TASK 5.2: ASSESSMENT AND POTENTIAL FOR VEGETATIVE REMEDIATION OF SELECTED BROWN MARSH AND DISTURBED SITES IN THE BARATARIA AND TERREBONNE BASINS – AERIAL SEEDING DEMONSTRATION

BY

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EXECUTIVE SUMMARY

Spartina alterniflora, also known as smooth cordgrass or oystergrass, is a dominant clonal grass in tidal salt marshes along the Gulf of Mexico and the eastern United States coastline. In Louisiana, *S. alterniflora* dominates vast expanses of coastal salt marshes and provides critical habitat and nutrition for many avian, fish, and invertebrate species.

In 2000, Louisiana experienced a sudden and extensive die-back of large areas of *S. alterniflora* dominated saline marsh. While patchy areas of marsh die-back are not uncommon, the size and rapidity of plant mortality of the current die-back was unprecedented. The areas most heavily affected were the salt marshes of the Barataria and Terrebonne Basins between the Mississippi and the Atchafalaya Rivers. Early estimates indicated that approximately 390,000 acres of salt marsh within the Barataria-Terrebonne Basins were impacted.

Task 5 – *Brown Marsh Remediation Trials* under the oversight of the Louisiana Department of Natural Resources' Coastal Restoration Division was developed and designed to assess a number of restoration initiatives and is divided into four subtasks. This study, *Seeding Demonstrations - subtask 5.2* was designed to assess the potential for large-scale vegetative restoration by seed applications, and to determine the effectiveness of using seeding as a method to restore degraded or highly disturbed marsh to vegetative equivalency of natural marsh.

The goals of this study are: (1) to advance methods for rapid, non-invasive, and economically obtainable remediation; and (2) to determine the potential for large-scale vegetative restoration via seed applications and seed effectiveness in re-establishing vegetative cover on critically impacted sites. The objectives of the study are: (1) to collect, process, and store large quantities of wild seed; (2) to assess seed germination and viability throughout the harvest and storage processes; (3) to determine applicable aerial seeding rates, seed carrier, bulk mixing method, aerial seeding calibration, and to contract with an aerial applicator; (4) to aerially seed multiple study sites; (5) to measure seeding, germination, seedling, and mature plant success rates; and (6) to assess one-year productivity measurements.

Subtask 5.2 was conducted over a two year period beginning in the fall of 2000 and completed in the fall of 2002. Elements of the study are grouped into three broad categories with a number of subtasks within each category. The three primary categories are: Seed Elements (four subtasks); Calculation and Seeding Methods (six subtasks); and Treatment Measurements (four subtasks).

RESULTS

Seed Elements

We collected *S. alterniflora* seed from two sources, wild seed from native marshes and cultivated seed from managed ponds. In 2000 and 2001, using three crews of three to five members over a

two week period, we hand collected enough seed to adequately implement an aerial seeding demonstration. In 2000, we collected and processed 440 pounds of clean seeds from 4 sites and in 2001 we collected and processed 980 pounds of clean seed from 3 sites. We maintained seed by collection lots and measured three seed variables that are typically good indicators of seed quality and are used when calculating and applying seed rates; we measured seed weight, germination, and viability by collection site.

We found seed produced from cultivated ponds was significantly heavier (indicating greater endosperm development) and that cultivated seed had a greater germination and viability rate than that of wild seed. In addition, we determined that a cold-wet stratification improved overall germination across seed type and that a minimum stratification period should be at least 90 days.

Calculations and Seed Preparation

To calculate an aerial seeding rate for *S. alterniflora*, we developed a template based on seeding-rate models used for rice culture and aerial seeding in Louisiana. To compensate for a lower germination differential between *S. alterniflora* and rice varieties, we modified the seeding formula by using a pure-live-seed factor as opposed to the germination percentage, and determined a marsh seeding rate of 43 lb/ac^2.

S. alterniflora is a relatively large seed, it is thin-walled and relatively light in weight. Because of its recalcitrant nature, seeds are stored aqueously and become cohesive, forming sticky clumps when drained. To compensate for the excessive seed moisture and clumping, we blended seeds and an inert clay (palygorskites) carrier using a clean, small-drum, motorized cement mixer to form a carrier-mix that separated seeds, absorbed excessive moisture, provided additional weight, and facilitated uniform seed distribution when released aerially. Seeds and carrier were mixed at a 1:2 ratio of seed-to-carrier by weight and mixed approximately 1,200 pounds of seed/carrier in 2001 and slightly under 3,000 pounds in 2002 for distribution.

We developed a contract with Air Farms, an agricultural aerial applicator for aerial seeding. Four sites were selected for aerial seeding over a two year period. Three sites were seeded on April 17, 2001, and one additional site the following year on March 8, 2002. The three sites seeded in 2001 were two, 3-acre brown marsh sites, Bayou Lafourche and Lake Felicity, and a 3-acre control pond at the PMC in Golden Meadow. The 2002 year study site was a 25-acre created-marsh at Port Fourchon. All of the sites in both years were seeded with 43 pounds/acre of *S. alterniflora* seed.

Treatment Measurements

Site Seeding Density and Distribution

We felt that one of the more significant accomplishments of the study would be to successfully distribute a predetermined quantity of seed efficiently and in mass. Obtaining good spatial seed distribution, while controlling the density of seeds within a given unit area, is the first step

towards broad-scale acceptance of aerial seeding by the restoration community. The target number of seeds for all sites was 89/ft^2 and the density of seeds we achieved across all 4 sites was consistently uniform with an average of 87±12 seeds/ft^2. Average seed density at three of the sites, Bayou Lafourche, Lake Felicity, and Port Fourchon were ±2% of the target number (~2 seeds/ft^2) while density at the PMC was 17% (~13 seeds) short of the target. We did not make any spatial measurements relative to seed dispersal, however, we did make ocular assessments of seeding patterns at every sample, and did not find a single occurrence of clumping or irregular patterns of seed concentrations.

Site Germination

Field germinations were taken at 30 and 60 day intervals at each treatment and adjacent unseeded control site. Seeds were determined to be germinated if either the plumule (shoot) or radicle (primary or seminal roots) were visible at 30 or 60 days. Net germination for the seeded areas at marsh sites and at the Plant Materials Center averaged 134±33 per ½m^2 after 30 days. We found some germinations in the three control sites, but they were infrequent and their numbers very low (<1.0 per ½m^2 quadrat). The average across 60 samples was 0.7 germinations per ½m2. The low number of control seeds indicates that there was limited natural distribution or drift of *S. alterniflora* seeds into the control sites from surrounding communities, and provides additional evidence that *S. alterniflora* seeds do not persist in saline marshes and there is little, if any, annual *S. alterniflora* seed derived from seedbanks found in saline soils.

In calculating the *S. alterniflora* aerial seeding rate, we used the higher of two germination results we obtained from our controlled germination test. Clipped seeds, which circumvented dormancy inhibitors, provided a 54% germination rate, while unclipped seeds provided a 26% germination rate. We hypothesized that the viability test (clipped seeds) was a more accurate measure of a seed-lot's true germination potential. Although this may still be accurate, it appears that germination results from unaltered seeds (26%) may be the more accurate estimator of field germination potential as it closely mimicked the actual field results that we obtained. Therefore, we significantly underestimated the number of seeds per unit needed to obtain a predetermined field germination.

Seedling Survival

Seedling survival measurements were taken 90 days after aerial seeding. Seedlings were scored as "surviving" if the plantlets had elongated past their primary shoot and protective coleoptile (first shoot leaf), were upright, anchored, and were developed to at least their third leaf stage. The number of surviving seedlings found within the control sites was very low. The average across 60 samples was 0.3 seedlings per ½m$^{2.}$ Within the seeded treatment sites net surviving seedlings varied slightly across sites, with means ranging from 3.3±1.8 to 5.3±2.1. Converting site averages of surviving seedlings from ½m^2 to ft^2 illustrates the low net ratio of seeds sown to surviving plants. For example, the average seeding rates at the 3 marsh sites was 468 seeds/m^2

which produced 4 surviving seedling per ½m^2. Converted seeds and seedlings to square feet produces 87 seeds/ft^2 and 0.7 seedling/ft^2, less than 1 seedlings/ft^2 of marsh seeded.

Productivity Assessments

One of the goals of this study was to quantify the application of aerial seeding as a means to accelerate the functional development and vegetative equivalency of created or rehabilitated *S. alterniflora* marshes to that of natural marshes. To determine productivity, several measurements of live standing biomass were taken at the end of the first growing season to assess vegetative abundance at aerial seeded sites, adjacent control sites, as well as an equivalent adjacent reference marsh. We sampled over a one week period beginning in early November, a time generally considered to be peak biomass accumulation. Samples were collected from the seeded marsh sites at Bayou Lafourche, Lake Felicity, and Port Fourchon, 3 adjacent controls sites, and 3 adjacent reference marshes. Measurements taken at each site were species composition, stem density, stem height, stem diameter, inflorescence production, and total biomass expressed as dry weight.

Species Composition

Relative to species composition, we found across all treatments that all sites were virtually monospecific in plant species composition. That is, we found no other species other than *S. alterniflora* in any of the 135 samples collected and analyzed.

Stem Density

Stem densities were analyzed with a two factor analysis of variance that tested for differences by treatments (control, reference, and seeded) and by site.

Pairwise comparisons showed that the density of seeded plots was not significantly different from the reference plots, indicating that in a single year, the seeded plots had achieved densities indistinguishable from the reference marsh, with about 100 stems per ½m^2. In contrast, the unseeded control plots had only 6-12 stems per ½m^2, significantly less than the seeded and reference plots. The low frequency of plant occurrence within the control sites is consistent with other studies and supports our findings that there is limited outside seed transport even in the presence of surrounding *S. alterniflora* marshes; and, that there is a low occurrence of a viable *S. alterniflora* seedbank in saline marsh soils. As demonstrated in the control sites, there is little natural foundation material found in highly disturbed or dredge-sediments soil; consequently, vegetative cover is generally sparse, uncertain, and slow to establish. Reference sites were not significantly different from each other, however, treatment sites were, varying from 76 to 128 stems.

Stem Height and Diameter

Seeded plots and reference plots had similar plant heights, about 1.0m, with the seeded plots about 15% taller. In contrast, plants in the control plots were drastically smaller, varying from

19 to 35 cm in mean height across site. Although stem heights varied widely among treatments, stem diameters were relatively uniform among treatments. An ANOVA showed statistically significant differences, given the large sample size. Control stems had slightly larger diameters (9.2mm) than seeded stems (8.8mm) or reference stems (8.5mm), but again, these differences are very small biologically.

Biomass

Above ground biomass was determined for the unseeded control plots, the aerially seeded plots, and the mature reference marshes. Dry weight biomass showed significantly large variation across treatments (control, reference, and seeded) and some variation across sites and all treatment means were significantly different from each other. In general, the seeded plots equaled or exceeded the reference plot means, although in the case of Port Fourchon, the seeded plot significantly exceeded the reference mean. In all cases, the control mean biomass was much smaller than the seeded or reference biomass, usually on the order of 20-25% of the seeded and reference plots.

One possible hypothesis that may explain the significantly larger robust growth at the seeded sites not seen at the control and reference marshes involves nutrient accumulation and competitive release. Both effects (nutrient enhancement and competitive release), either individually or as interactions, provided a compensatory growth not observed or measured in the reference marshes. Control plots, of course, lacked seeds to take advantage of the condition.

CONCLUSIONS AND RECOMMENDATIONS

Vegetative wetland restoration on a landscape-scale is a relatively new restoration concept. Historically, plants produced by rhizomes, tubers, or rootstock and used in conjunction with sediment enhancement, marsh creation, bay terracing, or other sediment related engineering practices have been successful but cost-inefficient. Although whole-plant restoration has proven to be more reliable and requires less establishment time than seed, it is also exponentially more expensive than seed and has significant limitation in its area of application.

The lack of information related to seed viability, germination, and seedling growth requirements has prevented an accurate determination of seeding rates for most wetland species. In addition, except for a few common species, the biology of wetland plants is poorly understood or not well documented.

Using *Spartina alterniflora* seed as a method for reestablishing vegetative cover on highly disturbed soils in saline marshes is both efficient and effective. Even with the relatively low return of mature plants to seeds sown, seeding still provides a greater plant density per measure unit area than the traditional means of establishing whole-plants after one growing season. However, seed harvesting in bulk is still limited to hand collections and will continue to severely

limit the number of acres that can be seeded annually. Currently, the only source of *S. alterniflora* seed is from native saline marshes.

The study of wetlands seeding as a restoration tool should continue to be a high priority because of its importance in developing more efficient technologies for reducing wetland loss in coastal Louisiana. Developing successful seeding systems that can be implemented in Louisiana's deteriorating marshes can play a major role in this process, yet information that will maximize the likelihood for success is still lacking. Continued seeding experiments will add to the knowledge base and will provide coastal wetland project planners, designers, and builders with additional management strategies that will better incorporate vegetative diversity and productivity into wetland restoration engineering.

TASK 5.2: ASSESSMENT AND POTENTIAL FOR VEGETATIVE REMEDIATION OF SELECTED BROWN MARSH SITES IN THE BARATARIA AND TERREBONNE BASINS

INTRODUCTION

Spartina alterniflora, also known as smooth cordgrass or oystergrass, is a dominant clonal grass in tidal salt marshes along the Gulf of Mexico and the eastern United States coastline. Stands of *S. alterniflora* are primarily maintained by vegetative reproduction, and ramets are usually interconnected by below-ground rhizomes (Dai and Wiegert, 1996). In Louisiana *S. alterniflora* dominates vast expanses of coastal salt marshes and provides critical habitat and nutrition for many avian, fish, and invertebrate species.

In the spring of 2000, fishermen, biologist, and resource managers noticed a "browning" in certain areas of coastal marsh in south Louisiana. While patchy areas of marsh die-back are not uncommon, the size and rapidity of plant mortality of the current die-back was unprecedented. The areas most heavily affected were the salt marshes of the Barataria and Terrebonne Basins between the Mississippi and the Atchafalaya Rivers. Though not exclusive to the Barataria-Terrebonne estuary, the die-back was most severe within these basins with an estimated one-third of the marsh in the estuary being affected (Linscombe and Chabreck, public communication, 2003). The Barataria-Terrebonne Basins are among the most fragile of Louisiana's eroding coastal wetlands and have some of the highest rate of land loss in the nation, a staggering 487,000 acres since 1930.

Smooth cordgrass is the most abundant plant species in the affected salt marshes; consequently, any factors that affect these plants have the capacity for wide area impact. Early over-flight surveys showed approximately 390,000 acres of impacted salt marsh within the Barataria-Terrebonne Basins. Of this amount, 105,000 acres (164 square miles) were classified as severe and the remaining 138,000 acres (215 square miles) were classified as stressed (LACoast, public communication, 2003). The most critical statistic obtained from these early surveys was that less than 40% of the affected marsh appeared healthy and that the land loss potential resulting from the die-back phenomenon would add significantly to the already estimated 25 square miles of coastal land that erodes each year in Louisiana. Figure 1 are photographs taken in mid-summer and later-winter of 2000, and exemplify brown marsh conditions.

The most optimistic view in 2000 was that the die-back would recover quickly if the dead areas were recolonized by living plants still in the area. However, inspections of roots and rhizomes (underground stems) (Figure 2) taken directly from die-back sites indicated that this event was

not simply a die-back of the above-ground plant materials, but involved death of a significant portion of the below-ground portions of the plant as well. This observation suggested that

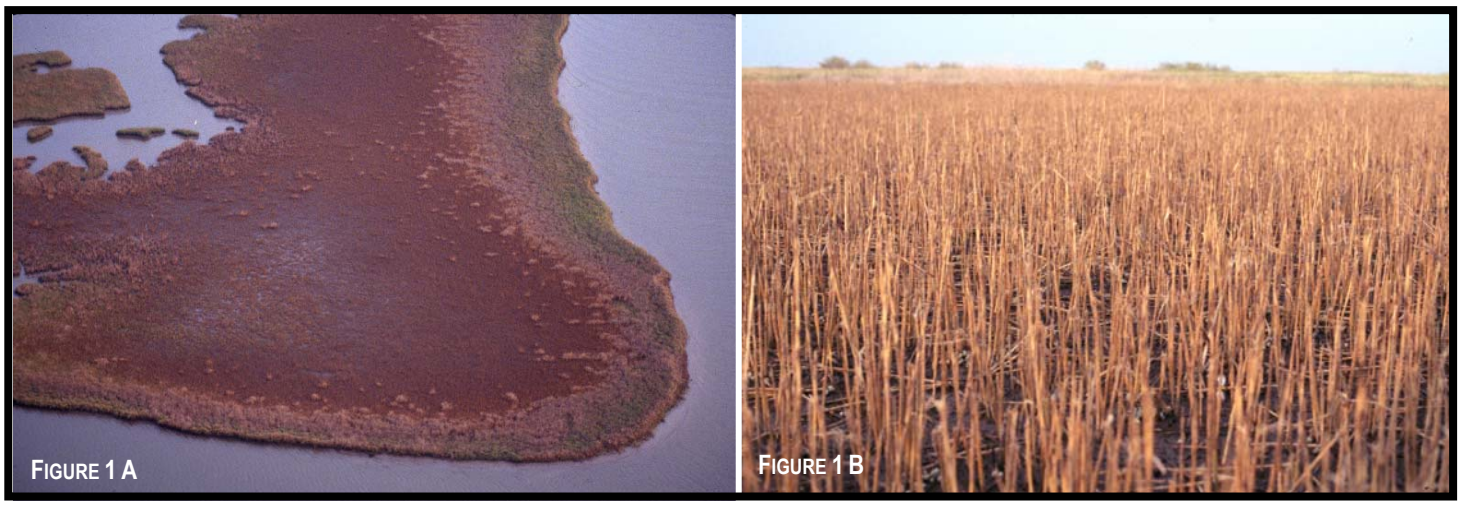


FIGURE 1

The large scale die-back that occurred in 2000 was termed "brown marsh" due to its unique reddish-brown signature. Figure 1A is a low altitude photograph of an impacted marsh in mid-summer 2000; the color gradient from high-bank (reddish-greenish) to the interior (deep rust) was characteristic of brown marsh sites in the Barataria-Terrebonne Basin and is indicative of the relative vegetative health. Figure 1B is a ground-level photo taken in the interior of an impacted marsh. Site characteristics were uniformly high mortality distinguished by leafless standing dead culms; note the total absence of any green stems. By late winter 2000 (Figure 1C) all the above-ground biomass had deteriorated and exported out of the marsh, leaving a large expansion of bare, loose, and unconsolidated mudflats. Standing-dead stems in mid-summer (fig.1B) provided some physical protection from sediment loss, but were reduced to surface stubble (Figure 1D) by late winter providing little, if any protection from surface erosion.

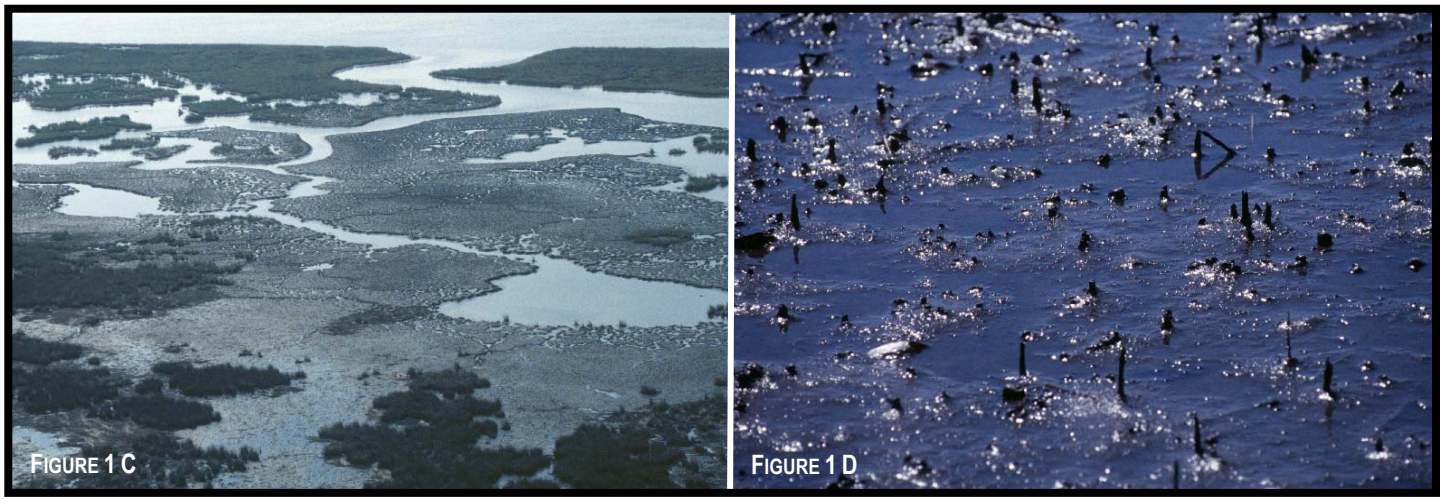


FIGURE 2

To assess below-ground mortality we harvested a large number of aboveground plant samples and belowground root cores from twenty impacted sites. The degree of mortality increased along a gradient starting at the high-bank shorelines and increased inland, with the greatest (near 100%) mortality found near the center of the die-back site. Figure 2A is representative of the basal area and standing crop that we sampled; note the blackened stem crown and the brown, brittle fibrous roots below. Both indicators that the plant had been dead for several months. Rhizomes from the majority of plants we sampled were hollow and limp, lacking any support tissue. Examination of the axillary buds along the rhizomes showed no budding or secondary nodal reproduction. Figure 2B is one of approximately 180 30cm cores we took to assess root/rhizome viability. Note the short collapsed rhizome fragments throughout the core profile. In the center of die-back sites we found virtually no living material in any of the samples that were taken.

FIGURE 2 A

FIGURE 2 B

recovery could be minimal to non-existent in many of the high mortality areas, as *S. alterniflora* seed is not typically maintained in saline marsh seedbanks. Consequently, recovery would be limited to existing surviving rhizomes of stressed plants within the die-back zones, from edge expansion from surrounding healthier communities, and from random seed and plant fragments that might drift in from remote locations.

If the die-back continued and recovery did not take place in a timely fashion, large portions of Louisiana's salt marshes (and to a lesser extent brackish marshes) would be threatened through large-scale marsh loss, resulting in rapid subsidence, erosion of unstable marsh soils, vegetation to open water conversion, and an increased threat of coastal community flooding and property damage. In addition, the die-back phenomenon could have profound consequences on Louisiana's ongoing restoration efforts. Losing a band of salt marsh the width of the die-back area would not only undermine the eco-structure within the region, it could potentially have moved the Gulf of Mexico many miles closer to south Louisiana communities.

GOAL AND OBJECTIVES

Task 5 – *Brown Marsh Remediation Trials* under the oversight of the Louisiana Department of Natural Resources' Coastal Restoration Division were developed and designed to assess a number of restoration initiatives and is divided into four subtasks. This study, *Seeding Demonstrations - subtask 5.2* was designed to assess the potential for large-scale vegetative restoration by seed applications, and to determine the effectiveness of using seeding as a method to restore degraded or highly disturbed marsh to vegetative equivalency of natural marsh.

Although plants have been used in agriculture to prevent soil erosion and to increase agronomic productivity for decades, there is limited information on the application and efficacy of wetland plants, particularly seed, to control wetland loss. Bioremediation, in conjunction with other wetland loss prevention measures, offers an excellent adjunct to construction of physical structures alone. Plants have proven to be a self-sustaining environmentally sound technique to controlling land loss. Under the right environmental conditions plants can persist indefinitely, are dynamic, and can expand or contract in response to changing conditions. Hard-structures on the other hand are static, have a limited life span, predictably require repair and maintenance, and generally are appreciably less cost-effective to install.

Despite the advantages of using vegetation for wetland loss and restoration, a number of inherent biotic and logistic problems, such as poor seed production, seed sterility, seed availability, the high cost of producing and establishing container vegetation, and the remoteness and inaccessibility of most wetland sites, have limited the widespread and large-field applications of

wetlands bio-remediation techniques. A number of these limitations specific to clonal and seed performance are currently being addressed in on-going biotechnology, plant breeding, and recurrent selection studies conducted by the Louisiana State University Agricultural Center and the USDA Natural Resources Conservation Service. Two areas of particular interest relative to this study are genetically improved genotypes to optimize plant performance in wetland restoration, and systems development for rapid and economic establishment of vegetative cover across extensive areas, i.e., seeding. However, proven wetland restoration methods using seed as the primary means of plant establishment are still several years away from large-scale application.

The goals of this study are therefore: (1) to advance methods for rapid, non-invasive, and economically obtainable remediation; and (2) to determine the potential for large-scale vegetative restoration via seed applications and seed effectiveness in re-establishing vegetative cover on critically impacted sites. The objectives of the study are: (1) to collect, process, and store large quantities of wild seed; (2) to assess seed germination and viability throughout the harvest and storage processes; (3) to determine applicable aerial seeding rates, seed carrier, bulk mixing method, aerial seeding calibration, and to contract with an aerial applicator; (4) to aerially seed multiple study sites; (5) to measure seeding, germination, seedling, and mature plant success rates; and (6) to assess one-year productivity measurements.

STUDY SITES AND EXPERIMENTAL DESIGN

Site Selection

Four sites were selected for aerial seeding over a two year period (Figure 3). Three sites were seeded in the spring of 2001 and one additional site in the spring of 2002. The three sites seeded in 2001 were two 3 acre brown marsh sites (Bayou Lafourche and Lake Felicity) that suffered almost total mortality of their existing *S. alterniflora* community in 2000 as a result of the brown marsh die-back. The third 2001 site was a 3 acre pond located at the USDA Natural Resources Conservation Service Plant Materials Center (PMC) in Golden Meadow, Louisiana. The 2002 year study site was a 25 acre portion of a 250 acre created marsh at Port Fourchon. The Port Fourchon site is a beneficial-use sediment project, constructed in 1999 over a relic submerged *S. alterniflora* marsh, and had remained virtually void of vegetation prior to seeding.

The two brown marsh sites (Bayou Lafourche and Lake Felicity) were selected because of the extent of *S. alterniflora* mortality (near 100%), and the existence of extensive baseline site data being generated as part of other associated brown marsh studies. The Bayou Lafourche and Lake Felicity sites were 2 of 23 intensively surveyed brown marsh and control sites included as part of the Status and Trends subtask 6: Salt Marsh Dieback in Coastal Louisiana – *Survey of Plant and*

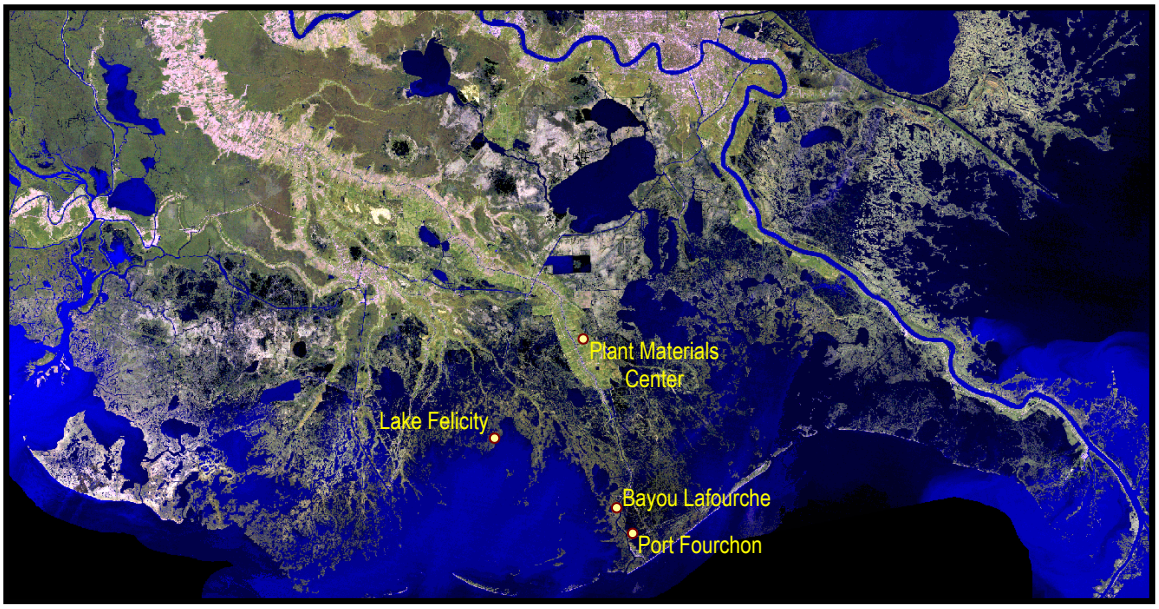


FIGURE 3
Vicinity Map – Aerial seeding 2001 and 2002 study sites

Soil Conditions at 23 Sites in Barataria and Terrebonne Basins, June 2000-September 2001 (McKee, Mendelssohn, Materne; 2003).

The Golden Meadow site was a non-marsh managed pond, selected to provide control over water depth, duration, and frequency. In addition, there were resident staff located at the PMC who could monitor study elements at a greater frequency than that of the brown marsh sites. The Golden Meadow Plant Materials Center has also produced colonial *S. alterniflora* materials for many years under artificial conditions, and had significant experience with the propagation and management of the species.

The Port Fourchon beneficial-use sediment site was selected because it represents one of the more common large scale marsh creation methods currently being employed in Louisiana. Marsh creation sites, left unplanted, often result in sparse patchy mixtures of plants and barren areas that provide little, if any, soil loss protection. Consequently, it is common for some type of artificial plant establishment to be implemented in conjunction with dredge-sediment projects. The Port Fourchon site offered an excellent opportunity to assess the effectiveness of plant establishment on a large-scale using seed as an alternative to expensive vegetative plantings.

METHODS

Subtask 5.2 was conducted over a two year period beginning in the fall of 2000 and completed in the fall of 2002. Elements of the study are grouped into three broad categories with a number of subtasks within each category. The three primary categories are: Seed Elements (four subtasks); Calculation and Seeding Methods (six subtasks); and Treatment Measurements (four subtasks).

Table 1 is a timeline that summarizes the study elements by group, subtasks, and implementation month across the life of the study. Each of the subtasks are further defined and discussed in the Methods section that follows.

TABLE 1 – STUDY ELEMENTS AND TIMELINE

Year/Month	2000		2001												2002											
	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
I Seed Elements																										
(a) Collection	■												■													
(b) Processing		■	■											■	■											
(c) Storage			■	■	■										■	■	■									
(d) Germination & Viability		■	■	■	■	■								■	■	■	■	■								
II Calculations and Methods																										
(a) Seeding Rate					■												■									
(b) Carrier Assessment					■												■									
(c) Mixing Methods					■												■									
(d) Securing Contractor				■												■										
(e) Aerial Calibration					■												■									
(f) Aerial Seeding						■												■								
III Treatment Measurements																										
(a) Seed Distribution						■												■								
(b) Site Germination							■	■											■	■						
(c) Seedling Survival									■												■					
(d) Productivity Assessments													■												■	

I. SEED ELEMENTS

Subtask 1a: Collection

A number of laboratory and field studies (Mooring, Cooper, and Seneca 1971; Broome, Woodhouse, and Seneca 1974) have shown that germination of *S. alterniflora* seed is significantly influenced by site variables. Site selection is critical as it has been shown that quality, as well as quantity of seed varies greatly from one stand of *S. alterniflora* to another. It is generally accepted that the best quality and greatest seed production are found on young vigorous plants, and those qualities are normally associated with recently colonized sites. It has been our observation that marsh enhancement, through the use of dredge-sediments produce exceptionally vigorous stands of *S. alterniflora* for three to five years after sediment placement. Consequently, all of the sites selected for seed harvest for this study where collected from young, sediment-enhanced marshes, or from cultivated ponds.

Seed harvest for both the 2001 and 2002 seeding plots were collected beginning the second week of November and harvested for a two week period. Seeds for the 2001 seeding plots were collected from two sediment-enhanced marshes, one in Cameron Parish adjacent to Brown Lake

and the other in Lafourche Parish adjacent to West Belle Pass. Two additional collections were made from cultivated research plots, one at the USDA Natural Resources Conservation Service Golden Meadow Plant Materials Center and the other at the Louisiana State University Agricultural Center Rice Research Station. Seeds harvested for the 2002 plot were also collected from the same marsh in Cameron Parish as the previous year, from Grand Terre, a sediment-enhanced bay island east-northeast of Grand Isle, Louisiana, and again from the Golden Meadow Plant Materials Center. Seed collections were made concurrently across sites within year, and seeds were maintained in separate lots by collection site. Figure 4 is a vicinity map showing seed collection sites for both years.

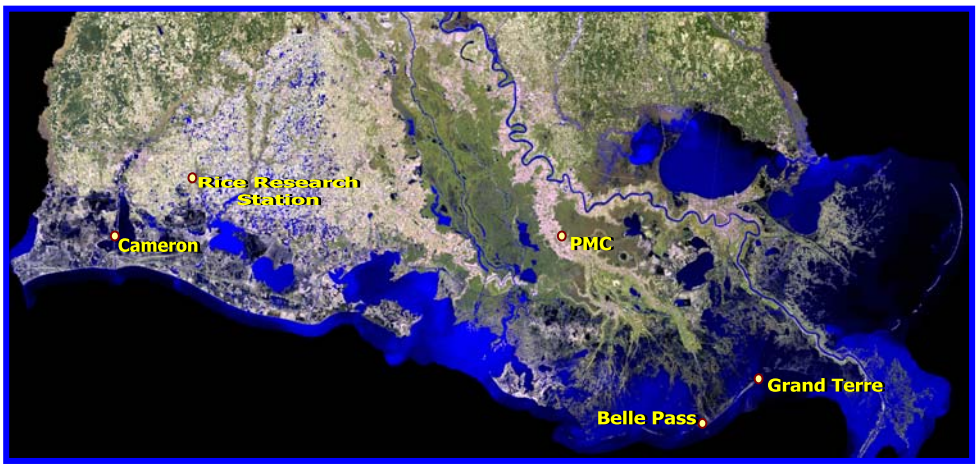


FIGURE 4
Vicinity Map – Seed harvest 2000 and 2001 collection sites.

Seed collections began at the first sign of seed shattering in the marsh. Flowering sequence in *S. alterniflora* originates at the distal tip of the inflorescence and proceeds basally. Consequently, the first seeds to shatter are those located at the seedhead tip. We used tip-shattering as a field indicator to begin harvesting. Seeds were hand harvested by cutting the entire inflorescence just below the bottom floret and placing them in 60 gallon plastic bags for transportation and storage (Figure 5).

Subtask 1b and 1c: Seed Processing and Storage

Germination studies have shown that harvesting seed as near to maturity as possible will yield the highest germination percent. Also, seed viability can easily be maintained for several months after harvest if seeds are properly stored (Broome, Woodhouse, and Seneca,1974). However, it is often necessary to compromise on complete seed maturity since many seeds will be lost due to natural shattering if harvesting is delayed too long.

An acceptable alternative method for harvesting seed prior to natural shattering is to clip the entire seedhead and store them in a high moisture environment under cool (40°F – 50°F) temperature for 30 to 60 days. This post-harvest storage is generally termed *after-ripening* and insures a continuation of maturation of immature seed. Seed moisture in the after-ripening phase is critical and should be maintained at 34%-36% (dry weight basis) to maintain viability (Chappell and Cohn, 2005). After-ripening also accelerates the deterioration of the seed pedicel,

FIGURE 5

Figure 5A is typical of the open marsh seedheads harvested in both collection years. Open marsh collection sites were all sediment enhanced marshes and produced robust and dense seedheads, as we observed stark contrasts in the number of inflorescences and seed production between normal (un-enhanced) and sediment enhanced marshes. Although enhanced marshes produced a greater number of seeds per unit weight, they also produced a larger number of empty, aborted, and non-viable seeds.

Figure 5B is the end product of a multi-stage process beginning with hand collecting and ending with storage. Although the procedures yield adequate seed for the seeding experiments, all of the several processes were labor intense. Note in Figure 5B that seeds remain clumped and cohesive even after the free water has been removed.

rachilla, and other points of seed attachment, and greatly facilitates separating seed from the rest of the inflorescence.

Spartina alterniflora seeds are both dormant and recalcitrant (Chappell and Cohn, 2005) and require post-harvest treatments to continue seed maturation and to break seed dominancy. It is generally accepted that a cold-wet stratification (2°C - 5°C) for at least 30 days is necessary to break dormancy. Seeds can be held under these conditions for approximately eight months; however, seeds will begin germinating even under cold-wet storage conditions if held too long. Most researchers estimate the effective shelf-life of *S. alterniflora* seed at approximately nine months from harvest (Broome, Woodhouse, and Seneca, 1974; Plyler and Proseus, 1996; Chappell and Cohn, 2005).

For the purposes of this study, we used the following after-ripening and clean-seed storage procedures for both the 2001 and 2002 seed collections: (1) harvested intact seedheads were thoroughly wetted and placed in tagged 60 gallon plastic bags and separated by seed lot (collection site); (2) storage bags were lightly perforated to allow for excessive moisture to drain and to facilitate periodic rewetting of the seedheads during storage; (3) bags were spread out in a single layer on a concrete floor, positioned out of direct sun light, and stored at ambient atmospheric temperature; (4) seedheads were checked at five day intervals after the first 30 days to determine the degree of seed release from their panicles; (5) hand shattering began 45 days after the beginning of the after-ripening process.

Seeds were shattered by tapping the seedhead on a plastic sheet laid on the ground, or in some cases, tapped against the inside wall of a drum. Once the panicles and seeds were separated, seeds were sieved through a standard seed cleaning screen to separate seed from chaff and other debris. Individual seed lots were shattered, cleaned, and stored separately. Seeds were immersed in a 0.8 to 1.0% saline solution, stored in eight gallon tubs with snap lids, and placed in refrigeration units at 2°C - 5°C.

Subtask 1d: Germination and Viability Assessments

We conducted germination and viability testing throughout the collection and storage phases of the study and considered these tests central to a number of key study elements. By determining a baseline germination at the time of collection and retesting at 30 day intervals, we were able to monitor seed viability throughout the various phases of the study. It was our intention to calculate a seeding rate for the study sites based on pure-live-seed and not bulk weight. Consequently, determining a reasonably accurate seed germination percentage for the seeds collected was critical.

To assess germination we followed (with minor modifications) a number of standardized protocols developed by Mooring, Cooper, and Seneca; (1971), and Broome, Woodhouse, and Seneca; (1974). We randomly selected 100 seeds immediately after harvest and again at 30, 60, 90 and 120 days post-harvest intervals. Seeds were soaked in a 20% Clorox solution for 15 minutes and then rinsed in distilled water for 15 minutes. Seeds were then evenly distributed on commercial grade (Anchor) germination paper that had been saturated with distilled water. A second saturated sheet was used as a cover to keep seeds from shifting and to insure a good seed-moisture interface. Each individual sample and replicate was placed in a separate, lidded, clear container with a small amount of distilled water to maintain hydration. Containers were then placed in a germination chamber with alternating thermoperiods of 8 hours at 35°C and 16 hours at 18°C. Seeds received no supplemental lighting other than when seeds were uncovered for germination counts. Germination counts were made at 7, 14, 21, and 28 days, and we used the presence or absence of an epicotyl (shoot) as a germination indicator. Germination tests were replicated three times by sample period and collection site (Figure 6).

Germination testing alone, however, does not always provide an accurate measure of *S. alterniflora* seed viability. It is well documented that *S. alterniflora* seeds possess dormancy mechanisms involving chemical inhibitors that influence germination (Carrick and Plyler, 1994; Plyler and Proseus, 1996; Chappell and Cohn, 2005). Tetrazolium testing (TZ test) is commonly used to determine viability in many agronomic crops. However, we found TZ testing problematic in conjunction with *S. alterniflora* seed because of the considerable greening found in the embryo most of which is attributed to an elongated and large scutellum (Walsh, 1990; Chappell and Cohn, 2005). To determine viability, we used a cut or nicking test that removed approximately one-forth of the seed's distal tip prior to plating. This test is commonly used on agronomic seeds of known dormancy (Venuto, 1999) and had proven to work well in other viability studies (Ryan, 2003). We followed the same testing frequency and germination methods for viability as that outlined for germination, with the exception that we reduced the sample size to 50 seeds per replica because of the labor required to excise individual seeds.

It should be noted; that unlike most documented germination tests conducted since the early 1970's, we randomly selected our test seed from unsorted lots. That is, where most researchers painstakingly selected to include only whole filled seed, our primary interest was in determining a "relative" germination percent for the entire collection. Since separating empty, aborted, or damaged seed from filled seed is impracticable when working with large quantities of bulk seed, the relative percent of non-viable seed is factored into a pure-live seed formula when calculating seeding rates. Consequently, the germination test results from this experiment represent germination percentages by site collection for unsorted non-select seed.

FIGURE 6

Figures 6A through 6E are examples of germination and storage methods. Figure 6A contrasts clipped and non-clipped germination tests to assess viability and germination of different seed collection lots. We deemed a seed viable (Figure 6C) at the appearance of either the epicotyl (shoot) or radicle (root), although the shoot was almost always the first structure to appear. Seeds were stored in 8 gallon snap-lid containers in an aqueous solution at 2 to 5°C.

Figure 6B is part of the 2000 seed collection prior to being drained and mixed. Although the only accurate method to quantify germination and/or viability is through controlled germination testing, some informal observation relative to germination potential can be made by looking at the number of floating seeds. Although we did not conduct any formal experiments on "floaters," we observed a greater number of floating seed in the open marsh collection lots than the cultivated. This relationship between the number of floating seeds and germination was later supported by the control led germination test results. Figure6D is one container from the Cameron Parish collection lot and Figure 6E is a container from the Rice Research Station collection. The Rice Research Station seed lot was 24% higher in germination than that of Cameron. It is reasonable to assume that seed buoyancy is a function of seed density, and density a factor in viability. Consequently, floating seed may be a quick and easy means of separating viable and non-viable seed prior to application.

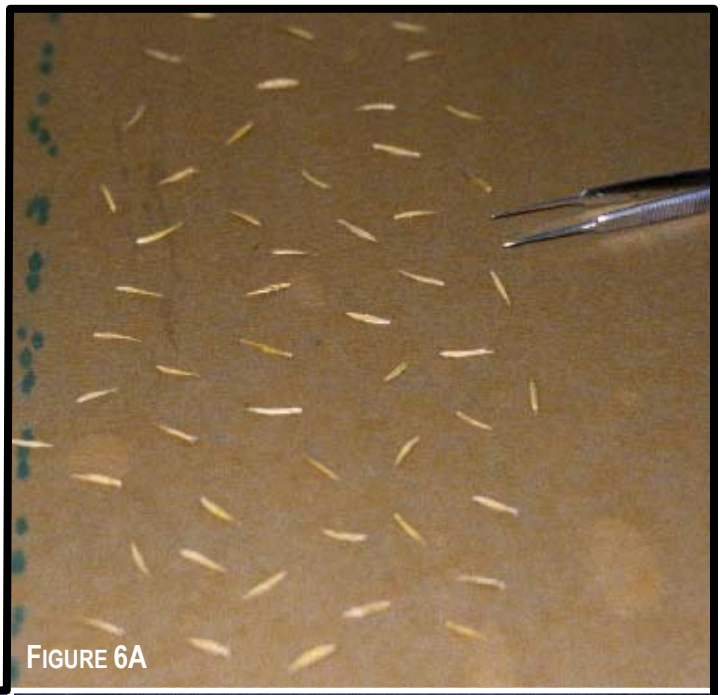


FIGURE 6A

FIGURE 6 B

FIGURE 6 C

FIGURE 6 D

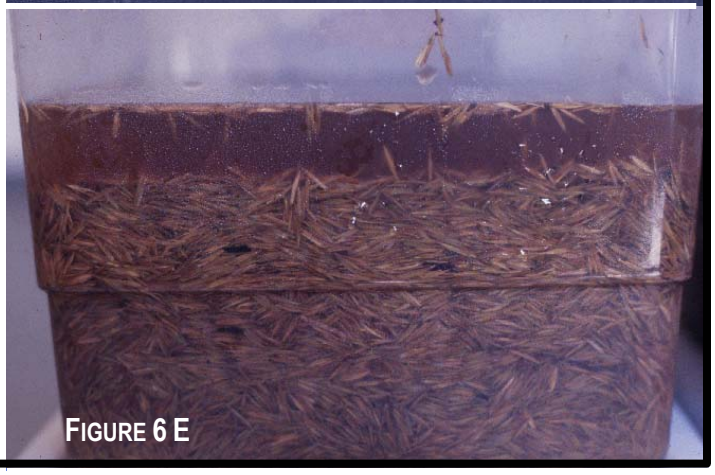


FIGURE 6 E

II. Calculations and Methods

Subtask 2a– Seeding Rate

Using *S. alterniflora* seed to establish vegetative cover in an uncontrolled marsh environment has been successfully demonstrated a number of times, dating back to the early 1970's. However, the extent of plots established by seed were always relatively small, were almost always seeded by hand, generally included a soil preparation treatment, such as scarification and covering, and seeds were always applied at ground level. Although these studies demonstrated that seeding is possible, they still required logistical access with equipment to the site and mobility around the site. This type of seeding method would preclude a great number of marshes in Louisiana that are simply inaccessible, or too fluid to physically support a ground-based seeding operation.

Aerial application of rice seed (*Oryza sativa*) is the primary means of planting rice in Louisiana and virtually all aspects of aerial seeding are well established. Louisiana farmers, on average plant 350,000 to 400,000 acres of rice annually by air (Linscombe, 2001). Rice and *S. alterniflora* are similar in a number of features – both are grasses, both are physiologically adapted to aquatic and semi-aquatic environments, and both produce large quantities of seed, although rice seed is significantly larger and heavier than that of *S. alterniflora*.

Given the similarities between the two species and the rich history of aerial rice culture, it seemed reasonable to develop a *S. alterniflora* seeding template from well established rice seeding rates. Elements used to calculate seeding rates for rice are: (1) a predetermined germination percentage; (2) a known number of seeds per pound; (2) a target number of seeds per square foot; (3) an expected field germination rate; and (4) an expected seedling survival rate per square foot. When all the target figures are met, plant density within the field is sufficient to produce an acceptable crop. Table 2 outlines the variables associated with aerial seed rice and their expected field performance in water culture.

Table 2 – Rice Seed Variables and Values

Variable	Values
*Germination %	80 to 90%
Number of seeds per pound	16,000 to 21,000
Seeding rate – lbs/ac^2	120 to 130
Target seeds/ft^2	50 to 60
Expected field germination – % of target seeds	60 to 65%
Expected germination (# of seed)	32 to 35
Seedling survival to maturity – % of germinated seed	25 to 35%
Number mature plants/ft^2	10 to 12

Here is an example of calculations used to determine the aerial seeding rate for an 80% germination rice variety. The calculations assume a seed number of 19,000 seeds per pound and a target seed density of 55 seeds per square foot in the field. The calculations will produce a per acre seeding rate and will estimate seed germination, seedling survival, and number of mature plants per square foot.

Calculations:

55 *(target seeds/ft^2)* x 43,560 *(ac ft^2)* = 2,395,800 *(seeds/ac^2)*

2,395,800/19,000 *(seeds/lb)* = 126 *(lb of seed/ac^2)*

55 *(target seeds/ft^2)* x .62 *(assumed field germination %)* = 34 *(number of field germ seeds/ft^2)*

34 x .32 *(assumed seedling survival %)* = 11 *(mature plants/ft^2)*.

We were satisfied that the rice model would serve as a basic template to develop a *S. alterniflora* seeding rate. However, we were concerned about the germination differential between the two species. *Spartina alterniflora* germination rates are considerably lower than rice; consequently, the target number of seeds/ft^2 used in rice culture may be an under estimation of that which is needed under marsh environments. To compensate for the germination differential, we added a pure-live-seed (PLS) component to the model that would increase the target seed number proportionally to that of the germination percent.

Using the same rice variables, but including the PLS element, the formula and results change as follows:

55 *(target seeds/ft^2)* x 43,560 *(ac ft^2)* = 2,395,800 *(seeds/ac^2)*

2,395,800/19,000 *(seeds/lb)* = 126 *(lb of seed/ac^2)*

126/.80 *(% germination)* = 158 *(seeding rate in lb/ac^2 based on pure live seed)*

(158 *(lb/ac^2)* x 19,000 *(seeds/lb))*/43,560 *(ac ft^2)* = 69 *(actual seeds/ft^2)*

69 x .62 *(assumed field germination %)* = 43 *(number of field germ seeds/ft^2)*

43 x .32 *(assumed seedling survival %)* = 14 *(mature plants/ft^2)*.

The results increase the seeding rate by 25% (from 126 lb/ac to 158 lb/ac); increase the target seed density by 25% (from 55 seeds/ft^2 to 69 seeds/ft^2); increase the estimated field germination by 26% (from 34 seeds to 43 seeds); and increase the estimated seedling survival by 40% (10 seedlings to 14 seedlings).

The only component in the *S. alterniflora* seeding model that was not part of the original study is an assessment of seeds per pound. We have since included a seed count component to the study and have a reasonable estimation of seed production.

Subtask 2b and 2c: Seed Carrier and Mixing Methods

Although *S. alterniflora* has a relatively large seed it lacks significant endosperm density; consequently, seeds are generally light in weight relative to their volume. In addition, because *S. alterniflora* is stored in an aqueous solution, seeds tend to adhere to each other, forming sticky clumps when drained. To compensate for the excessive seed moisture and to separate clumps, we experimentally developed a blend of seed and carrier that separated seeds, absorbed excessive moisture, had sufficient weight to resist floating, and facilitated uniform seed distribution when dispersed aerially at a 100 miles per hour.

In addition to experimenting with seed-carriers, we also develop a mechanized blending method and established a seed-to-carrier ratio (based on weight) that consistently proved to be an acceptable mixture (Figure 7).

FIGURE7

It took a three year trial and error period, spanning numerous material and equipment combinations, to develop a successful seed-carrier blend. Hand mixing (Figure 7A) is safe and produces little damage to the seed; however, it is very labor intense. Mechanically, a soil blender (Figure 7B) is an excellent piece of equipment on several fronts; they are readily available, motorized, and generally have a large hopper. However, the blade design (at least on ours) rubs the seed against the drum walls causing an unacceptable amount of seed damage. A cement mixer (Figure 7C) has the same mechanized advantages of a soil blender, but a different paddle design and a more convenient drum opening (Figure 7D). We found a cement mixer an excellent piece of equipment that blends seed and carrier with virtually no seed damage, provides good visibility of the mix while blending to facilitate adjusting moisture content, and produces an excellent well mixed, fluffed blend (Figure 7E).

FIGURE 7 A

FIGURE 7 B

FIGURE 7 C

FIGURE 7 D

FIGURE 7 E

Subtask 2d and 2e: Aerial Contractor and Plane Calibrations

Once in flight, there is no accurate method for determining how much seed has been distributed, or how much remains in the plane's hopper at any one time. As seeding requirements of the study were specific to site, it was necessary to develop an agreement with an experienced aerial contractor who would be willing to work with small seed loads and willing to make multiple trips to and from the marsh in order to ensure an accurate seed distribution.

Once we had an agreement, we provided the contractor with a sufficient amount of seed/carrier mix and worked collaboratively to calibrate the plane. Calibration (seed distribution) is a function of air speed, hopper opening, seed material size, and seed material weight. Calibration was determined by calculating the amount (by weight) of material released, over a control time, with a specific hopper opening, at a given air speed. We repeated this release, timing, and weight measurement until we had a consistent seeding rate within our target range. In addition, we determined that while in flight the seed/carrier mix tended to compact in the hopper, so an agitator was built into the hopper to obtain the most accurate and consistent seed delivery.

Subtask 2f: Aerial Seeding

Over a two year period, we seeded four different sites (Figures 8 and 9); three in the spring of 2001 and one in the spring of 2002. We used the Lafourche Parish airstrip at Golden Meadow as a staging area for refueling and loading. Golden Meadow was the nearest airstrip and a short straight-line distance to all of the marsh sites. Seeds were mixed and weighed the afternoon prior to seeding and stored in moistened burlap sacks. Seeding started at about 8:00 in the morning and was completed by 12:00 p.m. We specifically selected this timeframe to coincide with a low tide cycle.

The pilot was provided with GPS coordinates of each site and did not have difficulty locating them in the marsh. We provided two flagmen on the ground at each site at the time of seeding. It was the flagmen's responsibility to mark the flight line, move to the next swath width between passes, and flag the beginning and ending points of the seeding path for the pilot. Seeding from an airplane is rapid and an accomplished pilot can seed one acre in a straight-line distance (assuming a 30 foot swath width) in under 10 seconds.

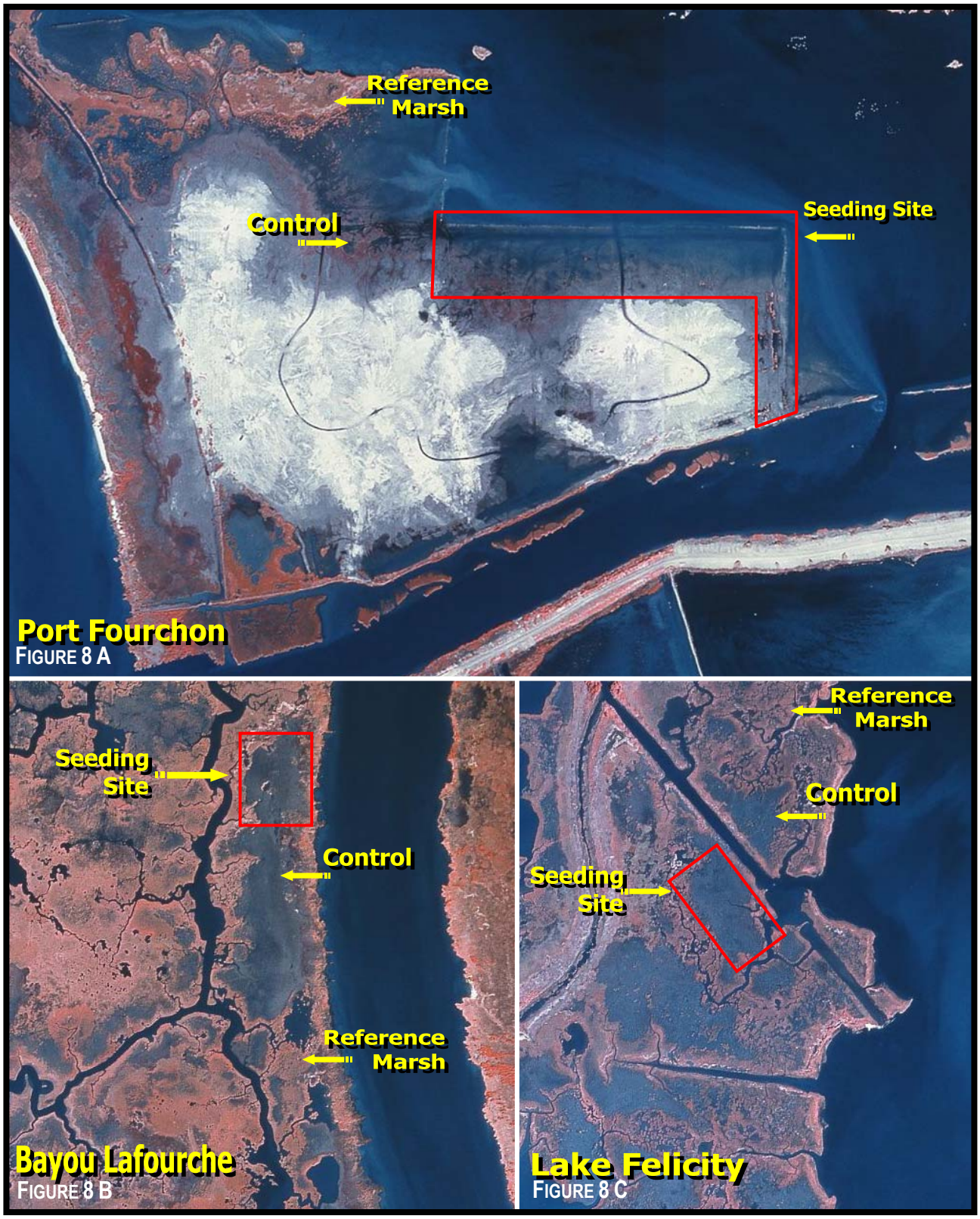


FIGURE 8

Aerial imagery of three of the four seeded sites. Not show is the NRCS Plant Materials Center in Golden Meadow, La.

FIGURE 9 A

FIGURE 9 B

FIGURE 9 C

FIGURE 9

Figures 9A through 9C are three of the four aerial seeding sites. Figure 9A is the PMC control pond, you can see the protection levee in the background and the leading edge of the pond in the foreground; Figure 9B is the Bayou Lafourche brown marsh site. Note the discrete edge of relatively healthy marsh in the background and the dead marsh in the foreground. We used PVC markers to design a ~30' swath flight line. Figure 9C is the northern retention levee at Port Fourchon, note the community of Fourchon in the background.

Figure 9D shows the distribution and flow pattern from the plane's hopper. Once free from the fan, a combination of air speed and prop-wash facilitated seed dispersal and provided an even and consistent 30' swath path on the ground.

FIGURE 9 D

III. TREATMENT MEASUREMENTS

Subtask 3a: Site Seed Density and Distribution

Seed measurements were taken immediately after aerial application at each site. Samples were taken using a ½m^2 quadrat and a sampling frequency of 15 at the Bayou Lafourche, Lake Felicity, and the PMC treatment sites. Control sites for Bayou Lafourche and Lake Felicity were both sampled at the same time and at the same frequency. We did not establish a control at the PMC site, as the pond's retention levees would preclude any drift or random chance seed occurrence. In addition, there were no other *S. alterniflora* plants within the immediate area of the PMC treatment site. Figure 10 is an example of typical seed distribution and special patterns obtained at all seeded sites.

The Port Fourchon site and control were sampled using the same quadrat measurement but at a greater frequency given their larger sizes. The Port Fourchon site is eight-fold larger than the brown marsh sites, consequently, we increased the sample frequently from 15 to 30 samples.

Seed density per unit measure and spatial distribution were the primary variables that we measured under the seed distribution subtask. Seed density measurements provided a quantitative assessment of the effectiveness of several study methods, such as: (1) how effective was the seeding rate template at estimating target seed densities; (2) how effective was the seed-carrier in providing uniform seed distribution; (3) how effective was the aerial calibration for seed release; and (4) does *S. alterniflora* possess seed characteristics that lend themselves to aerial distribution?

Subtask 3b: Site Seed Germination

Seed germination measurements were taken at 30 and 60 day intervals at each treatment and control site. We used the same sample unit size, frequency, and methodology for seed germination measures as that for seed distribution counts.

Seeds were determined to be germinated if either the plumule (shoot) or radicle (primary or seminal roots) were visible at 30 days (Figure 11). Seedlings were counted along with shoot/root as indicators at 60 days. We did not attempt to count non-germinated seeds within the sample unit for the purpose of determining a per sample germination percent. It was obvious that an accurate count of total seeds per sample was impossible to assess, as a significant number of seeds were partially to completely buried even within the first 30 days. Therefore, calculation of percent germination was based on true germinations divided by mean seed density at each site.

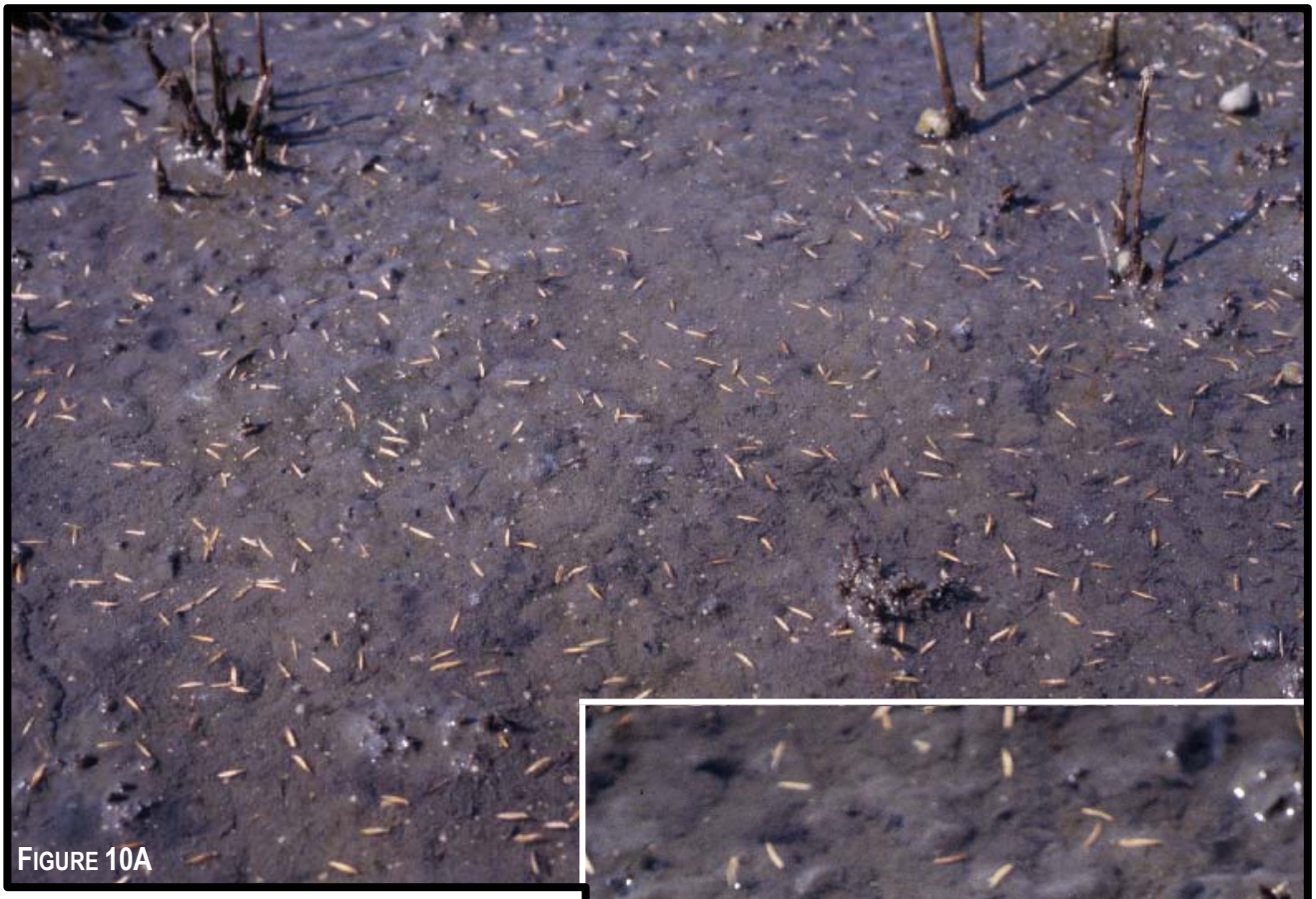


FIGURE 10A

FIGURE 10 B

FIGURE 10

Figures 10A and 10B are two photographs showing seed density and distribution patterns at the Bayou Lafourche seeding site. The Bayou Lafourche site is representative of the density and patterns that were obtained at all four seeding sites. Note the even, unclumped distribution in Figure 10A. This was consistent throughout all four sites. Figure 10B is a close up showing soil conditions and seed placement. Bare moist soils are the ideal site conditions because seeds adhered to surface quickly, generally remained in place, and were lightly embedded following the first tidal cycle without being smothered.

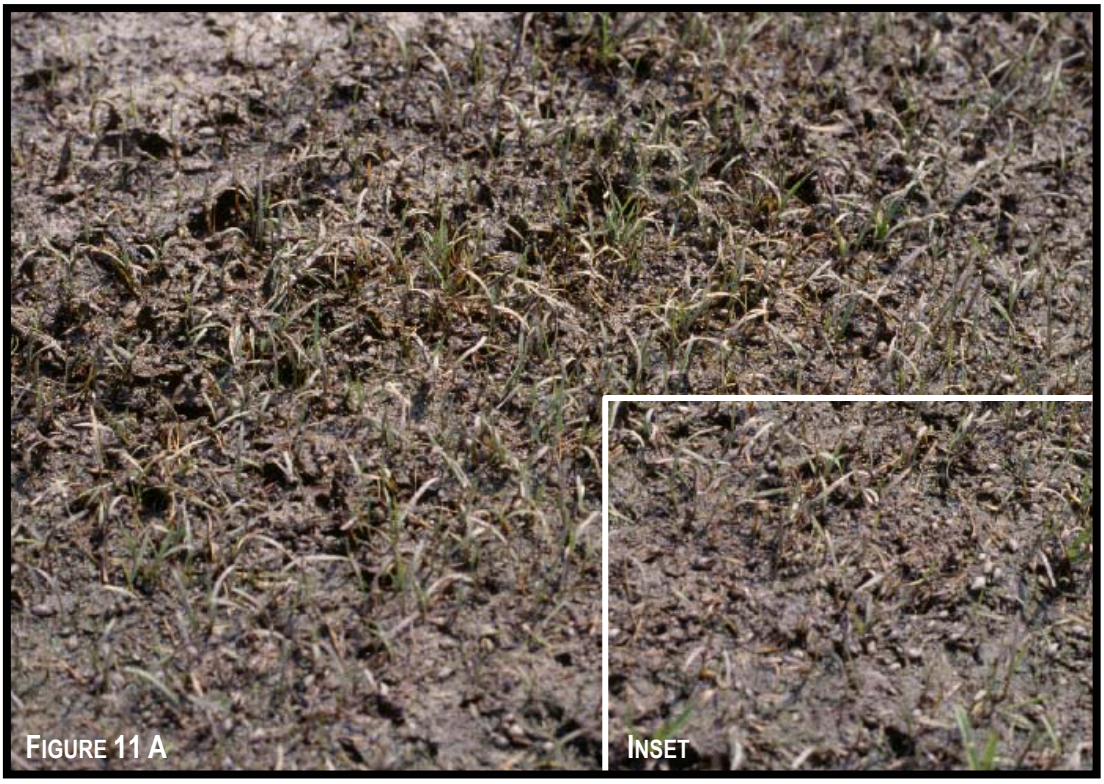


FIGURE 11

Figures 11A and 11B (with insets) are site germination photographs taken at the same site, but at slightly different elevations, showing variability of moisture regimes. In Figure 11A (broad view), note the fairly large number of prominent un-germinated seeds commingled with seedlings. Insets 11A and 11B are close ups showing germination at 30 days. Note the variability of germination, from two-leaf standing seedlings to early shoot and root elongation. Seedlings and early germinated seed in both of the inset photographs would have been deemed germinated and counted.

Subtask 3c: Seedling Survival

Seedling survival measurements were taken at 90 days post-seeding. We used the same sample unit size, frequency, and methodology for seedling counts as that for distribution and germination.

Seedlings were determined as surviving if the plantlets had elongated past their primary shoot and protective coleoptile (first shoot leaf) stage, were upright, anchored, and were developed to at least their third leaf stage (Figure 12). Seeds that were still in various stages of germination, and seedlings that were obviously chlorotic or damaged were not counted.

Subtask 3d: Productivity Assessments

Several productivity measurements were taken at the end of the first growing season to assess species composition and relative abundance. We sampled over a one week period beginning in early November, a time generally considered to be peak biomass accumulation. Samples were collected from the two brown marsh sites at Bayou Lafourche and Lake Felicity, the dredge-sediment site at Port Fourchon, site controls, and reference marshes. Measurements taken at each site were species composition, cover value, and standing crop biomass. No productivity measurements were taken at the Plant Materials Center at Golden Meadow as sampling was terminated after the second germination measurement at the PMC, as there were no surviving plants beyond a very young seedling stage.

Percent cover by species was visually estimated using a ½m^2 quadrat divided into quarter quads for improved visual separation. The Bayou Lafourche and Lake Felicity sites (3 acres each) were divided into approximately 3 equal subplots (approximately 1 acre each) and 5 sampling points were randomly selected within each of the 1 acre subplots. The larger Port Fourchon site (25 acres) was divided into approximately 5 equal subplots (approximately 5 acres each) and 6 sampling points were randomly selected within each of the 5 acre subplots. This provided samples sizes of 15 and 30, respectively, for the treatment sites. Figure 13 shows early summer growth. We saw little additional plant mortality from seedlings surviving to this growth stage.

To assess relative abundance, we used a clip-plot method (Mueller-Dombois and Ellenburg, 1974) to obtain above-ground biomass. Standing crop was harvested by clipping plant materials within a ½m^2 quadrat at approximately 1 cm above the soil surface. Clipped material was placed in plastic bags, labeled, and transported back to the lab for separation.

Under laboratory conditions, individual samples were separated by species and by presence or absence of a stem inflorescence. The length and basal diameter of 10 selected culms were measured and the total number of stems by species were counted. Clipped material was placed in labeled brown kraft bags, weighed, dried at 75°C to a constant weight, and re-weighed.

FIGURE 12

Figures 12A and 12B are seedling (90 day) survival photographs taken at two different sites. Figure 12A was taken at Lake Felicity and Figure 12B at Port Fourchon. Note the difference in soil textures between the two sites. Soil texture at the Lake Felicity site was primarily a silty clay muck, while some (but not all) of Port Fourchon contained large concentration of hemic, relatively un-decomposed organic matter. These large, blocky, coarse particles are dark brown to black in color and are generically referred to as "coffee grinds." Although these high organic areas provided excellent sites for germination, they proved to be problematic in later plant growth stages. Also, note the reduced number of seedling per unit measure compared to previous photographs showing large number of seed and germinating seedlings. Figure 12A inset, represents a seedling of interest at the 90 day seedling survival assessment; that is, a seedling that is upright, well anchored, and developed to at least the three-leaf stage.

FIGURE 13

Figure 13A is an aerial taken in mid-summer at the Lake Felicity seeded site. Individual plants are still somewhat discernable, but many are beginning to coalesce into larger units. Although seedlings are fully developed at this time, there are a number of indicators that separate seedlings from tillers regenerating from residual rhizomes. Note in Figure 13B the single long stem with closely grouped shorter lateral stems. The longer stem is the original primary shoot that elongated from the seed and the shorter secondary stems are from newly developing rhizomes. Also, the underground portion of a seedling (at this stage) is a is a compact unit of mostly fibrous roots with little evidence of rhizome development. Figure 13C compares a developing seedling (on the right side) with a single new tiller from a weakly surviving plant on the left. Note the dead stem from the previous season still standing and attached at the rhizome.

RESULTS AND DISCUSSION

Subtask 5.2 – Seeding Demonstration was designed and implemented to assess the potential for developing and expanding a seed-based restoration program in Louisiana. *Spartina alterniflora* is the primary and dominant plant species in Louisiana's saline marshes and is heavily used in association with marsh enhancement projects. The prevailing method for artificially establishing *S. alterniflora* is by clonal division, a problematic procedure on several levels. For example, at $2 to $8 per transplant, the expense associated with a vegetative restoration project of any magnitude would quickly become cost prohibitive. In addition, the vast majority of Louisiana's saline marshes are remotely located and are environmentally sensitive to damage from equipment.

Aerial seeding *S. alterniflora* offers an attractive, cost efficient, and non-invasive alternative to current planting technology. However, virtually every aspect of *S. alterniflora* seed handling in quantity is tenuous and needs further defining before seeding will be widely accepted.

In this section we will present our results and observations by the three primary groups that encompass the 14 subtasks, beginning with initial seed collection through productivity measurements. We will end the section with conclusions and a number recommendations.

I. SEED ELEMENTS

Seed Collection and Storage Elements

There are two potential sources from which an adequate supply of *S. alterniflora* seed could be obtained; wild seed from native marshes, or cultivated seed from managed ponds. The vast majority of natural *S. alterniflora* marshes in Louisiana are inaccessible and would be irreparably damaged with the introduction of heavy harvesting equipment. There are, however, large areas of highly productive marsh with firm soils. Most of these are dredge-sediment (created) marshes and generally have soils with an atypically high mineral content. For example, there are two large areas in Cameron Parish at Black Lake, Grand Terre in Jefferson Parish, the Port Fourchon mitigation sites, West Belle Pass in Lafourche Parish, and numerous sites that the Army Corps of Engineers use as sediment disposal sites. One or more of these marshes could be dedicated and managed for producing foundation seed.

In addition, *S. alterniflora* has been artificially cultivated in managed ponds at the Natural Resources Conservation Service's Golden Meadow Plant Materials Center and at the Louisiana State University AgCenter Rice Research Station for years. It has been our experience that even relatively small acreage of cultivated *S. alterniflora* will produce a significant amount of seed. Since water levels are artificially controlled in production ponds, a short draw-down prior to harvest can greatly facilitate mechanical harvesting and minimize plant/pond damage.

In both 2000 and 2001 we were able to hand collect enough seed to adequately implement an aerial seeding demonstration. Three crews of three to five members collected seeds over a two week period. A small portion of the seed was mechanically harvested using a flail harvester. Seeds harvested mechanically were handled and processed in the same manner as hand harvested seedheads.

In 2000, we collected and processed 440 pounds of clean seeds from 4 sites; the Black Lake sediment cells, the Louisiana State University AgCenter Rice Research Station, the Natural Resources Conservation Service Plant Materials Center, and the West Belle Pass dredge-sediment site. In 2001, using the same methods we collected and processed 980 pounds of clean seed from 3 sites; the Black Lake sediment cells, the Natural Resources Conservation Service Plant Materials Center, and the Grand Terre dredge-sediment site. Although we had not yet developed our experimental seeding rate in 2000, we felt confident that approximately 500 pounds of clean seed would be adequate for a multi-site, multi-acre test.

Throughout the harvesting process, we noted a significant contrast in plant density and apparent seed quality between wild seed collected from open marsh and seed collected from cultivated pond sites. Although we maintained seed by collection lots, we did not attempt to determine seed production on a per unit basis such as, seeds per seedhead. We did, however, measure three seed variables that are typically good indicators of seed quality, and are almost always factors when calculating and applying seed rates. We measured seed weight, germination, and viability by collection site.

Seed Weight

If we assume that seeds with fully developed endosperm (filled) are heavier than empty or immature seeds, then we would expect lower seed/gram from sites with greater quality seed. We compared seeds/gram at 4 sites in year 2000 and 3 sites in year 2001. There was no significant difference between years, suggesting that at least for these two years, there were no flooding or other climatic factors that affected seed quality.

Therefore, we treated years as replicate collections, such that we had 7 different site-years; 3 cultivated site-years from the Plant Materials Center (2 years), 1 year from the Rice Research Station, and 4 site-year collections of open marsh (wild seed) from Cameron (2 years), West Belle Pass (1), and Grand Terre (1). An analysis of variance across the seven sites showed highly significant differences ($p<0.0001$), with the three cultivated collections all being alike and different from the four wild collections in pairwise comparisons (LSD t-tests, $p<0.05$). Wild seed varied from 231 to 242 seeds/gram, whereas cultivated seed varied from 140 to 156 seeds/gram (Figure 14).

Overall, we concluded that seeds harvested from cultivated sites averaged 150 seeds/gram versus seeds harvested from open marsh which averaged 235 seeds/gram. Consequently, the wild collection seed lots had 58% more seeds/gram than seeds harvested from cultivated ponds (Figure 14). Fewer seeds/gram means heavier seeds exhibit greater endosperm development which may represent greater seed viability.

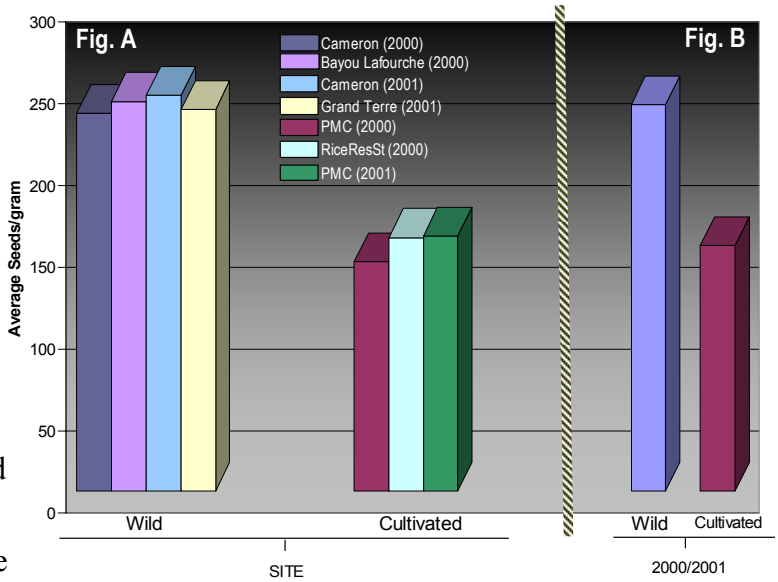


FIGURE 14
Seed Density Summary – Average number of seed/gram; (Fig. A) collection site by habit across year, (Fig. B) average seeds/g habit across both site and year.

Seed Viability and Germination

Although seed density may be a reasonable estimator of seed quality, percent germination is still the primary standard for seed quality assessment. As discussed earlier, *S. alterniflora* seeds are both dormant and recalcitrant; consequently, standard germination testing alone may not be the best measurement to approximate seed quality, as it perhaps underestimates seed viability.

The germination analysis involved an extensive effort based on a factorial design testing several different experimental factors, previously described in detail in the Methods section. The main factors were as follows: (1) seed collections varied by year, 2000 and 2001; (2) seed source varied by site, with wild seed from open marshes and cultivated seed from experimental ponds; (3) seed varied by treatment, as clipped and unclipped; and (4) stratification varied from none to 30, 60, 90, and 120 days. In total we tested 4,800 seeds for germination.

A preliminary analysis showed that seed collection year as a main effect had no significant effect and no interaction effect with other factors. Consequently, the analysis was simplified to retain the three remaining factors – seed source, seed treatment, and storage (stratification). An overall analysis of variance showed that all three factors had significant effects on germination ($p<0.0001$). In addition, there were significant interactions among each pair of the three factors. We present the results in order of the importance of the factors. First, seed germination treatment (clipped and unclipped), appeared to have a greater effect on germination than seed source or storage. The percent germination of clipped seed (36.6±14.9) was much greater than unclipped seed (13.6±9.8). This was true for both years and across all seed source sites (Figure 15).

Second in importance was seed source. The percent germination of cultivated seed (30.4±19.2) from the PMC and the Rice Research Station was greater than open marsh seed (20.0±12.0). This was true in both collection years, 2000 and 2001. Equally important as seed source was stratification. There was a positive relationship between increased stratification (storage) time and germination. Percent germination increased slightly from 0 to 30 days, rapidly from 30 to 60 days, and slowing again between 60 to 120 days produced an S-shape slip curve shown in Figure 16.

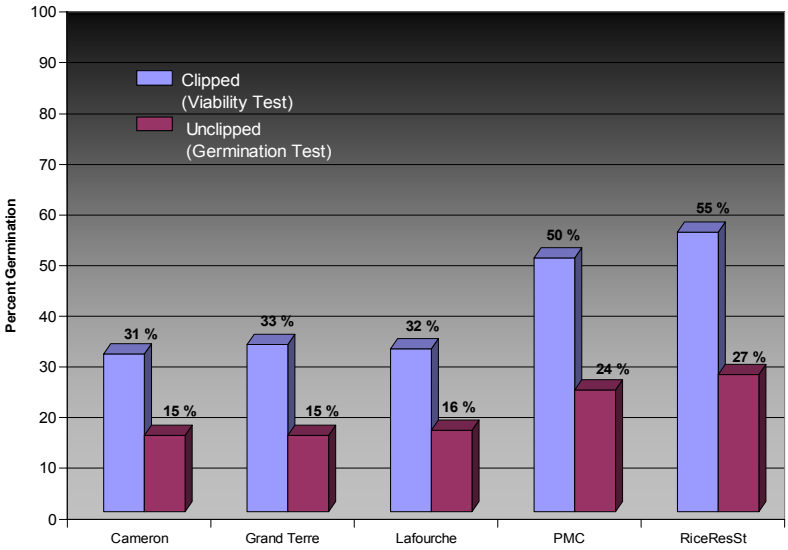


FIGURE 15
Germination By Seed source – Seed germination by collection site and seed treatment (clipped and unclipped) t across year.

In addition to the three main effects, the statistical analysis showed small interactions among treatments. Again, these can best be visualized in Figure 16, where cold storage increased germination for both clipped and unclipped seeds, differing, however, in the rate at which they increase. Germination in the clipped treatment increased at a relatively constant rate from 30 to 90 days, whereas germination within the unclipped treatment increased rapidly from 30 to 60 days, but proportionally less from 60 to 90 days. We mention these interactive effects as they are statistically significant, but in addition, have some biological importance. For example, cold storage for unclipped seeds could be terminated at 60 days without much loss in germination, whereas, stratification should be continued for 90 days for clipped seeds. However, the most significant results were found in main effect treatments, not in the interactions. The main effects were (1) clipping produces significantly larger germination results (54% for clipped seed), (2) followed by seed source (45% with cultivated seed), then by cold-wet storage (35% at 120 day stratification).

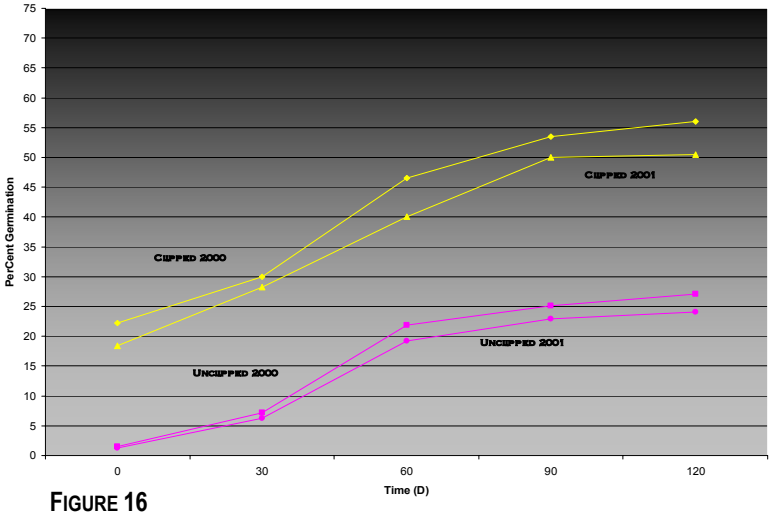


FIGURE 16
Stratification Effect – Seed germination by seed treatment (clipped and unclipped) year, and stratification period, across collection site.

II. Calculations and Methods

Calculations and Seed Preparations

As discussed earlier in the *methods section* of this report, we were satisfied that the rice model typically used to calculate aerial seeding rates would serve as a basic template to develop a *S. alterniflora* seeding rate. However, we were concerned about the germination differential between the two species. We estimated that *S. alterniflora* produces approximately 90,000 seeds per pound based on the average of 100 random samples that were weighted and counted. In addition, we estimated that the 2000 year seed lot had a viability rate of approximately 56%. We based this conclusion on the germination rates obtained from clipped seeds across the 4 collection sites. Consequently, as *S. alterniflora* germination rates are considerably lower than our rice model at 80%, the target number of seeds/ft^2 (~55) used in rice culture may be an under estimate when seeding in a marsh environment. To compensate for the germination differential, we proposed to include a pure-live-seed (PLS) component to the rice model formula that would increase the target number of seed proportionally to compensate for *S. alterniflora's* lower germination rate.

Modifying the seed rice formula to include a PLS compensation, the formula and resulting *S. alterniflora* seeding rate is as follows:

50 *(target seeds/ft^2)* x 43,560 *(ac ft^2)* = 2,178,000*(seeds/ac^2)*

2,178,000/90,00 *(S. alterniflora seeds/lb)* = 24 *(lb of seed/ac^2)*

24/.56 *(% germination)* = 43 *(seeding rate in lb/ac^2 based on pure live seed)*

(43 *(lb/ac^2)* x 90,000 *(seeds/lb))*/43,560 *(ac ft^2)* = 89 *(actual seeds/ft^2)*

Seed Carrier and Blending Methods

Although *S. alterniflora* is a relatively large seed, it is thin-walled and relatively light in weight. Because of its recalcitrant nature, seeds are stored aqueously and become cohesive, forming sticky clumps when drained. To compensate for the excessive seed moisture and clumping, we blended an inert clay with the seed to form a carrier-mix that separated seeds, absorbed excessive moisture, provided additional weight, and facilitated uniform seed distribution when released aerially. We experimented with a number of organic and mineral materials before settling on absorbent clay as a carrier. Palygorskite is a group of absorbent clays that include montmorillonite, bentonite, and diatomite. They are inexpensive, readily available in bulk, and available under a number of commercial trade names.

We blended seeds and carrier using a clean, small-drum, motorized cement mixer. We had used other motorized mixing equipment in the past, such as soil blenders, but found that they damaged

the seed as the rotating paddle scrapped the drum wall. Cement mixers use a different paddle design and proved to be equally efficient, providing an excellent mix, with absolutely no damage to the seed. In addition, the front-open design of the hopper provided simple access to the blend as it mixed. Consequently, it was easy to continually monitor the moisture content of the mix, adding additional water when excessively dry, or additional absorbent when too moist.

We used a 1:2 ratio of seed-to-carrier by weight. As most commercial clays are pre-packaged in 1, 5, 10, 25, 40, and 50 pound units, mixing by weight proved to be consistent and expedient. Seeds were weighed and mixed the afternoon prior to seeding and stored in moistened burlap sacks. We mixed approximately 1,200 pounds of seed/carrier in 2001 and slightly under 3,000 pounds in 2002 for distribution.

Aerial Contractor and Plane Calibrations

One year earlier, in March of 2000, the LSU AgCenter and the NRCS Plant Materials Program through a cooperative agreement, completed a small aerial seeding demonstration at Grand Terre Island. A fairly large relic of marshes on the Barataria Bay side of Grand Terre had been filled with dredge-sediments the previous year and was virtually void of vegetation. The LSU AgCenter established a contract with Air Farms, an agricultural aerial applicator to fly *S. alterniflora* seed at Grand Terre as part of a demonstration field day. Air Farms subsequently agreed to contract for the aerial application the following years, and provided aerial and logistical support services for both the 2001 and 2002 study sites.

Since we had experience with Air Farms using literally the same seed material and methods from the previous year, calibrating the aircraft for the 2001 study was relatively quick and easy, as we only needed to make minor adjustments to increase the seeding rate. To accomplish this we provided Air Farms with approximately 200 pounds of a ryegrass-clay mix and counted seeds as previously discussed in the Methods section.

Aerial Seeding

Four sites were selected for aerial seeding over a two year period. Three sites were seeded on April 17, 2001 and one additional site the following year on March 8, 2002. The three sites seeded in 2001 were two 3 acre brown marsh sites, Bayou Lafourche and Lake Felicity, and a 3 acre control pond at the PMC in Golden Meadow. The 2002 year study site was a 25 acre created-marsh at Port Fourchon. Sites boundaries were pre-marked with flagging and two flagmen were present at each site to mark flight lines during seeding. The pilot carried only enough material to seed one site at a time, returning to the airfield to reload between applications. All of the sites in both years were seeded with 43 pounds/acre of *S. alterniflora* seed.

Seeds were flown at low tide to facilitate seed/mud contact. It has been our experience that, even though filled seeds will sink, seeds flown directly into water tend to stay in suspension. Floating seeds, depending on water depth, wind, and tidal energy tend to concentrate in drift lines, resulting in irregular and poor spatial distribution. However, even light unfilled seed, when dropped on exposed mud are easily embedded and are quickly covered with a shim of sediment following the first tide cycle.

III. Treatment Measurements

Site Seeding Density and Distribution

The relative success at which we can distribute predetermined quantities of seed efficiently and in mass, is one of the more critical elements of this study. Obtaining good spatial seed distribution while controlling the density of seeds within a given unit area, is the first step towards broad-scale acceptance of aerial seeding by the restoration community.

Seed density per unit area is the primary variable that we measured under the seed distribution subtask. Seed measurements were taken immediately after aerial application at each site. Samples were taken using a ½m^2 quadrat and a sampling frequently of 15 at the Bayou Lafourche, Lake Felicity, and the PMC treatment sites. Control sites for Bayou Lafourche and Lake Felicity were both sampled at the same time and at the same frequency. We did not establish a control at the PMC site, as the pond's retention levees would preclude any drift or random chance seed occurrence. In addition, there were no other *S. alterniflora* plants within the immediate area of the PMC treatment site. The Port Fourchon site and control were sampled using the same ½m^2 quadrat but at a greater frequency given its larger sizes. The Port Fourchon site is eight times larger than the brown marsh sites; consequently, we increased the sample frequently from 15 to 30.

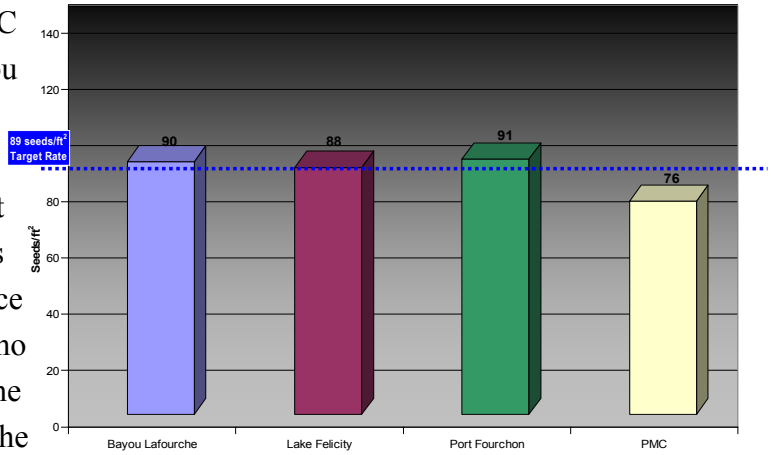


FIGURE 17
Seeding Site Density – Average number of seeds/ft^2 obtained by site, plotted against the target 89 seeds/ft^2 (broken line).

The density of seeds across all 4 sites was consistently uniform with an average of 87±12 seeds/ft^2. However, there were significant variations among site means ($p<0.0006$). Results from the least significant difference (LSD t-test) divided sites into two groups based on similarity of means within site and differences between sites. Pairwise comparison of the means showed

that the Plant Materials Center was significantly different from the three marsh sites (LSD t-test, $p<0.05$), but the three marsh sites were not different from each other (Figure 17).

Assessing a quantitative measure of spatial distribution for each density sample was beyond the scope of this study. We did, however, make ocular assessments of seeding patterns at every sample, and did not find a single occurrence of clumping or irregular patterns of seed concentrations. Seeds were uniformly and evenly distributed across every sample taken Figure 10).

The target number of seeds for all sites was 89/ft^2. Average seed density at three of the sites, Bayou Lafourche, Lake Felicity, and Port Fourchon were ±2% of the target number (~2 seeds/ft^2) while density at the PMC was 17% (~13 seeds) short of the target. Figure 17 contains average density by site plotted against the target number projected for each site (broken line).

Site germination

We assumed that there would be a germination differential between our controlled germination tests and actual site germination under marsh conditions. The degree of difference, and the amount of variability among sites were two important measurements that would influence future seeding models.

We obtained the field germination results using the following methodology. Germination measurements were taken at 30 and 60 day intervals at each treatment and adjacent unseeded control site. We used the same sample unit size, frequency, and methodology for seed germination measures as that used for seed distribution counts. Seeds were determined to be germinated if either the plumule (shoot) or radicle (primary or seminal roots) were visible at 30 or 60 days; seedlings were also counted along with shoot/root as indicators at 60 days.

The data were analyzed by subtracting the unseeded control germinations from the seeded germinations, yielding a net germinations count. We found some germinations in the three control sites, but they were infrequent and their numbers very low (<1.0 per ½m^2 quadrat). For example, of the 60 sample points taken across 3 control sites, we found only 29 samples that contained 1 or more seeds. The average across 60 samples was 0.7 germinations per ½m^2, with a minimum of 0 and a maximum of 3 germinated seeds across all samples. In total, there were only 40 germinating seeds found in 60 control samples across all sites. The low number of control seeds indicates that there was limited natural distribution or drift of *S. alterniflora* seeds into the control sites from surrounding communities, and provides additional evidence that *S. alterniflora* seeds do not persist in saline marshes and there is little, if any, annual *S. alterniflora* seed derived from seedbanks found in saline soils.

Net germination for the seeded areas at marsh sites and at the Plant Materials Center averaged 134±33 per ½m^2 after 30 days. An analysis of variance (ANOVA) showed no variation among the four sites (p=0.10). However, germination after 60 days dropped slightly from the 30 day count to 130±49 per ½m^2. An ANOVA of the 60 day germination data showed significant variation among sites (p<0.0001) with the Plant Materials Center being different (Figure 18) from the three marsh sites (p<0.05).

One obvious explanation for the reduction in PMC germination from 30 to 60 day was a competitive effect resulting from a proliferation of freshwater seedlings that advanced rapidly between 30 and 60 days samples. In addition, it is reasonable to assume that fresh environments (such as the PMC) would support a greater fungal population than that found in marine environments, consequently having the potential for greater pathogenic mortality.

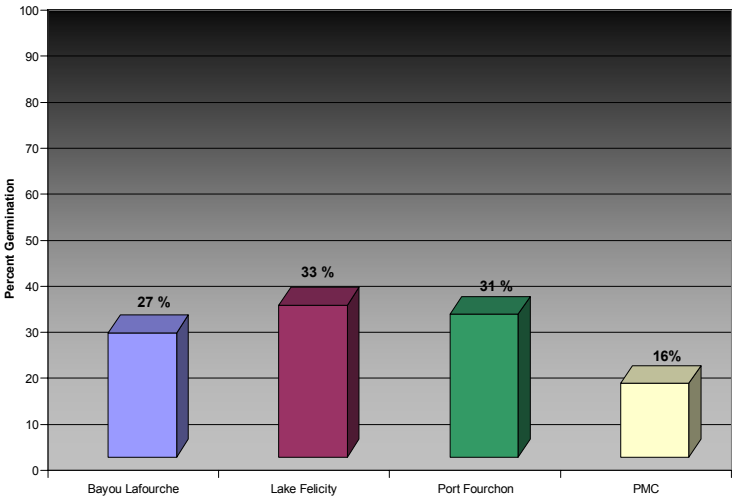


FIGURE 18
Seeded Site Germination – Average germination by seeded site at 60 days.

In calculating the *S. alterniflora* aerial seeding rate, we used the higher of two germination results we obtained from our controlled germination test. Clipped seeds, which circumvented dormancy inhibitors provided a 54% germination rate, while unclipped seeds provided a 26% germination rate. We hypothesized that the viability test (clipped seeds) was a more accurate measure of a seed-lot's true germination potential. Although this may still be accurate, it appears that germination results from unaltered seeds (26%) may be the more accurate estimator of field germination potential as it closely mimicked the actual field results that we obtained. Therefore, we significantly underestimated the number of seeds per unit needed to obtain a predetermine field germination.

Seedling Survival

Seedling survival measurements were taken 90 days after aerial seeding. Seedling samples were not taken at the Plant Materials Center at Golden Meadow, as sampling was discontinued after the second germination measurement following high seedling mortality associated with extensive freshwater plant competitors. We used the same sample quadrat size, frequency, and methodology for seedling counts as that for germination counts except that seedling survival was defined more rigorously than germination. Seedlings were scored as "surviving" if the plantlets had elongated past their primary shoot and protective coleoptile (first shoot leaf), were upright, anchored, and were developed to at least their third leaf stage. Seeds that were still in various

stages of germination, and seedlings that were obviously chlorotic or damaged were not counted. Again, we subtracted the number of seedlings in unseeded control sites from the number in the aerially seeded sites to obtain a net surviving seedlings—i.e., the number of surviving seedlings directly attributable to aerial seeding. The number of surviving seedlings found within the control sites was very low. Of the 60 sample points taken across 3 control sites, we found only 19 samples that contained 1 or more seedlings. The average across 60 samples was 0.3 seedlings per ½m^2 with a minimum of 0 and a maximum of 2 from all samples; there were only a total of 25 seedlings found in 60 samples across all control sites.

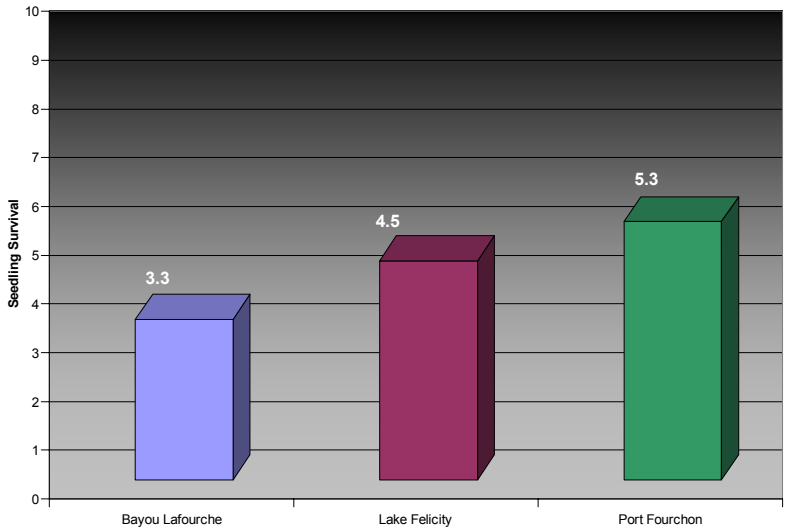


FIGURE 19
Seedling Survival – Number of surviving seedlings per ½m^2 by seeded site.

Net surviving seedlings varied slightly across the three seeded marsh sites (ANOVA, p=0.01), with means ranging from 3.3±1.8 to 5.3±2.1 as shown in Figure 19. There were no site differences in a pairwise comparisons (LSD t-test, p=0.05). Converting site averages of surviving seedlings from ½m^2 to ft^2 illustrates the low net ratio of seeds sown to surviving plants. For example, the average seeding rates at the 3 marsh sites was 468 seeds/m^2 which producing 4 surviving seedling per ½m^2. Converted seeds and seedlings to square feet produces 87 seeds/ft^2 and 0.7 seedling/ft^2, less than 1 seedlings/ft^2 of marsh seeded. See Table 3 for individual seeded site figures.

TABLE 3 Seedling Survival and Conversion

Variable	Site		
	Bayou Lafourche	Lake Felicity	Port Fourchon
Net Surviving Seedlings/½m^2	3.3	4.5	5.3
Net Surviving Seedlings/ft^2	0.61	0.83	1.0
% Seedling Survival			
Based on Seeds/Site	0.69	0.94	1.09
Based on Germination/Site	1.49	1.54	1.42

Without further experiments we can only hypothesize as to factors that may contribute to the low seedling survival given the relatively high density of seed distribution. However, it is

noteworthy to make two observations on soil conditions that we observed while conducting the seedling sample; one is related to high organic content and the other to algal bloom.

In very high organic soils (at least in the surface layer), healthy and robust seedlings, still attached at their root tips (Figure 20), were lodged and laying flat on the soil surface in large numbers. We speculated that energy from tides or wind at low water, were sweeping the loose, buoyant, and unconsolidated organic particles away from seedling roots until they could no longer support their aboveground biomass, and lodged over. We did not see any seedlings in any stage of "righting" themselves, but did observe several downed seedlings in varying stages of mortality, indicating that downed seedlings were not recovering. In addition, we found large areas of algal bloom (Figure 20) smothering germinating seeds and seedlings as the algae dehydrated. These two factors and the extent of the area that they impacted were large enough to significantly effect seed germination and surviving seedling percentages.

Productivity Assessments

For decades, agricultural aerial seeding has been demonstrated to be one of the most efficient and cost effective methods for establishing vegetative cover over large areas. However, as a wetlands restoration tool, the availability and applicability of plant materials has impeded its usefulness in coastal marshes.

The goal of this study was to quantify the application of aerial seeding as a means to accelerate the functional development and vegetative equivalency of created or rehabilitated *S. alterniflora* marshes to that of natural marshes. To determine productivity, several measurements of live standing biomass were taken at the end of the first growing season to assess vegetative abundance at aerial seeded sites, adjacent control sites, as well as an equivalent adjacent reference marsh. We sampled over a one week period beginning in early November, a time generally considered to be peak biomass accumulation. Samples were collected from the seeded marsh sites at Bayou Lafourche, Lake Felicity, and Port Fourchon, 3 adjacent controls sites, and 3 adjacent reference marshes. Measurements taken at each site were species composition, stem density, stem height, stem diameter, inflorescence production, and total biomass expressed as dry weight. To assess relative abundance, we used a clip-plot method (Mueller-Dombois and Ellenburg, 1974) to obtain data on stems, inflorescences and aboveground biomass. *Spartina alterniflora* standing crop was harvested from ½m^2 quadrats by clipping plant materials at approximately 1 cm above the soil surface. Clipped material was placed in plastic bags, labeled, and transported back to the lab for separation and measurement.

FIGURE 20

There was a steady decline in the number of seedlings that survived to maturation. Although it would be reasonable to assume a degree of natural mortality caused by pathogens, birds, and other predators associated with plants established from seed, we observed a number of other biotic and abiotic elements that significantly influenced seedling survival at some sites. For example, loose and shifting surface organics (Figures 20A and inset) undermined large number of seedlings until they lodged over and died; note the withered seedlings laying over in the inset. In addition, large areas of algal mats (Figures 20B and inset) smothered a significant number of small seedlings; note the only seedlings visible are those along fissure openings in the algal mat.

Although there is some anecdotal evidence that marsh periwinkle (*Littoraria irrorata*) can and does inflict damage to S*partina alterniflora*, these two photographs (Figures 20C and Inset) represent one example of the type of concentration of herbivore and omnivore populations that use developing seedlings as a food source.

Under laboratory conditions, individual samples were separated by species, living and dead stems, and by the presence or absence of a stem inflorescence; dead stems, leaves, and other plant fragments were discarded from the sample. The length and basal diameter of 10 selected culms from each sample were measured, and the total number of stems by species were counted. Clipped material was placed in labeled brown kraft bags, weighed, dried at 75°C to a constant weight, and re-weighed.

BIOMASS RESULTS

All results were determined from collections made in November, approximately 9 months after the experimental sites were aerially seeded.

Species Composition

The dominant vegetation in the Louisiana's saline marshes is *S. alterniflora*. Within the intertidal zones and where mean salinity is ~20%, *S. alterniflora* occurs in nearly pure stands forming distinct ecological zones. There are a number of sympatric species such as *Distichlis spicata, Juncus roemerianus, Salicornia virginica* and *S. biglovii* that are often found intermixed in *S. alterniflora* communities, however, these species generally constitute only a small percentage of the total vegetation.

Relative to species composition, we found across all treatments that all sites were virtually monospecific in plant species composition. That is, we found no other species other than *S. alterniflora* in any of the 135 samples collected and analyzed.

Stem Density

Stem densities were measured for (1) treatment sites where aerial seeding was applied; (2) control sites that initially had no *S. alterniflora* resulting either from brown marsh die-back of existing marsh or from the formation of newly created marsh by dredge-sediments; and (3) reference marshes that had a full complement of *S. alterniflora* that were not impacted by brown marsh or other significant disturbances . Stem densities were analyzed with a two factor analysis of variance that tested for differences by treatments (control, reference, and seeded) and by site. The ANOVA shows a strong treatment effect ($p<0.0001$) and a smaller site effect. Pairwise comparisons showed that the density of seeded plots was not significantly different from the reference plots (LSD t-test, $p=0.32$), indicating that in a single year, the seeded plots had achieved densities indistinguishable from the reference marsh, with about 100 stems per ½m^2 (Figure 21). In contrast, the unseeded control plots had only 6-12 stems per ½m^2, significantly less than the seeded and reference plots ($p<0.0001$).

Clearly, there is some variation among sites that is much less than the effect of aerial seeding. Statistically, when we check the LSD comparisons for significant differences, we find that the control sites are different from each other and were consistently lower than both their respective reference and seeded sites. The low frequency of plant occurrence within the control sites are consistent with other studies and support our findings that there is limited outside seed transport even in the presence of surrounding *S. alterniflora* marshes; and, that there is a low occurrence of a viable *S. alterniflora* seedbank in saline marsh soils. As demonstrated in the control sites, there is little natural foundation material found in highly disturbed or dredge-sediments soil; consequently, vegetative cover is generally sparse, uncertain, and slow to establish. Reference sites were not significantly different from each other, however, treatment sites were ($p<0.01$), varying from 76 to 128 stems.

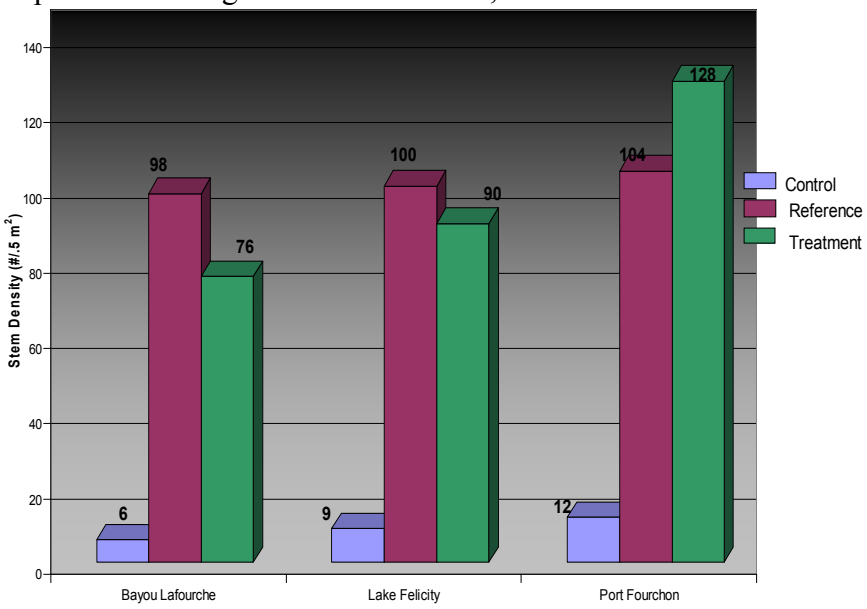


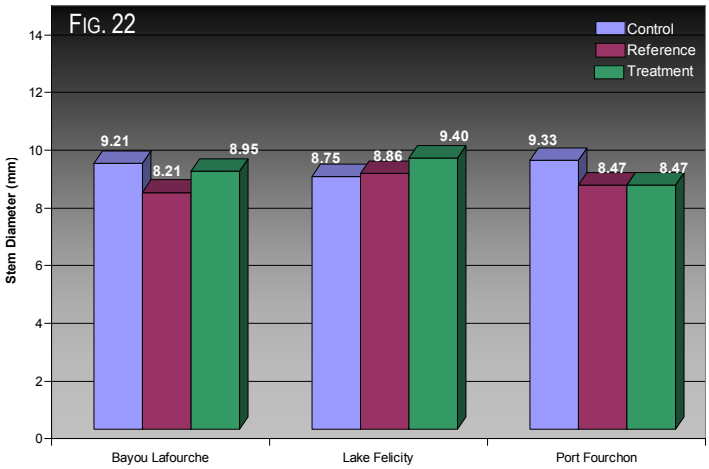
FIGURE 21
Stem Density – Compares averages number of stems produced by treatment within site

Stem Height and Diameter

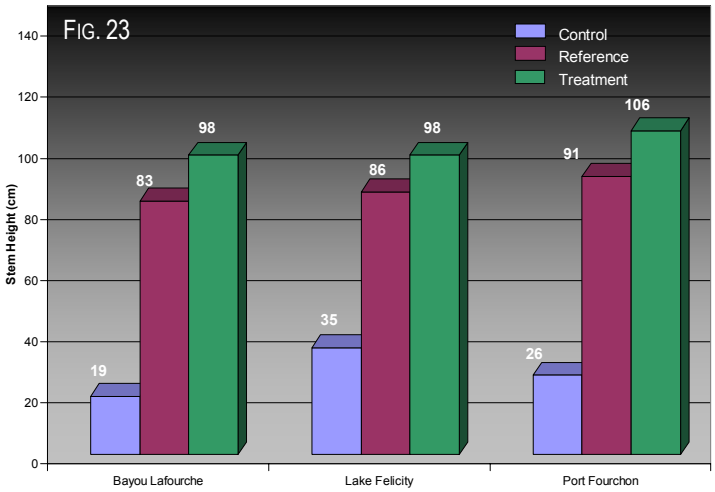
Stem height and diameter were measured for (1) treatment sites where aerial seeding was applied; (2) control sites that initially had no *S. alterniflora* resulting either from brown marsh die-back or newly created marsh; and (3) reference marshes that had a full complement of *S. alterniflora* and were not impacted by brown marsh nor been recently disturbed. These three sites were compared across treatment sites at Bayou Lafourche, Lake Felicity, and Port Fourchon.

Stem heights were analyzed with an ANOVA that tested for differences in stem height across treatments, across sites and across samples nested in sites. The ANOVA showed a strong effect of treatment ($p<0.001$). There was also a much smaller effect of site, that was statistically significant given the large sample sizes ($p<0.0001$), but biologically it was very minor.

The post-hoc comparison of means, utilizing the LSD t-test shows all three treatments (control, reference, and seeded) were different from each other ($p<0.0001$). Seeded plots and reference plots had similar plant heights, about 1.0m, with the seeded plots about 15% taller. In contrast, plants in the control plots were drastically smaller, varying from 19 to 35 cm in mean height across site (Figure 22).



Although stem heights varied widely among treatments, stem diameters were relatively uniform among treatments. An ANOVA showed statistically significant differences, given the large sample size ($p=0.0001$). Control stems had slightly larger diameters (9.2mm) than seeded stems (8.8mm) or reference stems (8.5mm), but again, these differences are very small biologically (Figure 23).



FIGURES 22 DIAMETER AND 23 HEIGHT
Stem Diameter and Height – Compares average stem height and stem diameter within treatment and seeded site.

Stem Inflorescence

Each *S. alterniflora* stem was assessed for reproductive activity and the inflorescence analysis is presented in two stages, absolute number of inflorescences per quadrat and percent of stems with inflorescences.

The absolute number of inflorescence were analyzed with an ANOVA that tested for differences in number across treatments and across sites. The ANOVA showed a strong treatment effect ($p<0.0001$). There was also a much smaller site effect, although it too was statistically significant given the large sample size ($p<0.0001$). The number of flowering stems in the aerially seeded plots, range from 63 to 85 stems/½m^2 (Figure 24). The LSD t-test comparing treatment means showed that the seeded plots were significantly different from both the reference and control plots ($p<0.0001$), but that the latter two were not different from each other ($p<0.34$).

The percent of inflorescence per quadrat were analyzed with an ANOVA and again showed a strong effect of the seeding treatment and a somewhat smaller effect of site ($p<0.0001$).

The percent results, of course, parallel to some degree the absolute number of inflorescences, but reveal some important differences. First, the aerially seeded plots are not only producing many more inflorescences than the reference and control plots, but their stems are producing seed at a very high rate, i.e., 72%. That implies that establishment in the year of aerial seeding will yield high seed production for additional local colonization. This may also mean, that in larger scale applications, the rate of aerial seeding can be adjusted downward according to the timeline for marsh restoration. Overall, we should expect that applications over larger marsh areas will achieve both economy of scale and economy of seeding by establishing plants.

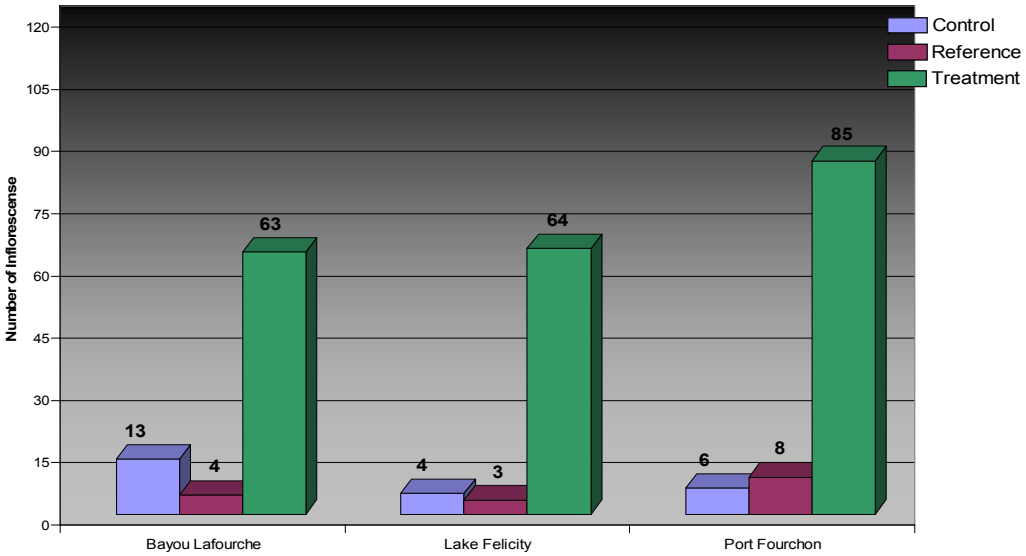


FIGURE 24
Inflorescence Production – Compares the average number of inflorescent stems within treatment and across seeded sites

Second, while the absolute number of inflorescences was similar in the reference and control plots, the percent with inflorescences was very different, 38% for the controls and 5% for the references. However, the higher percent in the controls is offset by the reduced number of stems produced, thereby yielding about the same number of stems with inflorescences for control and reference plots as shown in Figure 24.

Biomass

Above ground biomass was determined for the unseeded control plots, the aerially seeded plots and the mature reference marshes. Biomass, as dry weight and as wet weight, was measured from harvested plant material. As expected, these results tended to parallel those for stem density and stem height. Here, we present the results for dry weights only, as wet weights are a function of dry weights and there was a high correlation, as would be expected, between the two measurements ($R^2=0.99$). As in previous comparisons, we excluded any samples from the analysis that were completely void of any vegetation.

Dry weight biomass showed significantly large variation across treatments (control, reference, and seeded) and some variation across sites (ANOVA =p<0.0001). All treatment means were significantly different from each other (LSD t-test, p<0.005), as shown in Figure 25. In general, the seeded plots equaled or exceeded the reference plot means, although in the case of Port Fourchon, the seeded plot significantly exceeded the reference mean. In all cases, the control mean biomass was much smaller than the seeded or reference biomass, usually on the order of 20-25% of the seeded and reference plots. Biomass also varied by site, with Port Fourchon showing significantly larger biomass than Bayou Lafourche and Lake Felicity (p<0.0001) (Figure 25); however, there were no significant differences between Bayou Lafourche and Lake Felicity (p=0.63).

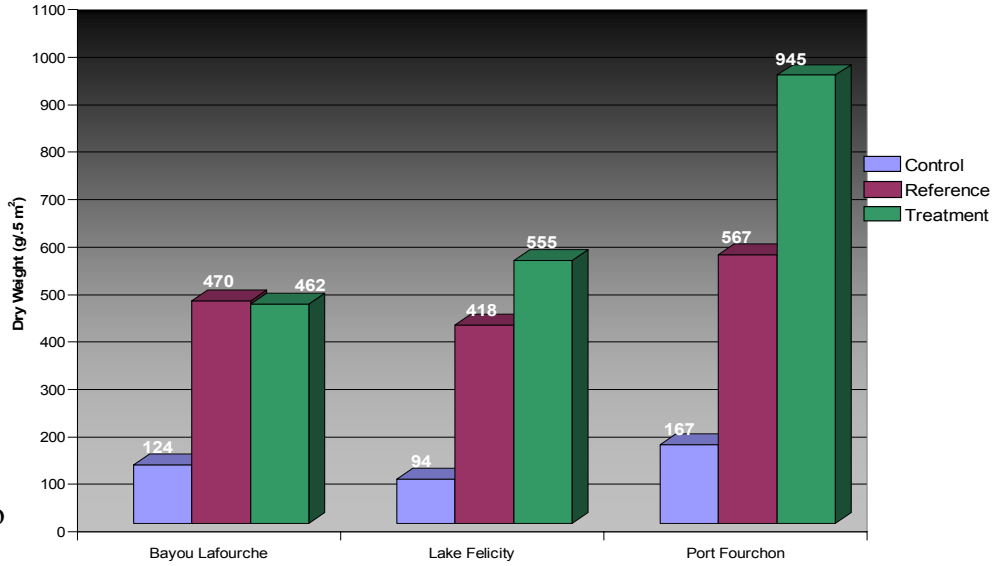


FIGURE 25
Biomass as Weight – Average dry weight production of aboveground biomass within treatment and across site.

Figure 26 is a summary chart showing the five productivity measurements grouped as control and seeded treatments across the three treatment sites. The barchart values represent the difference between the sample means subtracted from its respective reference mean and converted to percent difference. Positive

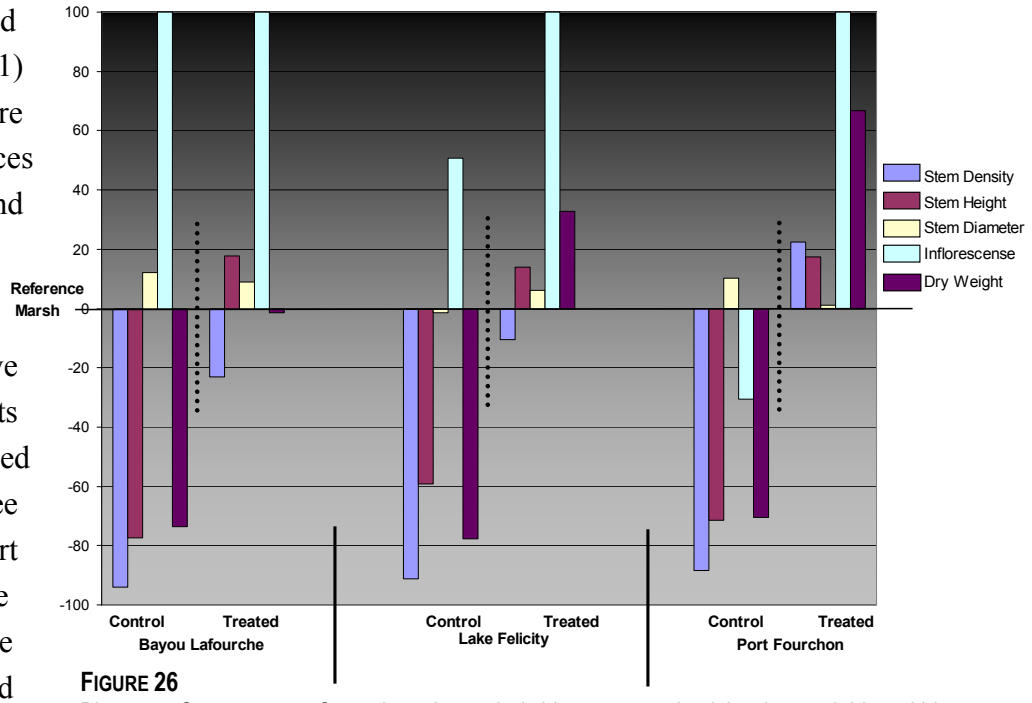


FIGURE 26
Biomass Summary – Control and seeded biomass productivity by variable within treatment and across site. Measurements are expressed as increase or decrease percentages relative to reference which is expressed as zero.

differences indicates greater productivity and negative differences indicate less productivity then their respective reference marshes. Sample differences are expressed as percent and plotted against the reference which is expressed as zero. One anomaly to the chart is that inflorescence values at the three seeded treatments and at one control treatment disproportionally exceeded that of the reference sites and had a range of 853 to 1,869%. Consequently, we choose to limit their value to 100% on the barchart.

Possible hypotheses that may explain the significantly larger robust growth at the seeded sites not seen at the control and reference marshes involve nutrient accumulation, competitive release and improved soil aeration. As both the seeded and control sites were completely void of vegetation for at least a year prior to the study, it is reasonable to assume that nutrient availability would vary significantly between treatments. That is, it is logical to assume there would be an enhanced nutrient-bank available to newly developing plants at both the seeded and control sites, not present at reference marshes. Coupled with increased nutrient availability, there was an obvious lack of vegetative competition within the seeded and control sites, thus allowing unrestricted and non-competitive growth. Both effects (nutrient enhancement and competitive release), either individually or as interactions, provided a compensatory growth not observed or measured in the reference marshes. Control plots, of course, lacked seeds to take advantage of the condition.

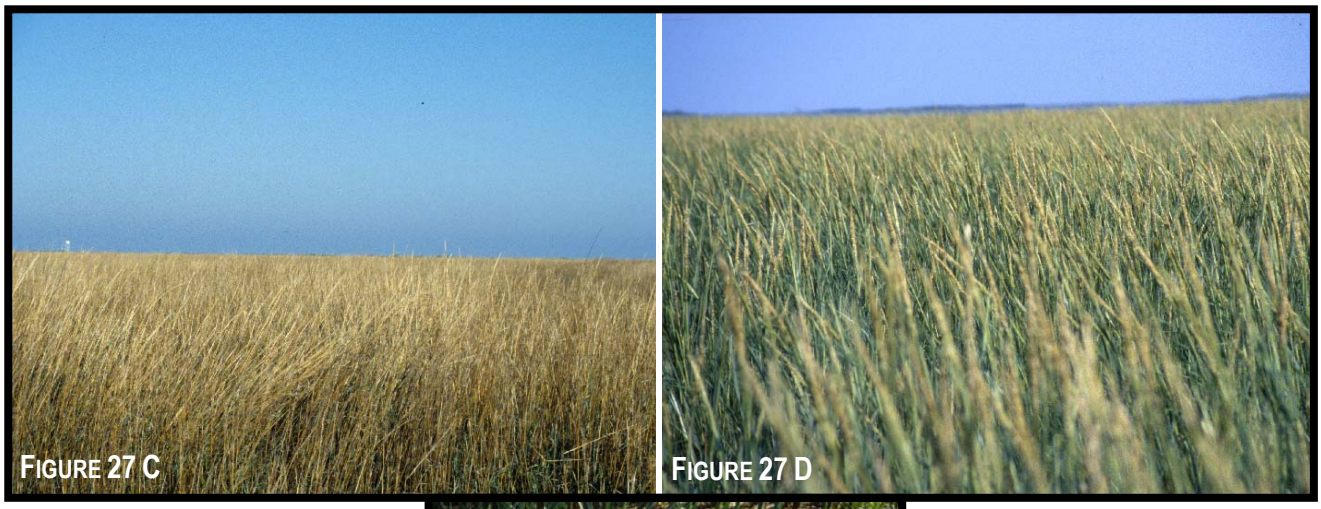
In addition, there is evidence that supports a positive relationship between robust *S. alterniflora* communities and increased soil oxidation and may further explain the biomass differential between the seeded, control, and reference sites. It is well documented that *S. alterniflora* typically exhibits variation in productivity and morphology within marshes. A reduction in sediment oxidation, nitrogen availability and uptake, interstitial NH_4, salinity, and hydrogen sulphide concentrations is common in more reduced marsh environments (Mendelssohn, McKee & Patrick, 1981) and is often associated with short *S. alterniflora* communities with lower standing biomass crop. Biomass production within the control sites was sparse and poorly developed, generating from random seed/rhizome fragments; whereas both the reference and seeded sites were moderate to robust plant communities. Studies have shown (Howes, Dacey, and Goehringer, 1986) that increased sediment oxidation removes reductants (primarily reduced sulphur compounds) and that robust growth of *S. alterniflora* is linked to plant-soil-hydrology interactions by increasing surface porosity. Consequently, it has been proposed that as plant density increases so does soil oxidation, setting up a positive feedback loop that affects plant nitrogen uptake, soil oxidation, plant water uptake and pore-water discharge and resulting in greater productivity.

Figure 27 is a series of photographs taken at the Port Fourchon seeded site that demonstrates the exceptional vegetative and seed production return following seeding. The top two photographs

(27A and 27B) are comparisons of the same area taken at the time of seeding (March 2002) and nine-months later at the end of the first growing season (December 2002). Figures 27C and 27D illustrates the abundance of inflorescences production in November 2002 (853% greater than the adjacent reference marsh) and stem density per unit area (December 2002). The last photograph shows new tiller production and seed concentrations in early spring the following year (March 2003).

FIGURE 27

Figures 27A (March 2002) and 27B (January 2003) were taken along the northern retention levee facing east southeast; note the community of Fourchon in the background. By the end of the first growing season, the Port Fourchon site had exceeded vegetative equivalency compared to its reference marsh. Figure 27C (December 2002) shows the density of the newly established *Spartina alterniflora*; note the Leeville tower in the background, and 27D (December 2002) shows exceptional inflorescence production. Figure 27E was taken in March 2003 (12 months post seeding), note the leading edge of tillers into the un-vegetated area and the dense seed mass within the developing tillers.



SUMMARY

Wetland deterioration is a serious environmental problem in coastal Louisiana with the average rate of wetland loss at $65km^2y^1$. The cumulative effect of decades of coastal wetland loss, exacerbated by tropical depressions, hurricanes, and anomalies such as the brown marsh die-back, has pushed Louisiana beyond a crisis edge, and is directly affecting its culture, infrastructure, industry, and food supply. Consequently, there is a need for a more comprehensive, widespread and accelerated program of coastal wetlands restoration and creation.

Vegetative wetland restoration on a landscape-scale is a relatively new restoration concept. Historically, plants produced by rhizomes, tubers, or rootstock and used in conjunction with sediment enhancement, marsh creation, bay terracing, or other sediment related engineering practices have been successful but cost-inefficient. Although whole-plant restoration has proven to be more reliable and requires less establishment time than seed, it is also exponentially more expensive than seed and has significant limitation in its area of application. The lack of information related to seed viability, germination, and seedling growth requirements has prevented an accurate determination of seeding rates for most wetland species. In addition, except for a few common species, the biology of wetland plants is poorly understood or not well documented. Consequently, the goal of this study was to assess the potential for *Spartina alterniflora* seed as a viable means to effectively vegetate large-scale marsh soils and to accelerate the functional development of saline marshes, i.e,. to develop planting methods that will lead to or reduce the time required for establishment of a vegetative productive wetland .

PERFORMANCE BENCHMARKS

This study was initiated in the fall of 2000 and completed in the fall of 2002. Significant benchmarks of the study were the successful completion of a number of subtasks grouped into three objectives.

OBJECTIVE ONE –

Objective one involved obtaining and managing bulk quantities of *S. alterniflora* seed to adequately implement an open marsh multi-site aerial seeding study.

SUBTASKS

Subtasks included: (1) organizing and implementing two large-scale bulk *S. alterniflora* seed harvest from both open marsh and cultivated ponds; (2) establishing procedures and methodologies to affect after-ripening, cleaning, and storing large quantities of seed; and (3) developing methods for monitoring germination and seed testing through a four-month holding and stratification period.

ACCOMPLISHMENTS

Accomplishment included: (1) bulk collection of *S. alterniflora* seeds in two consecutive years (2000 and 2001) from seven sites. Sites were further divided by wild and cultivated seed with

wild seed collections made at Black Lake in Cameron Parish (2000 and 2001),West Belle Pass in Terrebonne (2000), West Grand Terre in Jefferson Parish (2001). Cultivated seeds were obtained from the Golden Meadow Plant Materials Center in Lafourche Parish (2000 and 2001) and the Rice Research Station in Acadia Parish (2000); (2) successful completion of after-ripening, cleaning, and cold-wet stratification of approximately 1,400 pounds of clean *S. alterniflora* seed; (3) successful implementation of a germination and viability testing procedure to monitor seed viability through the storage and stratification period. Germination and viability samples were taken at 0, 30, 60, 90 and 120 days, assessed at 7 day intervals, and maintained for 28 days; and (4) determination of a *S. alterniflora* bulk germination rate (56%) that would be needed as part of the seeding-rate calculations.

Objective Two –

Objective two involved developing seeding-rate calculations and aerially applying *S. alterniflora* seed on 34 acres of treatment sites.

Subtasks

Subtasks included: (1) creating a seeding-rate model based on Louisiana rice culture; (2) developing methods and materials to facilitate aerial dispersal; (3) coordinating an aerial applicator to calibrate and modify aerial equipment to accommodate moist seed application; and (4) aerially applying seed/mix onto four treatment sites.

Accomplishments

Accomplishment include: (1) developing a *Spartina alterniflora* aerial seeding-rate model based on Louisiana rice culture. Seeding model components include germination (56%), seeds per pound (90,000), and a target number of seeds/ft^2 (89). Using these variables, we established a per acre marsh seeding rate of 43 lb/acre; (2) testing for an effective seed carrier (absorbent clay), an effective bending ratio (1:2 by weight), and an efficient mixing method (cement mixer); (3) successfully working with an aerial contractor to calibrate the plane and to determine modifications (fin removal and the addition of a hopper agitator); and (4) successfully aerially seeding four treatment sites, Bayou Lafourche (3 acres), Lake Felicity (3 acres), Plant Materials Center (3 acres), and Port Fourchon (25).

Objective Three –

Objective three involves two primary field data assessments. They were: (1) to quantitatively measure the accuracy of the seeding model to distribute a predetermined quantity of seed over a given unit area; and (2) to establish several baseline seed and biomass measurements needed to determine *S. alterniflora* productivity and how well it approached normal marsh equivalency within one growing season.

SUBTASKS

Subtasks included: (1) assessment of on-site seed distribution; (2) assessment of on-site seed germination; (3) assessment of on-site seedling survival; and (4) completion of six different measurements of plant productivity

ACCOMPLISHMENTS

Accomplishments included: (1) aerially seeding four sites, three in 2000 and one in 2001. We achieved a ±2% (~ 2 seeds) seeding accuracy at three of the four sites and a ±17% (~13 seeds) seeding accuracy at one site; (2) determining on-site germination rates at 30 and 60 days post-seeding at four treatment and four control sites and determined it to be low (~25 seeds or 29%) within the seeded treatments and ~0.7 seeds/m^2 within the control sites; (3) determining seedling survival at 90 days post-seeding, and again determining it to be low (~4 seedlings/m^2); (4) completing several aboveground biomass measurements (stem density, height, diameter, inflorescence, and dry weight biomass) of the seeded treatments and the control treatments to determine the relative productivity to that of reference marshes. We found that two of the seeded sites (Bayou Lafourche and Lake Felicity) equaled or slightly exceeded productivity of their respective reference marshes. However, the fourth site, Port Fourchon greatly exceeded its reference marsh in five out of six productivity measurements. The control treatments were under productive relative to their respective references at all sites.

CONCLUSIONS AND RECOMMENDATIONS

Using *Spartina alterniflora* seed as a method for reestablishing vegetative cover on highly disturbed soils in saline marshes is both efficient and effective. Even with the relatively low return of mature plants to seeds sown, seeding still provides a greater plant density per measure unit area than the traditional means of establishing whole-plants after one growing season. However, seed harvesting in bulk is still limited to hand collections and will continue to severely limit the number of acres that can be seeded annually. Currently, the only source of *S. alterniflora* seed is from native saline marshes. With a renewed interest in more rapid and less costly plant establishment techniques, cultivated ponds of *S. alterniflora* plants may provide a more efficient alternative source and advance the use of mechanized harvesting.

Procedures for cleaning and storing *S. alterniflora* seeds are adequate but need to be mechanized if significantly large amounts of seed are to become available. Additional studies should be undertaken to determine if the stratification (storage) period can be reduced without negatively affecting seed germination or if mechanically scarifying seed would increase functional germination. Existing aerial equipment is more than adequate to meet future demands of aerial application as the number of potential acres of saline marshes that could be aerial sown is a fraction of the agricultural acres flown annually.

The study of wetlands seeding as a restoration tool should continue to be a high priority because of its importance in developing more efficient technologies for reducing wetland loss in coastal Louisiana. Developing successful seeding systems that can be implemented in Louisiana's deteriorating marshes can play a major role in this process, yet information that will maximize the likelihood for success is still lacking. Continued seeding experiments will add to the knowledge base and will provide coastal wetland project planners, designers, and builders with additional management strategies that will better incorporate vegetative diversity and productivity into wetland restoration engineering.

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