle and averaged for all panicles that flowered at the same day.

¶ Seed viability were estimated by multiplying seed set with germination rate for each panicle and averaged for all panicles that flowered at the same date.



**Fig. 3.3. Filled seeds vs. unfilled seeds per panicle within flowering date in *S. alterniflora* grown at Baton Rouge, LA during 2000.**



**Fig. 3.4 Simple linear regression of total seed, unfilled seed, germination and seed set on flowering date in *S. alterniflora*.**

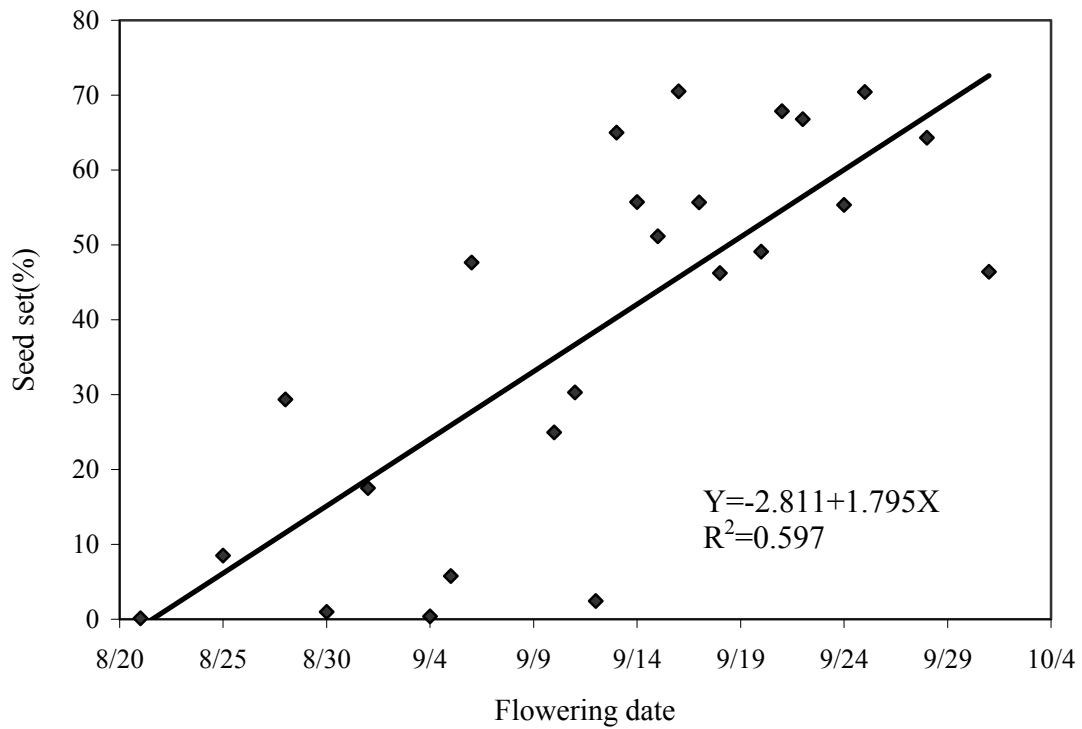
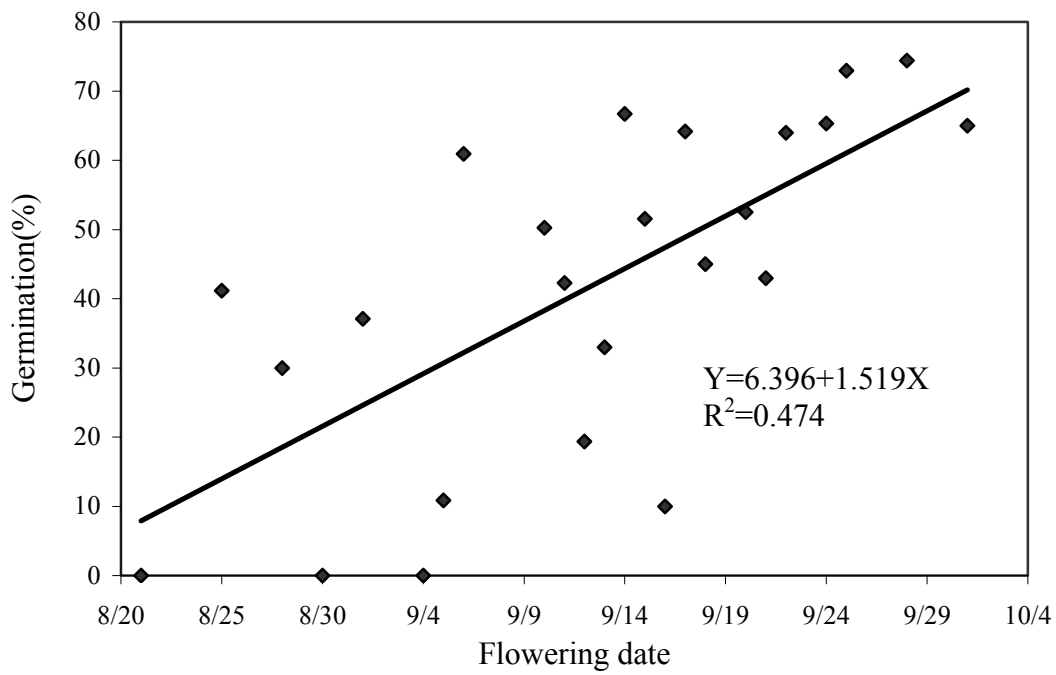


Fig. 3.4. Continued.

*S. alterniflora* inflorescences started emergence in July and ripened caryopses by November, approximately 12 weeks after emergence. This is a longer grain-filling period than that observed in many cereals. Because of long grain-filling period, inflorescences that emerged late after the flowering peak might have insufficient time to ripen caryopses. This could be the reason for large number of panicles with many empty spikelets in late flowering.

**Table 3.6. Simple linear regression analyses of reproductive traits on flowering date for *S. altrniflora* grown at Baton Rouge, LA during 2000.**

Reproductive traits	Simple linear regression	R-square
Unfilled seeds/panicle (no)	Y= 476.849-11.045X	0.786**
Kernel weight (g/1000 kernel)	Y= 1.299+0.054X	0.598**
Seed set (%)	Y=-2.811+1.795X	0.597**
Total seeds/panicle (no)	Y= 491.953-6.46X	0.488**
Seed viability (%) §	Y=-2.996+1.081X	0.484**
Germination (%)‡	Y=6.396+1.519X	0.474**
Filled seeds/panicle (no)	Y= 15.102+4.585X	0.385**
Panicle height (cm) †	Y= 149.408+0.771X	0.226*
Panicle length (cm)	Y= 26.142-0.052X	0.063
Seed weight/panicle (g)	Y= 0.7156+0.0047X	0.040

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

† Panicle height was measured from ground to the top of the panicle for the flowering shoot from which the panicle was randomly chosen for seed production study

‡ Germination was made on filled seeds for each panicle.

§ Seed viability were estimated by multiplying seed set with germination rate.

Mullins and Marks (1987) investigated flowering and seed setting in *S. anglica* in four growing zones, pioneer, transitional, mature, and invaded and found that mature zone produced the largest inflorescences and the pioneer zone produced the smallest inflorescences. Examination of the effects of temporal variations on inflorescences and subsequent seed setting showed there was a close relationship between the date of inflorescence emergence and the presence of filled spikelets within an inflorescence. Results of our study also showed a close relationship between seed production and

flowering date. Selection for plants that flower from early September to early October in Louisiana should result in better seed set and seed weight.

### **3) Correlation among Reproductive Traits in *S. alterniflora*.**

Correlation coefficients among reproductive traits were analyzed in this study (Table 3.7). Seed viability was significantly positively correlated with germination ( $r=0.87$ ), seed set ( $r=0.81$ ), kernel weight ( $r=0.70$ ), flowering date ( $r=0.60$ ), and seed weight/panicle ( $r=0.47$ ), indicating better seed production for *S. alterniflora* intended to be associated with higher seed set and germination, better seed weight, and later flowering date within the peak flowering period. Flowering date was positively correlated with kernel weight, seed set, germination, and seed viability. Within peak flowering period, late flowering plant (from middle September to early October) showed higher seed setting, germination, and heavier seeds. Plants flowering during this period can avoid much of the high temperature and humid weather in south Louisiana. However, flowering date was negatively correlated with total seeds/panicle, which indicated that seeds/panicle was reduced in later flowering due to shorter day length. It was observed that flowering was continued after the peak period, however, panicles were getting shorter and little seed set was observed in late flowering panicle.

Seed germination was significantly correlated with flowering date, seed set, kernel weight, and plant height. Plant that flowered during the flowering peak period tended to have better seed germination. Seed set was positively correlated with kernel weight, flowering date, seed weight/panicle, and panicle height. Kernel weight and flowering dates are two most highly correlated factors with seed set among traits

**Table 3.7. Pearson correlation coefficients between reproductive traits in *S. alterniflora* grown at Baton Rouge, LA during 2000.**

	Flowering date	Panicle height	Panicle length	Seed wt./panicle	Kernel wt /1000 kernel	Total seeds /panicle	Seed Set	Germination	Seed Viability¶
Tiller number†	0.21	-0.08	-0.39*	-0.33*	-0.04	-0.35*	-0.23	-0.03	-0.02
Flowering date		0.26	-0.27	0.13	0.67**	-0.56**	0.69**	0.52**	0.60**
Panicle height‡			0.37*	0.41**	0.44**	0.00	0.34*	0.36*	0.31
Panicle length				0.19	-0.06	0.28	-0.03	0.05	-0.03
Seed wt/panicle					0.55**	0.53**	0.55**	0.29	0.47**
Kernel wt./1000 kernel						-0.37*	0.82**	0.49**	0.70**
Total seeds/panicle							-0.22	-0.15	-0.20
Seed set								0.56**	0.81**
Germination§									0.87**

† Tiller number was number of tillers that set panicles per 929 cm<sup>2</sup>.

‡ Panicle height was measured from ground to the top of the panicle for the flowering shoot from which the panicle was randomly chosen for seed production study.

§ Germination was made on filled seeds for each panicle.

¶ Seed viability was estimated for each panicle by multiplying seed set with germination

investigated ( $r=0.82$  and  $0.69$ , respectively). Kernel weight should be considered for increasing seed set.

Tiller number per  $929 \text{ cm}^2$  was correlated either positively or not significantly with any other seed reproductive traits investigated. This is probably because plants had not grown enough tillers to affect seed productivity for the first growing season. As *S. alterniflora* ages and spreads, tiller density may become limiting factor affecting seed production.

Seed yield is a complex trait and influenced by many factors. In grain crops, yield components include number of reproductive units per unit area, number of grains per reproductive unit, and average weight per grain. Focus of selection may vary based on the growing environment and the plant species. Viable seed production for *S. alterniflora* is unpredictable across years in both native and introduced populations (Broome et al., 1974; Sayce, 1988). In order to avoid this problem in selection, it is suggested that the selected materials should be tested in different environments and years to test stability and adaptability before the release for wetland erosion control.

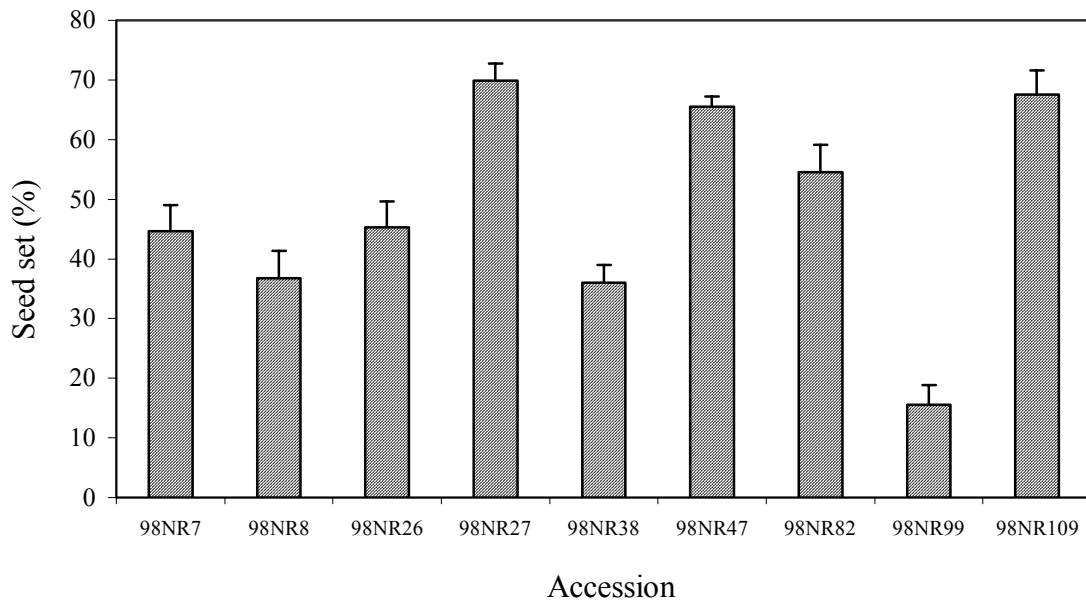
### **3.3.3 Plant Selection in *S. alterniflora***

#### **1) Performance of Selected Plants in 2000**

In the summer of 2000, plants were selected from south Louisiana native accessions. The selection was based on plant height growth, vigor, spreading ability, and disease resistance of individual plants. A total of 40 plants were selected from nine accessions. Among selected plants, 35 plants came from seven accessions with five plants being selected from each accession, four plants from one accession, and one plant from one accession.

There were significant differences among selected accessions for seed traits (Table 3.8). Accession 98NR27 had the highest seed set (69%) and germination (68%). 98NR99 had the lowest seed set (16%) and germination (23%). Among selected accessions, flowering date ranged from September 9th to September 4<sup>th</sup>; plant height ranged from 136 to 190 cm; panicle length from 22 cm to 28 cm; seed weight per panicle from 0.52 gram to 1.03 gram; kernel weight from 1.93 to 3.91 gram; total seeds/panicle from 219 to 449; seed set from 16% to 69%, germination from 23% to 68%, and viability from 8% to 51%. Considerable variation for important reproductive traits existed among and within selected accessions. Seed set for selected accessions varied from 16% in 98NR99 to 69% in 98NR27 (Fig. 3.5). Selection on desirable characteristics is an effective way to improve seed production and promote establishment for *S. alterniflora*.

Plant establishment is a comprehensive process. Apart from soil and environmental conditions, successful plant establishment is highly dependent on seed viability. Improvement of seed set is essential in a breeding program since a lot of empty seeds are found under natural conditions. Observation showed that seed set was highly related to flowering date during the peak flowering period. Early flowering panicles did not have good seed set. Panicles flowering during the peak flowering period had better seed set. Germination of filled seeds also showed that the panicle flowered during the peak period had better germination than those flowered earlier. These results indicated that selection for better seed production plants should focus on plants that flower during the peak period. After been selected in one environment, materials need to be grown across locations and years to identify the superior accessions with wide range of adaptation for future release.



**Fig. 3.5. Seed set for nine *S. alterniflora* accessions at Baton Rouge, LA, during fall 2000.**

## **2) Potential Materials for Plant Improvement**

To initiate a breeding program in *S. alterniflora*, it is necessary to collect and evaluate germplasm from different environments. Accessions collected from natural environment possessed many desirable traits such as disease resistance, salt tolerance, and vigorous growth characteristics and they constitute valuable genetic materials for the breeding program.

Plant selection was done in two stages in 2000. First, selection was conducted based on plant seedling vigor, spreading ability, growth, vigor, and disease resistance in the field. Forty plants were chosen from nine accessions. Second, plants with better seed production traits such as filled seeds/panicle, seed weight/panicle, kernel weight, seed set, were selected.

Plants having seed set above average were initially selected. Plants having better germination of filled seeds were then selected from among these plants (Table 3.9).

Germination and seed set were combined to estimate seed viability for the accessions. After selecting for the plants with above 50% seed set, selection was done for plants with above 50% germination rate. Kernel weight, seed weight/panicles, and total seeds /panicle were the next selection criteria. This selection process resulted in 13 plants from 5 accessions: 2 plants from 98NR7 and 98NR8 (collected from Lafourche), 2 plants from 98NR82 and 4 plants from 98NR47 (collected from Cameron) and 3 plants from 98NR27 (collected from Terrebonne). These selected plants will be used in future breeding for improving seed production ability.

Because of cross-pollinating nature of *S. alterniflora*, restricted selection on single plants may be difficult and have limited utility in population improvement programs. A population improvement program would provide a superior means to improve seed production traits in *S. alterniflora*. It is important to identify breeding objectives for a breeding program. Short term breeding objectives for *S. alterniflora* should be focused on selection of plants from native collections that have a high percentage of seed set, germination, and broad adaptability. Ecotype selection should be an effective way of improving *S. alterniflora* at first stage. By collecting, assembling, and evaluating germplasm, ecotype selection can lead to rapid development and release of superior cultivars. Spaced plant evaluation can provide an opportunity for the breeder to observe the useful phenotypic variation existing within and among accessions. Data collected from these evaluation trials can then be used to select superior accessions. Long-term objectives will be to develop improved populations by recurrent selection. Recurrent selection is a breeding method used to increase the frequency of desired alleles for

**Table 3.8. Means of reproductive traits for selected *S. altriniflora* accessions.**

Accession	Tillers†	Flowering date	Panicl height‡	Panicl length	Seed wt /panicle	Kernel wt /1000kernel	Total seeds /panicle	Seed set§	Seed viability§	
									cm	g
98NR7	18	9/10	180	28	0.97	2.31	440	46	48	21
98NR8	26	9/12	166	25	1.03	2.42	449	37	55	29
98NR26	20	9/14	166	25	0.89	2.67	368	46	44	21
98NR27	31	9/22	175	24	0.92	3.36	276	69	68	51
98NR38	32	9/24	182	24	0.52	2.40	219	36	56	22
98NR47	26	9/22	181	22	1.02	3.91	264	65	58	37
98NR82	25	9/14	190	25	0.99	2.77	337	56	42	30
98NR99	30	9/9	136	24	0.58	1.93	305	16	23	8
98NR109	19	9/17	167	28	0.97	3.82	253	68	58	40
LSD <sub>0.05</sub>	----	3	9	2	0.18	0.37	60	12	13	10

† Tiller number was number of tillers that set panicles per 929 cm<sup>2</sup>.

‡ Panicle height was measured from ground to the top of the panicle for the flowering shoot from which the panicle was randomly chosen for seed production study

§ Average of all panicles investigated for the accession.

¶ Germination was made on filled seeds for each panicle.

**Table 3.9. Means of reproductive traits for selected *S. alterniflora* plants at Baton Rouge, LA, in 2000.**

Plant	Tiller number†	Flowering date	Panicle height‡	Panicle length	Seed wt /panicle	Kernel wt /1000 kernel	Total seeds /panicle	Seed set§	Germination¶	Seed viability§
98NR7-8	16	9/16	170	31	0.97	2.87	368	70	11	5
98NR7-12††	17	9/8	188	28	1.01	2.37	425	64	63	40
98NR7-14	20	9/10	191	30	0.63	1.64	384	7	45	3
98NR7-15	18	8/29	157	26	0.89	1.49	596	25	61	20
98NR7-16††	20	9/17	194	27	1.32	3.19	427	64	58	37
98NR8-4	29	9/5	154	23	0.88	1.93	460	18	49	16
98NR8-5	21	8/29	154	24	0.63	1.45	437	9	38	7
98NR8-12	32	9/12	170	25	1.28	1.91	636	27	24	11
98NR8-13††	23	9/27	183	26	1.35	3.50	383	67	79	55
98NR8-17††	25	9/17	170	24	1.02	3.34	327	63	82	58
98NR26-3	14	9/23	138	20	1.08	3.10	346	82	37	30
98NR26-4	23	9/10	175	26	0.73	1.41	527	12	46	9
98NR26-10	20	9/13	176	25	0.83	2.69	308	29	43	11
98NR26-13	20	9/11	147	22	0.95	2.28	428	47	29	13
98NR26-18	25	9/16	191	30	0.88	3.85	229	60	63	40
98NR27-2††	30	9/21	182	22	1.13	3.04	365	79	67	55
98NR27-3††	30	9/23	167	23	1.14	3.65	309	75	81	66
98NR27-13	30	9/25	165	23	0.69	3.74	184	87	81	70
98NR27-19	29	9/22	159	24	0.75	3.19	234	56	54	31
98NR27-20††	34	9/17	200	27	0.91	3.18	290	50	59	30
98NR38-4	28	9/23	176	27	0.48	2.42	195	55	68	39
98NR38-7	31	9/22	183	25	0.81	2.35	342	29	36	11

**Table 3.9. Continued.**

Plant	Tiller number†	Flowering date	Panicle height‡	Panicle length	Seed wt /panicle	Kernel wt /1000 kernel	Total seeds /panicle	Seed set§	Germination¶	Seed viability§
98NR38-9	32	10/1	186	22	0.40	3.01	133	46	65	30
98NR38-16	36	9/20	184	21	0.39	1.84	205	12	57	8
98NR47-4††	27	9/22	160	20	0.86	3.15	274	70	51	35
98NR47-5††	28	9/25	177	23	0.92	3.88	235	63	81	52
98NR47-14	29	9/21	194	20	0.90	4.68	191	74	27	20
98NR47-16††	18	9/17	194	25	1.36	3.84	356	65	71	46
98NR47-18††	27	9/26	181	22	1.05	4.00	262	53	60	34
98NR82-3	23	9/21	201	23	1.36	3.69	369	83	30	25
98NR82-6††	27	9/23	189	23	1.18	3.38	348	80	70	57
98NR82-10	26	8/31	192	27	0.38	1.73	226	5	4	0
98NR82-19††	25	9/7	201	28	1.63	3.37	493	55	58	40
98NR82-20	24	9/19	167	22	0.40	1.70	247	55	50	28
98NR99-1	37	9/8	116	20	0.32	1.68	194	2	13	0
98NR99-2	31	8/30	151	25	0.89	1.80	493	2	5	0
98NR99-3	31	9/20	142	28	0.67	2.39	278	55	52	29
98NR99-4	33	9/11	132	22	0.47	1.77	269	1	10	0
98NR99-18	18	9/9	141	26	0.57	2.03	293	22	37	12
98NR109-11	19	9/17	167	28	0.97	3.82	253	68	58	40

† Tiller number was number of tillers that set panicles per 929 cm<sup>2</sup>.

‡ Panicle height was measured from the ground to the top of the panicle which was randomly chosen for seed production study

§ Average of all panicles investigated for the accession.

¶ Germination was made on filled seeds for each panicle.

†† Potential selections for future plant improvement.

particular quantitatively inherited characters by repeated cycles of selection. It can be used to assemble, evaluate, select, and intermate germplasm to produce the necessary base populations for recurrent selection.

Since *S. alterniflora* can be reproduced vegetatively and by seeds, it is suggested that comparison of seed production potential among vegetative clones and seeded plants be made. Seed production information would be obtained from such comparison in order to prepare for large-scale seed production.

## CHAPTER 4. SUMMARY AND CONCLUSION

*S. alterniflora* is a perennial grass species and is propagated both vegetatively and by seed. It exhibited protogynous flowering where stigmas exerted 2 to 5 days before the pollens shed in the same inflorescence. Soon after the inflorescence emerged from the sheath of the uppermost leaf, stigmas started exertion from the flowers at the upper 1/3 of the panicle and proceeded toward the base and also toward the top of the inflorescence. Anthers exerted later and followed the same sequence. Pollen shed was at maximum between 8:00 and 10:00 AM. Pollen was viable and stigmas were receptive after exertion. Pollen initiated germination within 15 minutes after contacting the stigma. After germination, the pollen tubes penetrated the stigma papilla, grew through the stigma branch and the central axis of the stigma. The pollen tubes reached the micropyle in 55 to 75 minutes after contacting the stigmas. Hybridization between different parents of *S. alterniflora* was possible without emasculation due to protogynous flowering and reduced tedious labor required for making these crosses.

*S. alterniflora* started flowering in early July and ended by the middle of October. The peak flowering period was between early September and early October in Louisiana. Most seeds matured by late November and started shattering from the plants. During the peak flowering period, seed set, kernel weight, and seed viability were positively related to flowering date. Selecting plants that flower during the flowering peak period might result in better seed production. Kernel weight, flowering date, seed weight/panicle, and panicle height were correlated positively with seed set.

Field investigation showed that seed set varied largely among accessions and the plants within accessions. The overall seed set was 47% with the range from 0 to 94% in

this study. This tremendous variation provided opportunities for selection of higher seed set. However, environmental factors affecting seed set needed to be considered when making selection.

Recurrent selection is a commonly used breeding procedure for genetic improvement of cross-pollinated species. It increases the frequency of desired alleles for quantitatively inherited characters by repeated cycles of selection. Short term breeding objectives for *S. alterniflora* should be focused on selection of plant from native collection that has high percentage of seed set, germination, and broad adaptability. This is the most practical and effective approach to initiate the breeding program in *S. alterniflora*. Several lines with improved characteristics were selected from this study and they would be valuable materials for utilization in recurrent selection program with an objective of developing improved *S. alterniflora* populations.

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