Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana
August 10, 2010

A Report of the:

Barataria-Terrebonne National Estuary Program

By:

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Prepared for the:

Environmental Protection Agency's National Estuary Program

EPA Tracking Numbers: 2001-22, 2004-10, 2006-07, 2007-15

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United States Department of Agriculture
NRCS Natural Resources Conservation Service

"Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana

Status Report

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Phase I – Part 1

Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana

Introduction

The reasons submerged aquatic vegetation (SAV) is important to Louisiana coastal ecosystems include the minimization of storm damage by reducing wave action, stabilization of sediments, improvement of water quality by absorption of nutrients and contaminants, and it provides critical habitat for wintering waterfowl and many commercially important fish species, (Zieman and Zieman 1989, Boustany 2003). Very little is known on the status of SAV throughout the coastal region of Louisiana. Unlike seagrasses, which include few species and inhabit very limited areas of the gulf coast in typically clear marine waters, estuarine SAV species are spread throughout the multitude of ponds and bayous of coastal Louisiana and include many species. These areas are often in locations difficult to access, difficult to view in the murky waters, and occur in the entire range of different habitat types. Because we do not have a broad understanding of the biology and ecology of the SAV communities, their importance has, until recently been overlooked. It is now recognized that SAV are important to the estuarine ecosystem and that techniques to propagate and plant need to be developed. Phase I objectives include the development of nursery propagation techniques for *Vallisneria americana* and *Ruppia maritima*, to determine the optimal growth conditions, type of container system to grow the plants in, growth media, proper handling and care of the plants, and development of standard operating procedures of the two species for transfer to the commercial growing trade.

V. americana **is important SAV in the Louisiana coastal ecosystem**

Procedure

The study was conducted at the USDA-Natural Resources Conservation Service (NRCS) Golden Meadow Plant Materials Center, Golden Meadow, LA in 2008-2009. Propagation tanks (4 ft x 8 ft) were fabricated from wood, and lined with a double layer of 10 mil poly sheeting, to produce a 270 gallon water holding capacity. *V. americana* and *R. maritima* plants were collected from native stands growing in fresh to brackish marsh (.3 ppt) in Lafourche Parish, LA near Clovelly Farms on 13 May 2008. Plants were divided into 360 individual propagules of each species and planted in a 4 inch pot. A mixture of peat moss and pine bark was used to fill 25% of the pot (bottom) with the remaining 75% filled with commercial grade fine sand. Plants were grown under greenhouse conditions from 14 May 2008 until 5 November 2008. *V.*

V. americana **was collected from Clovelly Farms, LA in May 2008.**

Planting *V. americana* at the USDA-NRCS Golden Meadow Plant Materials Center

americana and *R. maritima* were cut 2.5 cm on 5 November 2008 and uniform plants of each species were submerged into each water-filled propagation tank to a depth of 14 inches. Propagation tanks were maintained in the greenhouse with temperature ranges from 90 to 100°F during the day (+/- 10) to 75 to 85°F at night (+/- 10).

After submerging the plants into assigned propagation tanks, light was reduced by covering each tank with an artificial shade of 0 (control), 63 and 90% using a commercial shade cloth material. A single layer of 63% shade was doubled to achieve a 90% shade. A commercial dye (Aqua Shade®, applied biochemist) was also included as a shade treatment. Dye was added to assigned propagation tanks according to manufactures label for achieving a coloration of 25 ppm. Performance data included vigor (health of the plant), spread, leaves and plant height using a rating scale of 1 to 9 with 1 = best and 9 worst. Six plants were randomly selected for measurement. Plant height was determined by measuring from the media surface to average height of the plant. Average number of leaves per plant was determined by counting the number of visible leaves and dividing the sum by 6. Average spread, which is defined as the number of actively growing, vegetative buds on each plant, was determined by counting the number of healthy sprouts per plant and dividing the sum by 6. Plant performance measurements were made approximately every 2 weeks from December to May 2008-2009. Algal growth was not controlled in any of the propagation tanks to replicate a typical commercial propagation operation. Experimental design included 4 shade levels with 3 replications arranged in a completely randomized design.

Plants of *V. americana* submersed into propagation tank at the USDA-NRCS Golden Meadow Plant Materials Center.

Results and Discussion

No data was collected for the *R. maritima* due to poor survival at all shade treatment levels including the control after the plants were submerged in the propagation tanks. A preliminary test was conducted to determine if survival could increase using other planting media such as coconut fiber mat. However, this test produced poor results. *R. maritima* survival and growth is sensitive to low water quality and lighting (USGS, 2006; Verhoevin's, 1979). Algae were not controlled in the tanks and may have contributed to poor water quality and subdued lighting in the tank, leading to poor plant survival. Additional propagation techniques will be explored in the future to increase survivability by modifying harvest methods, light intensity, water temperature, and salinity.

V. americana response to shading is presented in figures 1-4. Plant height increased as light intensity decreased with shading and dye treatment (fig 1). Plant height was greatest for the 90% shade which ranged from 18-33 cm over the 10 week period.

However, the condition of the plants after week 3 is described as long, spindly, and exhibiting low vigor (fig 2). Furthermore, it is anticipated low light produced by 90% shade and dye treatment substantially reduced spread potential by limiting the production of spring buds and actively growing leaves at various evaluation dates (figs 3-4). Titus and Adams (1979) reported *V. americana* was tolerant of low light but responded to increasing light availability. Although we found *V. americana* to perform satisfactory where light was not limited, we also found it to perform poorly where light availability had significantly been reduced (90% shade and dye treatment).

Plant height measurements for 63% shade and dye treatment were similar but plant vigor for 63% shade was much greater than all treatments (fig 2). The 63% shade did not restrict aerial productivity, reduce plant vigor, or decrease active bud and leaf growth as did the 90% shade and dye treatment (fig 1-4). Consequently, the 63% shades provided the best plant performance when compared to the other actual shade treatments; however, results were similar to the control. These preliminary results suggest plant grown no shade up to 63% shade may allow for sufficient plant growth and development that will warrant additional studies in Phase II.

Conclusion and Summary

No data was collected for *R. maritima* due to poor survival soon after the study began. Additional propagation techniques for plant survival will be explored in the future. *V. americana* plant performance was severely decreased with 90% shade and dye treatment due to significant light reduction. The no shade treatment and 63% shade provided the best plant performance for *V. americana*. These treatments will be further evaluated in Phase II to document and refine propagation techniques for production of *V. americana* for commercial nursery trade. Ultimately, we intend to produce a product for making field deployment and plant establishment of *V. americana* possible in Louisiana coastal restoration projects.

Reference

Boustany. R.G. 2003. A prevegetated mat technique for the restoration of submersed aquatic vegetation. Ecological Restoration 21:2

Titus, J.E., Adams M.S. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria amerciana* Michx. Am Midl Nat 102:263-272

Zieman, J.C. And R.T. Zieman. 1989. The ecology of the seagrass meadows of the west coast of Florida: a community profile. U.S. Fish and Wildl. Serv. Biol. Rep. 85(7.25). 155 pp.

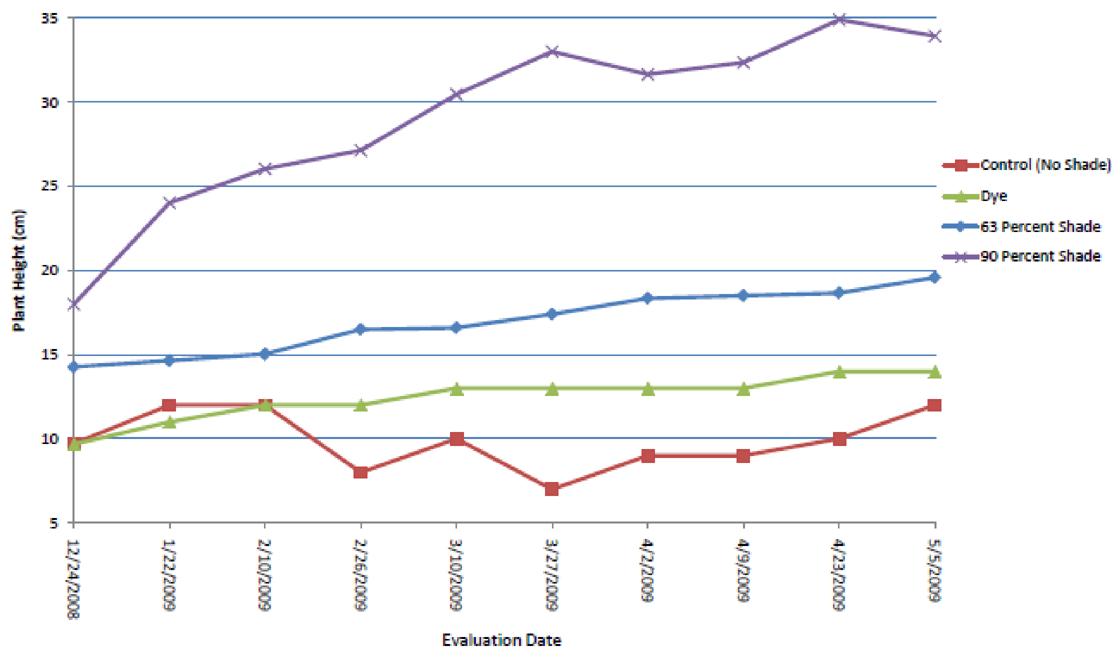

Figure 1. Plant height of *Vallisnaeria americana* as effected by various shade treatments. USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

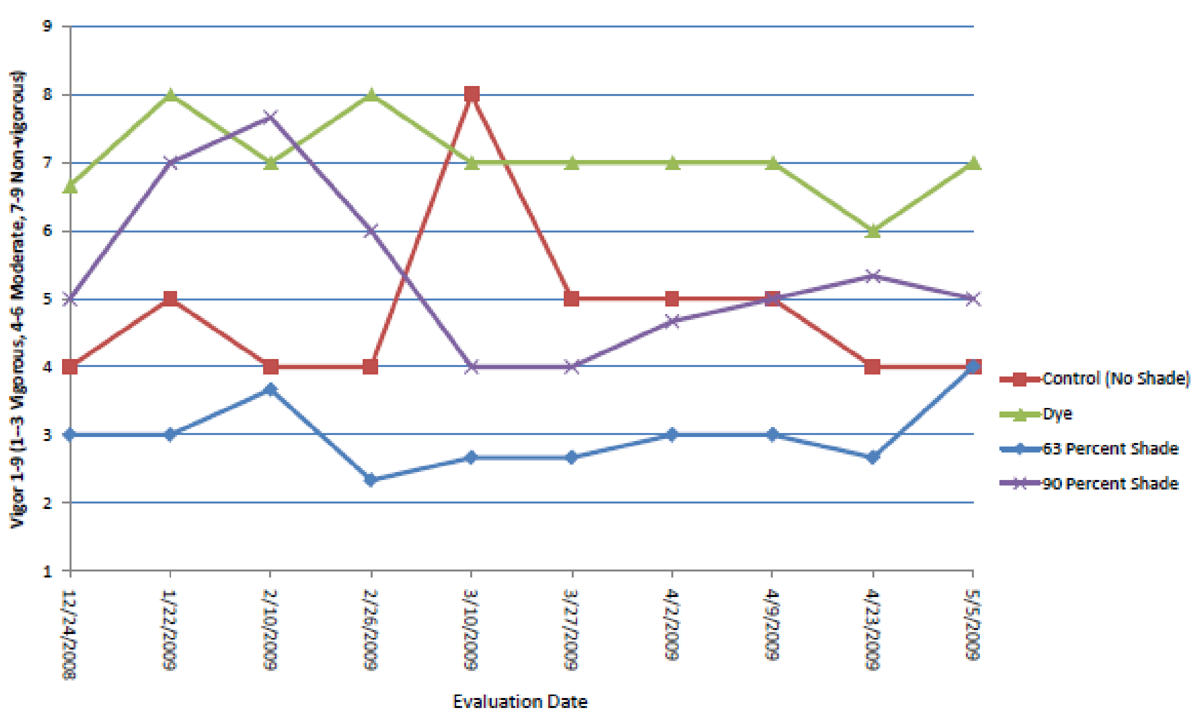

Figure 2. Plant vigor of *Vallisneria americana* as effected by various shade treatments. USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

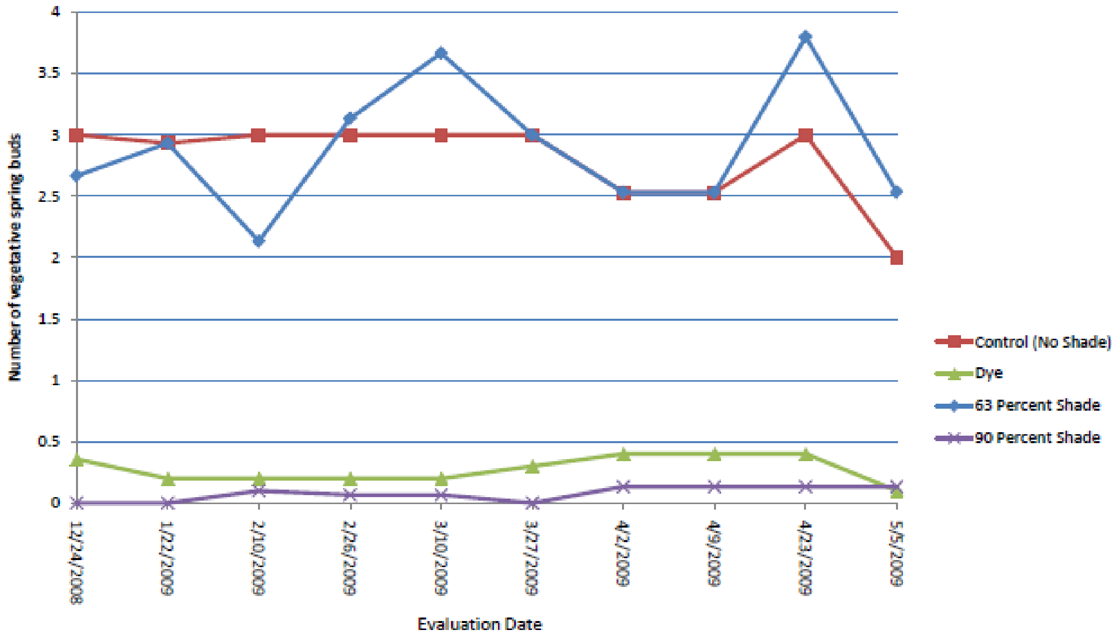

Figure 3. Number of spring buds produced by *Vallisneria americana* s effected by various shade treatments, USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

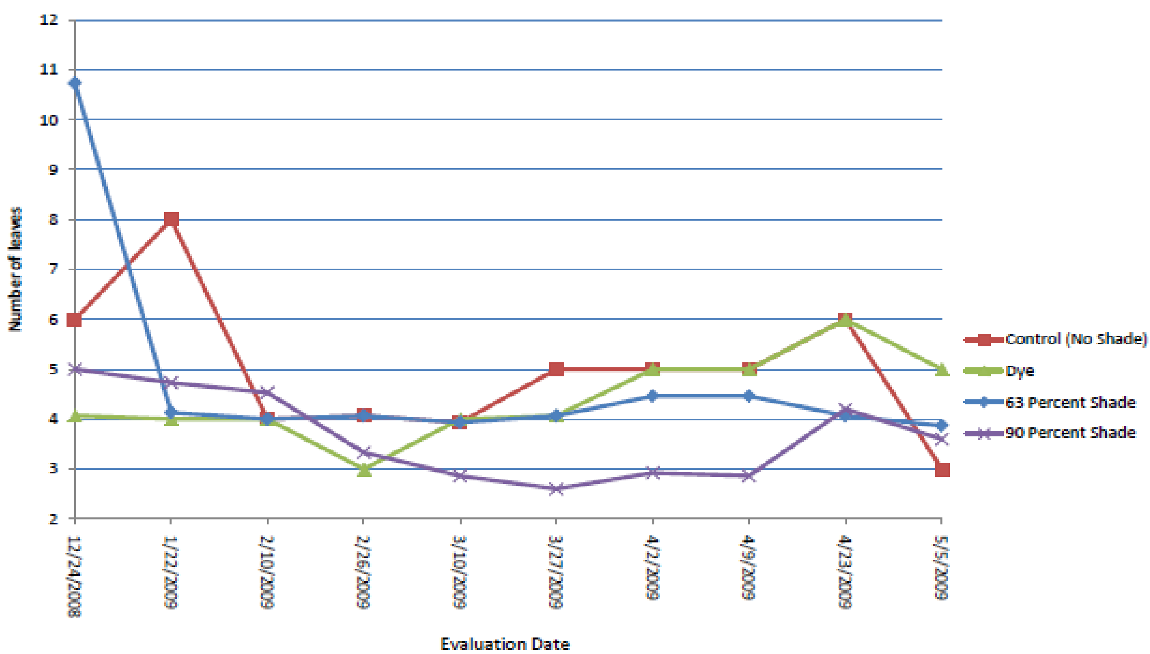

Figure 4. Number of actively growing leaves of *Vallisneria americana* as effected by various shade levels, USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

Phase I - Part 2

Comparison of the growth potential of *Vallisneria americana* grown under a 63% shade and without shade.

Introduction

Preliminary results from Part 1 suggest that the 63% shades provided the best plant performance when compared to the other actual shade treatments; however, results were similar to plants grown under no shade. To verify observations from Part 1, additional experiments were conducted to quantify initial results. Part 2 of the study will evaluate plants of *V. americana* grown in 4 inch pot under the 63% shade treatment and compare those to plants grown under no shade. Measurements used to compare the two treatments included plant height, number of actively growing leaves, and biomass measurements (total, leaf, and root).

Procedure

The study was conducted at the USDA-Natural Resources Conservation Service (NRCS) Golden Meadow Plant Materials Center, Golden Meadow, LA in 2009-2010. *V. americana* plants were collected from native stands growing in fresh to brackish marsh in Lafourche Parish, LA near Clovelly Farms on 8 February 2010. Local conditions at harvest site were; air temperature 16.2°C; water temperature 10.3°C; water ph 4.89; water salinity .3ppt; and dissolved oxygen 10.3 ppm and 91%L. Plants were divided into 90 individual propagules and planted in a 4 inch square plastic nursery container. A 50/50 mixture of peat moss and pine bark was used to fill each container 25% full with the remaining 75% filled with commercial grade fine sand. To alleviate problems associated with the wooden frame, sheet plastic lined tanks used previously. Tanks made of UV protected low density polyethylene (Rubbermaid ® brand) with a capacity of 300 gallon each were purchased for use. Plants were grown under greenhouse conditions from 12 February 2010 until 26 July 2010. *V. americana* were cut to 25 cm on 16 April 2010 and uniform plants of each species were submerged into each water-filled propagation tank to a depth of 55 cm. Propagation tanks were maintained in the greenhouse with temperature ranges from 90 to 100°F during the day (+/- 10) to 75 to 85°F at night (+/- 10). Algal growth was not controlled in any of the propagation tanks to replicate a typical commercial propagation operation. Experimental design included 2 treatments (63% shade and no shade) with 3 replications arranged in a completely randomized design.

Photo showing UV protected low density polyethylene (Rubbermaid ® brand) 300 gallon propagation tank

After submerging the plants into assigned propagation tanks, light was reduced by covering 3 tanks with an artificial shade of 63% using a commercial shade cloth material and 3 tanks uncovered 0% (control). Air temperature, water temperature, water ph, water salinity (ppt) and dissolved oxygen (ppm and %L) were recorded on a weekly basis, between the hours of 8:00am and 10:00am, to monitor environmental conditions under greenhouse production (Table1.)

Table 1. Average environmental conditions.

	Low	High	Average
Water PH	5.88	9.64	8.21
Water Salinity ppm	0.2	0.3	0.22
Dissolved oxygen ppm	1.467	10.205	5.195
Dissolved oxygen %L	16.67	97.35	57.66
Water Temperature	15.33	32.33	23.71

Performance data included plant height (cm), number of actively growing leaves, and biomass measurements (total plant, root weight, top growth weight) was taken to measure overall plant performance. Five plants were randomly selected from each tank for measurements. Plant height was determined by measuring from the media surface to average height of the plant. Average number of leaves per plant was determined by counting the number of actively growing leaves and dividing the sum by 5. Plant biomass measurements were made on 26 July 2010. Individual plants were removed from containers and were washed of any potting media. Wet weights of each whole plant were recorded. Individual plants were placed in paper bags and dried for 24 hr at 60°C. Total dry weights were recorded for each plant. Plants were than separated at the plant/root interface with root and top growth being weighted separately. All plant weights were recorded as average weight/plant in grams.

Dry biomass plant sample of *V. americana*

Results and Discussion

V. americana response to shading as compared to growth in no shade is presented in figures 1 and 2. Plant height increased as light intensity decreased with 63% shading (fig 1). Cutler (1980) reported that leaf elongation in rice was directly related to light and dark conditions. Plant height was greatest for the 63% shade which ranged from 77 to 87 cm when compared to plants grown in no shade which had an average height of 47.6 to 56.2 cm (fig 1). Plants grown under no shade produced considerable differences in the number of actively growing leaves.

V. americana grown under shade produced an average of 39.5 leaves per pot as compared to plants grown under 63% shade only produced an average of 14.4 leaves per pot (fig 1). Titus and Adams (1979) reported *V. americana* was tolerant of low light but responded to increasing light availability. Although we found *V. americana* to perform satisfactory where light was limited (63% shade), we also found it to produce a significantly greater number of healthy actively growing leaves when it was grown under no shade (fig 1).

Total biomass for plants of *V. americana* grown under 63% shade was significantly less at 2.76 g/plant when compared to plant grown under no shade which had an average weight of 9.55 g/plant (fig 2). Total root biomass comparing 63% shade to no shade was 0.97 g as compared to 3.91 g. Total top growth biomass comparing 63% shade to no shade was 1.78 g as compared to 5.66 g. These results suggest plant grown under no shade allow for sufficient plant growth and development for commercial production.

Conclusion and Summary

Overall performance for plants grown under 63% shade shown to have greater leaf height, but produced fewer leaves and significantly less plant biomass (only 28.9% of the total no shade biomass). Overall performance for plants grown without shade produced shorter leaf height, but overall biomass weights and number of actively growing leaves were significantly greater.

In conclusion, plants grown under 63% shade produced viable transplants, however plants grown under no shade demonstrated to produce a healthier and more vigorous transplant that would allow for production of *V. americana* for commercial nursery trade. Additional propagation techniques will be explored using biodegradable bags compared to plants grown in plastic pots.

Reference

Cutler, J.M., 1980. Dymanic Aspects and Enhancements of Leaf Elongation in Rice. Plant Physiology 66:147-152

Titus, J.E., Adams M.S. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria amercian* Michx. Am Midl Nat 102:263-272

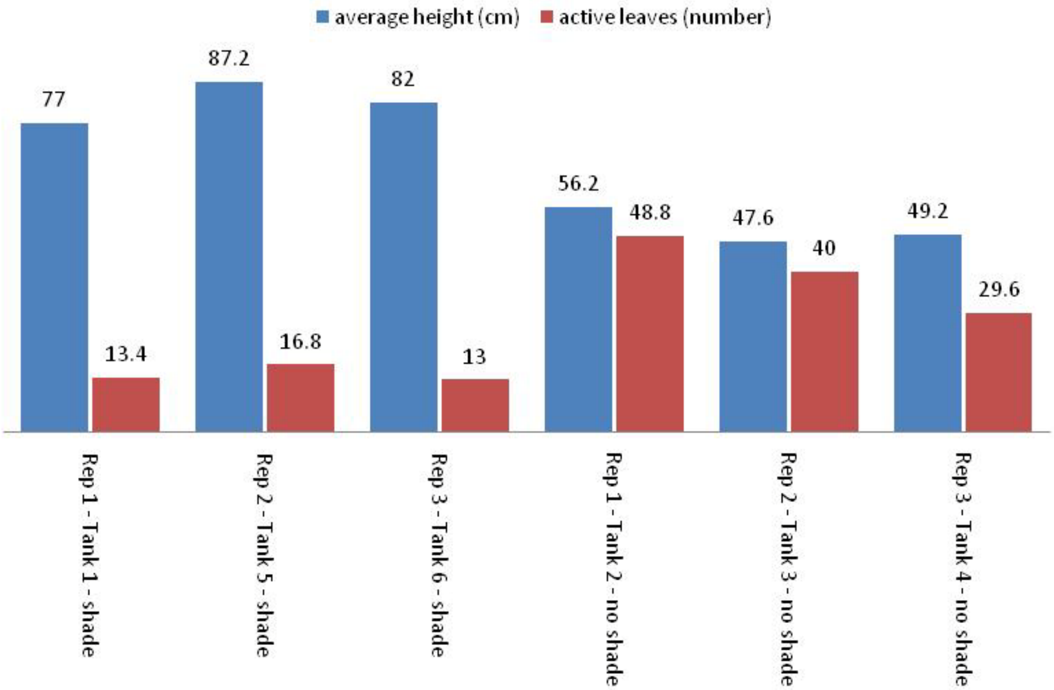

Figure 1. Number of actively growing leaves and average leaf height of *Vallisneria americana* grown in plastic pots as effected by 63% shade and no shade, USDA-NRCS Golden Meadow Plant Materials Center, February 2010 to July 2010

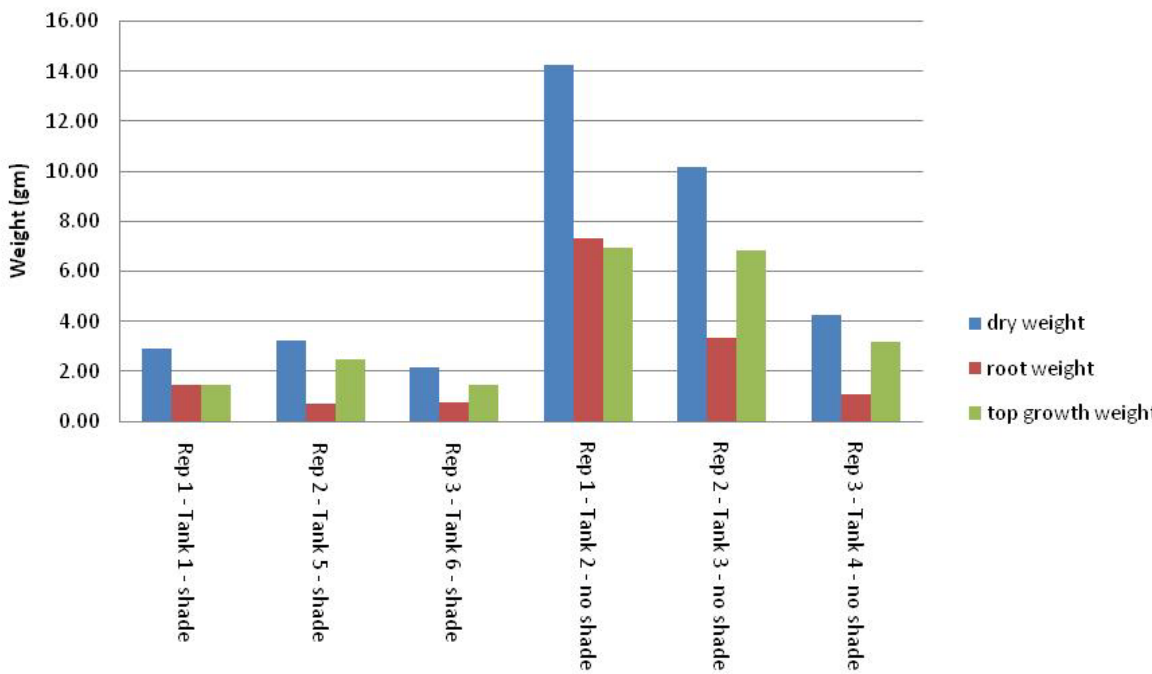

Figure 2. Average biomass weight of *Vallisneria americana* grown in plastic pots as effected by 63% shade and no shade, USDA-NRCS Golden Meadow Plant Materials Center, February 2010 to July 2010

Phase I - Part 3

Comparison of growth potential of *Vallisneria americana* grown in plastic pots and biodegradable bags.

Introduction

Traditional planting stock for *Vallisneria americana* have included the use of bare-root plants and small container grown plants. Both methods although widely accepted and successful have limitations associated with cost of production, transportation, and labor associated with field deployment. Part 3 of the study will evaluate additional container or planting systems that may be useful for the propagation, nursery production, and field deployment of *V. americana*. Alternative methods considered for this study included; fibrous matting materials and biodegradable burlap bags.

Results from Part 2 suggests that *V. americana* planted in 4 inch plastic pots produced under a no shade environment provided the best plant performance when compared to those grown under the 63% shade treatment. For this study selected container systems or propagations methods will be evaluated under no shade. Measurements used to compare treatments will include plant height (cm), number of actively growing leaves, and biomass measurements (total plant, leaf, and root weight). During initial screening investigations the fibrous matting materials proved temporarily unsuccessful results. Problems associated with the buoyancy of the material and the inability to successful attach *V. americana* plantlets into the material demonstrated the need for further long term investigations. Based on these initial finding the biodegradable burlap bag was selected as the preferred method to evaluate and compare with plants grown in 4 inch square nursery pots.

Procedure

The study was conducted at the USDA-Natural Resources Conservation Service (NRCS) Golden Meadow Plant Materials Center, Golden Meadow, LA in 2009-2010. *V. americana* plants used for the study were collected from native stands growing in fresh to brackish marsh in Lafourche Parish, LA near Clovelly Farms on 8 February 2010. Local conditions at harvest site were; air temperature 16.2°C; water temperature 10.3°C; water ph 4.89; water salinity .3ppt; and dissolved oxygen 10.3 ppm and 91%L. Plants were divided into 180 individual propagules and 90 planted in a 4 inch square plastic nursery container and 90 planted into a small 6 x 10 inch burlap bag. Plastic pots were filled 25% full with a 50/50 mixture of peat moss and pine bark with the remaining 75% filled with commercial grade fine sand. Each burlap bag was filled with approximately 2 cups of inert coarse sand. No fertilizers or soil amendments were given to either treatment. To alleviate problems associated with the wooden frame, sheet plastic lined tanks used previously. Tanks made of UV protected low density polyethylene (Rubbermaid ® brand) with a capacity of 300 gallon each were purchased for use.

Plants were grown under greenhouse conditions from 12 February 2010 until 26 July 2010. *V. americana* were cut to 25 cm on 16 April 2010 and uniform plants of each species were submerged into each water-filled propagation tank to a depth of 55 cm. Propagation tanks were maintained in the greenhouse with temperature ranges from 90 to 100°F during the day (+/- 10) to 75 to 85°F at night (+/- 10). Algal growth was not controlled in any of the propagation tanks to replicate a typical commercial propagation operation. Experimental design included 2 treatments (pot vs. biodegradable burlap bags) with 3 replications arranged in a completely randomized design.

Air temperature, water temperature, water ph, water salinity (ppt) and dissolved oxygen (ppm and %L) were recorded on a weekly basis, between the hours of 8:00am and 10:00am, to monitor environmental conditions under greenhouse production (Table 1).

Table 1. Average environmental conditions.

	Low	High	Average
Water PH	5.88	9.64	8.21
Water Salinity ppm	0.2	0.3	0.22
Dissolved oxygen ppm	1.467	10.205	5.195
Dissolved oxygen %L	16.67	97.35	57.66
Water Temperature	15.33	32.33	23.71

Performance data included plant height (cm), number of actively growing leaves, and biomass measurements (total plant, root weight, top growth weight) was taken to measure overall plant performance. Five plants were randomly selected from each tank for measurements. Plant height was determined by measuring from the media surface to average height of the plant. Average number of leaves per plant was determined by counting the number of actively growing leaves and dividing the sum by 5. Plant biomass measurements were made on 26 July 2010. Individual plants were removed from containers and were washed of any potting media. Wet weights of each whole plant were recorded. Individual plants were placed in paper bags and dried for 24 hr at 60°C. Total dry weights were recorded for each plant. Plants were than separated at the plant/root interface with root and top growth being weighted separately. All plant weights were recorded as average weight/plant in grams.

Results and Discussion

A comparison of growth characteristics of *V. americana* grown in 4 inch plastic pot and biodegradable burlap bags is presented in figures 1-5. Average leaf length measurements of plants grown in plastic pots were similar to those grown in burlap bags (fig 1). Average leaf length for the plants grown in plastic pots was 51 cm as compared with those grown in burlap bag reaching an average length of 38 cm (fig 1). Consequently, the plastic pots produced a greater number of actively growing leaves, 39.5 for pot as compared to 29.3 for the burlap bags (fig 2).

Total biomass yield for plants of *V. americana* grown in the burlap bags was significantly less at 4.09 g/plant when compared to plant grown in the plastic pot which had an average weight of 9.54 g/plant (fig 3). Total top growth biomass comparing the plastic pots to the burlap bag was 5.64 g as compared to 2.22 g (fig 4), and total root growth biomass comparing plastic pots to burlap bags was 3.9 g as compared to 1.87 g (fig 5).

Conclusion and Summary

Although results were somewhat similar the data suggest that plants of *V. americana* grown in 4 inch plastic pots produced a slightly more robust plant. Visual observations suggest that there may have been some residual nutrients in the peat moss and pine bark mixture use in the plastic pot. The inert commercial sand use in the bag was void of any beneficial plant nutrients. This would account for the slight increased in overall performance of plants grown in the plastic pots. To verify these similar results additional trials may need to be established using both methods with the incorporation of plant nutrients into the evaluation. However initial results suggest that both plant grown under in 4 inch plastic nursery pots and biodegradable burlap bag proved successful at establishing a marketable plant for commercial usage.

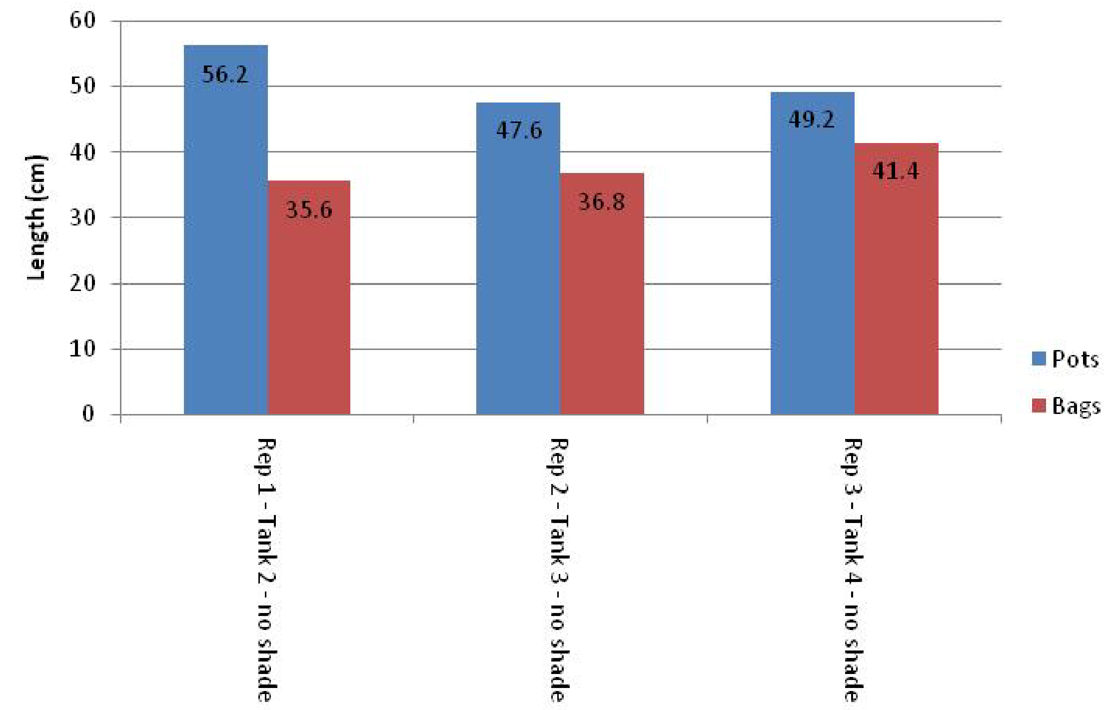

Figure 1. Comparison of average leaf length of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

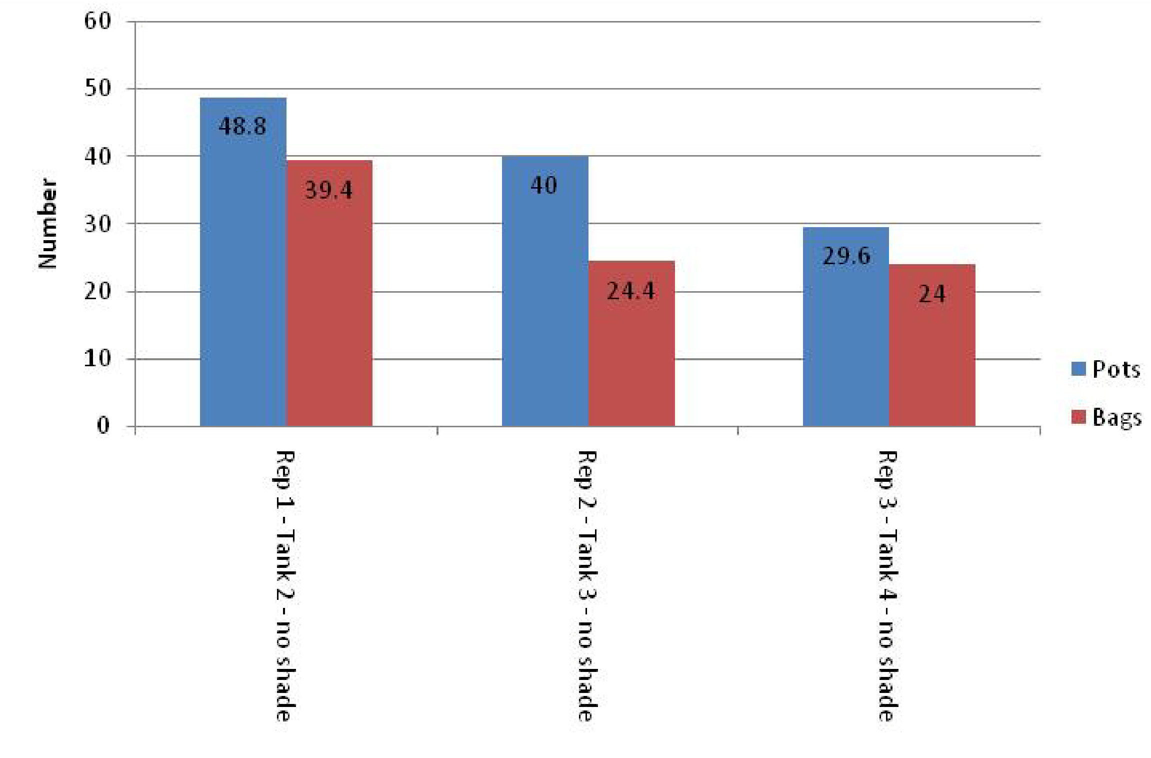

Figure 2. Comparison of the number of actively growing leaves of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

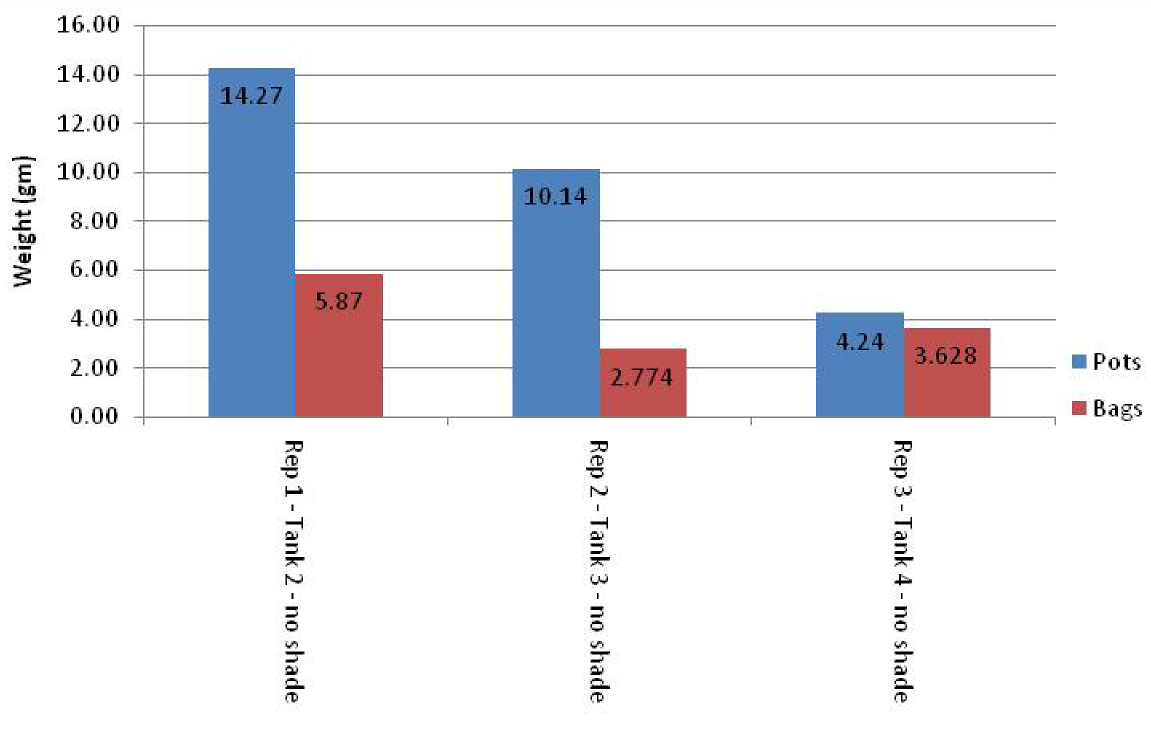

Figure 3. Cpmparison of total plant biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

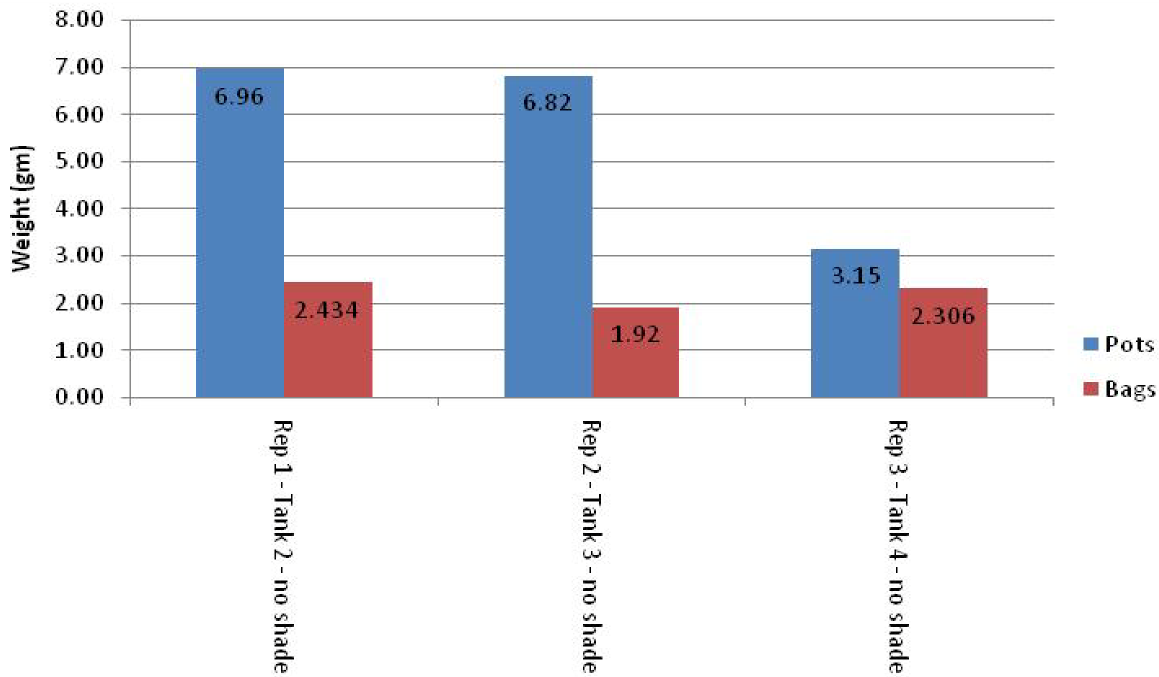

Figure 4. Comparison of top growth biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

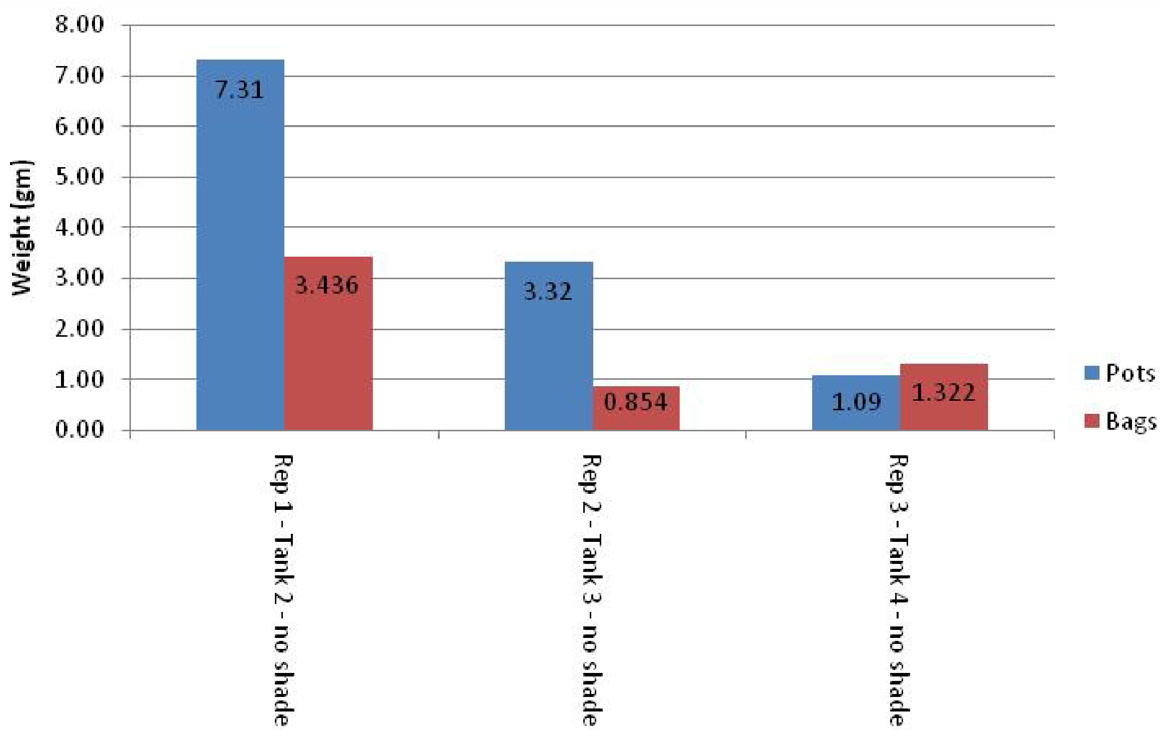

Figure 5. Comparison of root biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

Phase II

Submersed Aquatic Planting Techniques for Restoration in Coastal Louisiana

Introduction

Many attempts to reestablish Submersed Aquatic Vegetation (SAV) by methods of using plugs, peat pots, coconut erosion control mats, plastic pots, sods, wire mesh, seeds, and winter buds has had very little success. Many SAV restoration projects have failed as a result of poor selection of planting sites or plant material and incorrect use of planting methods. Results from Phase I determined that plants of *Vallisneria americana* grown under no shade had greater numbers of actively growing leaves and higher biomass measurements when compared to the shade treatments. Container systems evaluated under Phase 1 determined that plants of *V. americana* grown in 4 inch plastic pots and biodegradable burlap bags had similar biomass and growth measurements.

Phase II will evaluate and compare the field deployment of plants of V. *americana* grown in 4 inch pot and biodegradable bag under actual field conditions. Measurements used to compare the two container systems included; plant survival, % cover and average stem/leaf length.

Procedure

The study was conducted at the USDA-Natural Resources Conservation Service (NRCS) Golden Meadow Plant Materials Center, Golden Meadow, LA, and at two offsite locations in fresh/ brackish marsh in Lafourche Parish, LA near Clovelly Farms. Plants were grown under greenhouse conditions from 12 February 2010 until 26 July 2010.

Established plants grown under greenhouse conditions were taken to 2 selected field sites for evaluations. Fifteen plants from 4 inch pots and fifteen plants of biodegradable burlap bag were randomly removed from tanks and immediately placed in tubs filled with water for transport to the offsite evaluation area. Planting site was marked using PVC pipe to help identify the 6 ft x 6 ft plots for evaluation. Plants were carefully removed from the transport tubs and plastic containers were removed before plant was lowered into the water to be transplanted. Biodegradable bags were not removed during transplanting. Each plot contained 5 plants from either pots or biodegradable bags. Plants were space approximately 12 inches apart within row. Transplanting was completed on 2 July, 2010.

Field evaluations for the 2 sites were monitored on 5 August, 2010 approximately 30 days after planting. Each site was evaluated for plant survival, stem/leaf length (cm), and % plant cover. Due to depth of water and water clarity, plant survival was done by physically using hand to feel to verify plant was present and actively growing. Plant % cover was also determined using hands to feel plant spread within a 12 X 12 inch area around the plant.

Results and Discussion

Initial field evaluation of *V. americana* is presented in Tables 1-3. Plant survival was highest for plants grown in 4 inch pots for site 1 at 93.3 %. However plant survival was higher for burlap bags at site 2 at 80 % (fig 1). Percent cover for biodegradable burlap bags was slightly higher compared to 4 inch pot (fig 2).

Initial stem/leaf length measurements for 4 inch pots compared to biodegradable bags for site 1 and 2 were not significantly different during the first evaluation. Plants in biodegradable burlap bags in site 1 averaged a greater stem/leaf length compared to 4 inch pots in site 2, and pot in site 1 averaged a greater stem/leaf length compared to burlap bags in site 2 (fig 3)

Conclusion and Summary

These preliminary results are based on only one evaluation. Results show some minor differences, but at this stage insufficient data has not been recorded to present ending results.

Additional monitoring will be conducted every month to record additional plant data. Additional field deployment is also scheduled at 2 evaluation sites for September 2010. Ultimately, we intend to produce a product for making field deployment and plant establishment of *V. americana* possible in Louisiana coastal restoration projects.

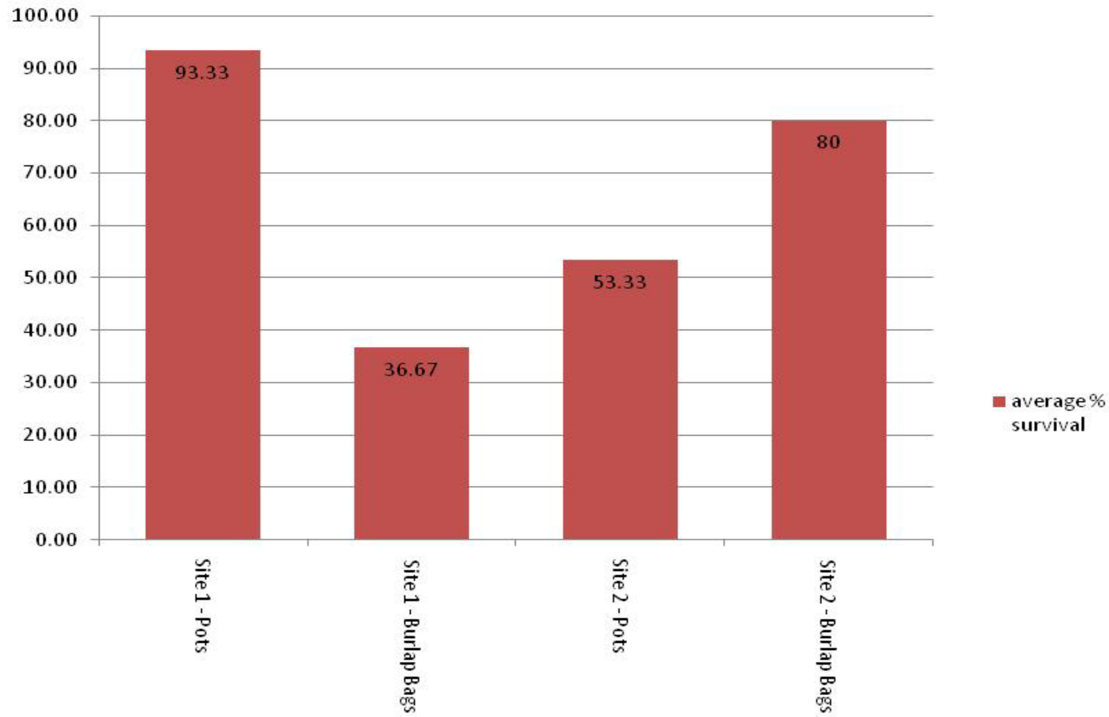

Figure 1. Average field survival of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

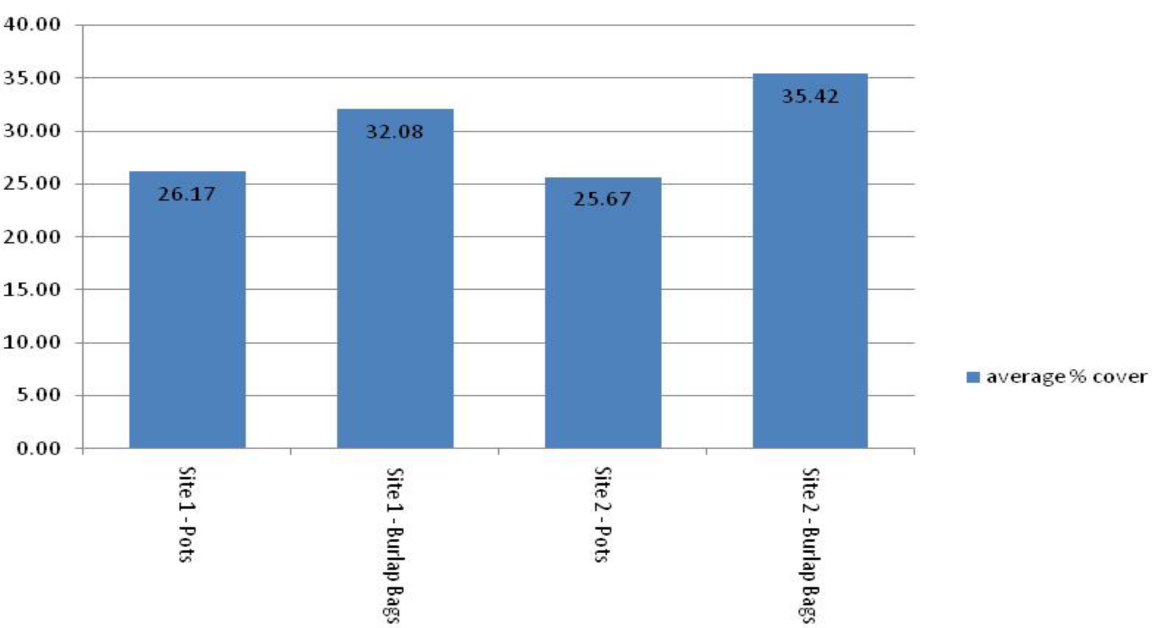

Figure 2. Average % cover of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

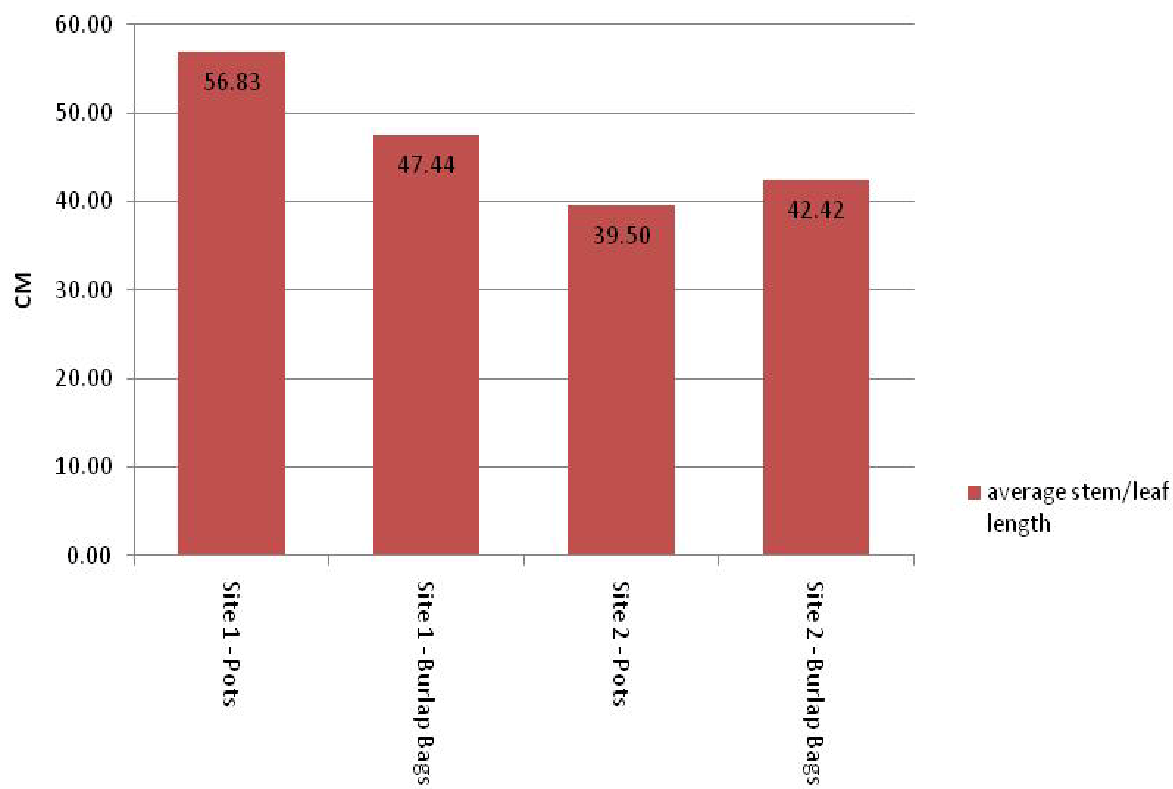

Figure 3. Average stem length of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

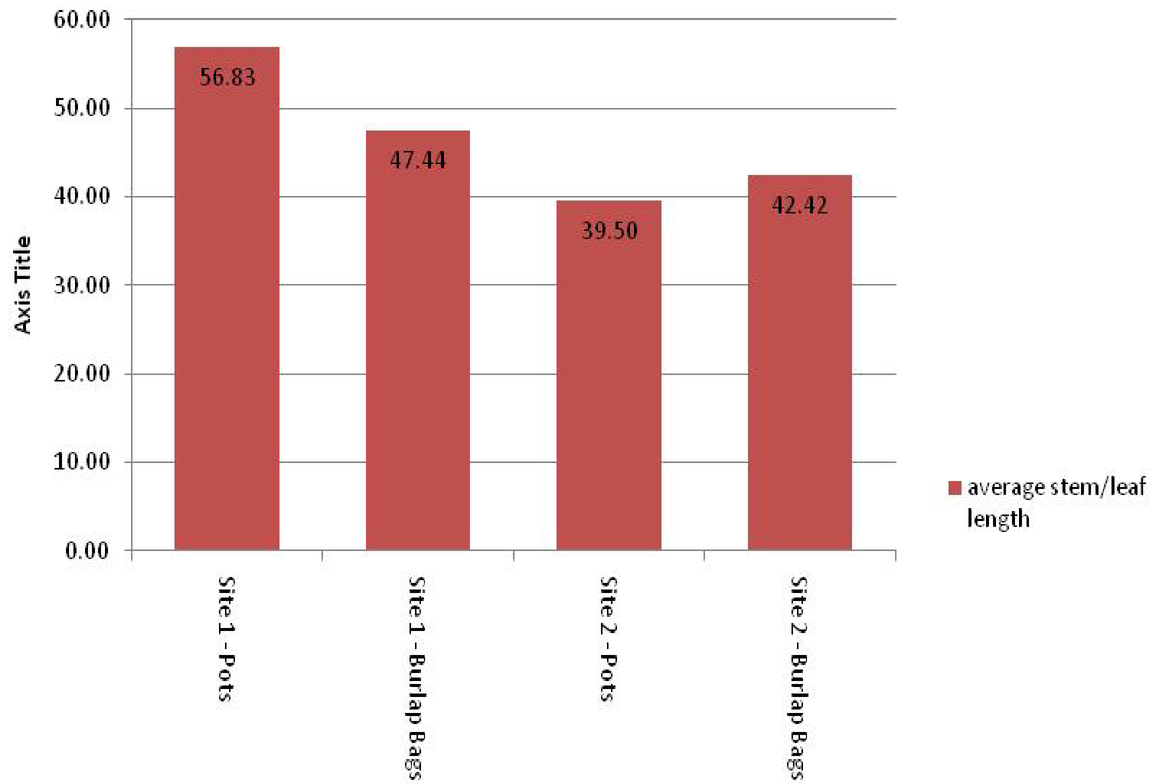

Figure 3. Average stem length of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana

QUALITY ASSURANCE PROJECT PLAN (QAPP)

Barataria-Terrebonne National Estuary Program (BTNEP) Project

Cooperative Agreement No.
CFMS Interagency Agreement No.
PMC Project No.

Submitted to:
Barataria-Terrebonne National Estuary Program
Nichols State University
P.O. Box 2663
Thibodaux, LA 70310

Submitted by
United States Department of Agriculture
Natural Resources Conservation Service
3737 Government Street
Alexandria, LA 71302

April 16, 2007
Draft

Section A: PROJECT MANAGEMENT

A.1 Title and Approval Sheet

Sampling and Quality Assurance Plan for

Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana

____ Date:

Steve Carmichael
State Resource Conservationist
Contract Manager
USDA, Natural Resources Conservation Service

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Richard Neill
Golden Meadow Plant Materials Center Manager
Principal Investigator
Golden Meadow Plant Materials Center
USDA, Natural Resources Conservation Service

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Ron Boustany
Natural Resources Specialist
Co-Principal Investigator
USDA, Natural Resources Conservation Service

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Terry Clason
Quality Assurance Officer
USDA, Natural Resources Conservation Service

____ Date:
Kerry St. Pé
Director
Barataria-Terrebonne National Estuary Program

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Dean Blanchard
Project Manager
Barataria-Terrebonne National Estuary Program

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Andrew Barron
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Barataria-Terrebonne National Estuary Program

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Betty Ashley
Project Officer
U.S. Environmental Protection Agency

____ Date:
Phil Crocker
Region 6, Aquatic Biologist
U.S. Environmental Protection Agency

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Olivia Balandran
Region 6, Acting Chief, Assistance Programs Branch
U.S. Environmental Protection Agency

A.2 TABLE OF CONTENTS

Project Management

Measurements and Data Acquisition

A.3 Distribution List

A final copy of this Quality Assurance Project Plan will be sent by the Project Coordinator to the following individuals:

Kerry St. Pe, BTNEP (Work Assignment Manager)
Dean Blanchard, BTNEP (Project Manager)
Steve Carmichael, NRCS (Contract Manager)
Richard Neill, NRCS (Principal Investigator)
Ron Boustany, NRCS (Co-principal Investigator)Terry Clason, NRCS (Quality Assurance Officer)
Andrew Barron, BTNEP (Quality Assurance Officer)
Betty Ashley, EPA (Project Officer)

A.4 Project/Task Organization

The organization of the project team for **Submersed Aquatic Vegetation Propagation and Planting Techniques for Restoration in Coastal Louisiana** is presented in A.4.1. A review of the primary staff and responsibilities for the management, quality assurance, and peer review is given below.

Project Management:

Contract Manager– - Steve Carmichael (State Resource Conservationist, USDA-Natural Resources Conservation Service, Alexandria, LA) is the Contract Manager has responsibility for the contractual and quality aspects of the project.

Principal Investigator – Richard Neill (Manager USDA-Natural Resource Conservation Service, Golden Meadow Plant Materials Center, Galliano, LA) is Principal Investigator of the project. He has responsibilities for directing project planning activities and is responsible for overall technical quality and consistency of all project activities and deliverables. Dr. Neill will also be responsible for developing release protocol for the project.

Co-Principal Investigator – Ron Boustany (Natural Resources Specialist, USDA- Natural Resources Conservation Service, Lafayette, LA) is Co-Principal Investigator of the project. He has responsibilities for directing project planning activities and is responsible for overall technical quality and consistency of all project activities and deliverables. Mr. Boustany will assist with experimental design and be responsible for preparation of the QAPP.

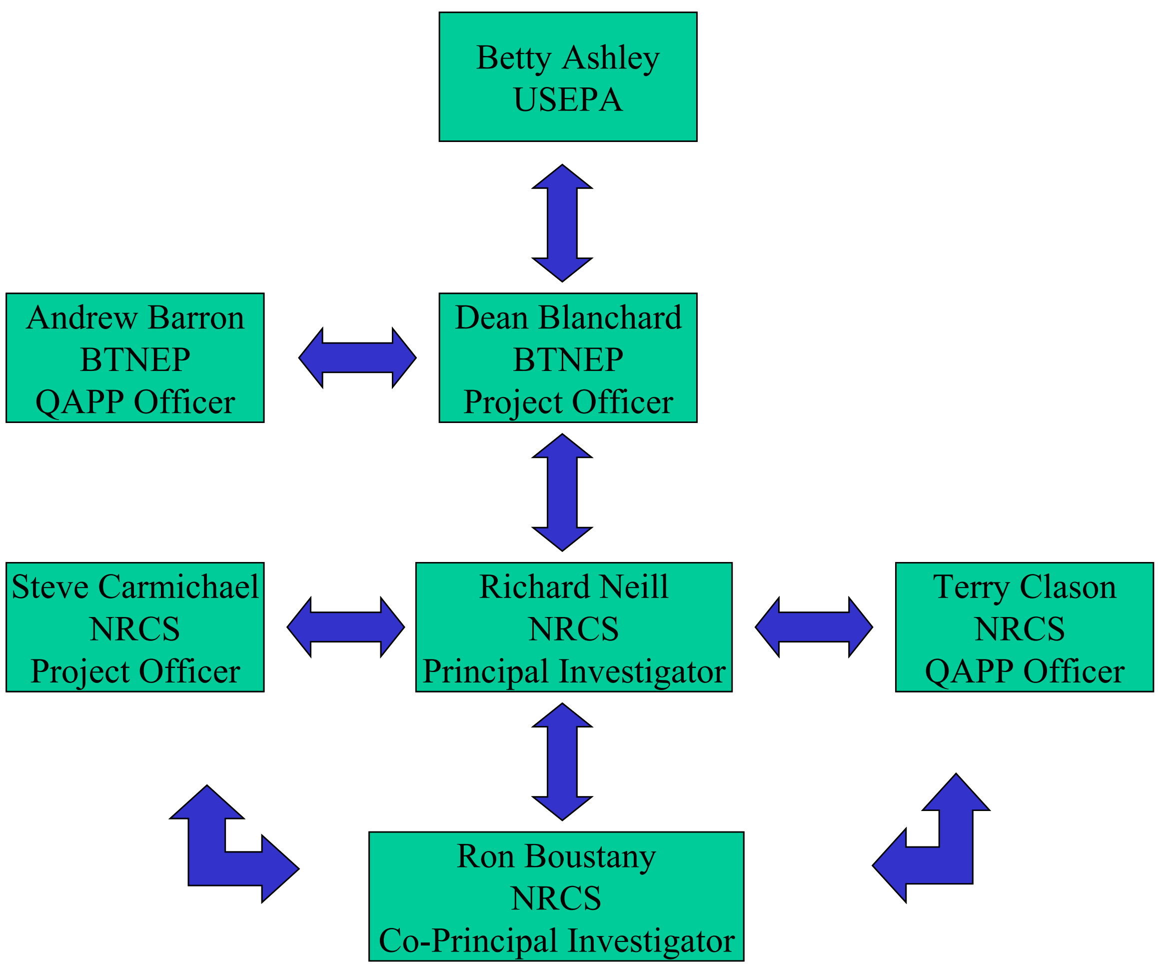
Quality Assurance Officer – Dr. Terry Clason (State Forester, USDA – Natural Resources Conservation Service, Alexandria, LA) is the Quality Assurance Officer. Dr. Clason and Steve Carmichael, NRCS State Resource Conservationist, will review the QAPP to insure that it meets all necessary quality assurance and quality control requirements.

Project Manager – Dean Blanchard (Thibodaux, LA) is the BTNEP Project Manager will monitor progress of NRCS in achieving the objectives of this project.
Quality Assurance Officer - Andrew Barron (Thibodaux, LA) BTNEP will review the QAPP to insure that it meets all necessary quality assurance and quality control requirements and will approve the QAPP when those requirements have been met.

Director - Kerry St. Pe', BTNEP, will review the QAPP to insure that it meets all necessary programmatic goals of National Estuary Program.

Project Officer - Betty Ashley (USEPA) will also review the QAPP and approves the QAPP when all applicable requirements have been met.

A.4.1 Project Organization Chart



A.5 Problem Definition/Background

A.5.1 Purpose/Background

The main focus of Louisiana coastal restoration efforts is generally the reestablishment of sustainable coastal wetland ecosystems that support a variety of important emergent wetland species. Submersed aquatic vegetation (SAV) is considered a critical component of sustainable coastal ecosystems in Louisiana however, very little is know about the methodology by which to restore SAV in Louisiana.

The purpose of this project is to establish criteria and methodology for restoration of SAV in Louisiana. The project will consist of two main areas of interests: 1) development of nursery propagation techniques and 2) development of field planting techniques. The nursery propagation will include the identification of important species for restoration and development of explicit methodology for growing the plants. The development of field planting techniques will evaluate various methodologies for planting SAV to determine which methods are most successful, cost effective, and applicable in coastal Louisiana.

A.5.2 Problem Statement and Background

The importance of SAV to Louisiana coastal ecosystems includes the minimization of storm damage by reducing wave action, stabilization of sediments, improvement of water quality by absorbing nutrients and contaminants, and provide critical habitat for wintering waterfowl and many commercially important fish species, (Zieman and Zieman 1989, Boustany 2003). Very little is known on the status of SAV throughout the coastal region of Louisiana. Unlike seagrasses, which include few species and inhabit very limited areas of the gulf coast in typically clear marine waters, the estuarine species that are spread out throughout the multitude of ponds and bayous of coastal Louisiana are more numerous. These areas are often in locations difficult to access, difficult to view in the murky waters, and occur in the entire range of different habitat types. Because of the lack of understanding of these species, the common recognition of the need to sustain and restore SAV communities in coastal Louisiana, and the lack of available restoration techniques available, it is necessary to begin to develop the necessary means by which to carry out successful SAV restoration projects.

A.6 Project/Task Description

A.6.1 Purpose/Background

OBJECTIVES

1. Nursery Propagation Development: Species will be identified, collected and propagated to determine the optimal growth conditions, type of container system to grow the plants in, growth media, proper handling and care of the plants, and development of standard operating procedures for transfer to the commercial growing trade.

2. Field Planting Techniques: The development of field planting techniques will be evaluated and standard operating procedures will be developed for transfer to managers and practitioners of restoration.

A.6.2 Description of the Work to be Performed

Nursery Propagation Development: Species will be identified that are considered ecologically important to coastal Louisiana and evaluated for feasibility to be used as a restoration species. Plants identified will be required to be native to Louisiana coastal ecosystems, commonly found throughout the target restoration area, are relatively easy to grow, and demonstrate a reasonably broad tolerance to varying environmental conditions. The selected species will then be collected and propagation methodologies will be developed at the NRCS Golden Meadow Plant Materials Center. Development of propagation will include determination of the optimal growth conditions, type of container system to grow the plants in, growth media, proper handling and care of the plants, and development of standard operating procedures for transfer to the commercial growing trade.

Field Planting Techniques: The development of field planting techniques will involve testing of various planting techniques that have been used to plant SAV in other areas of the country as well as development of new methodologies that will be tailored to the conditions of coastal Louisiana. Of particular interests is the imbedding of plants in supplemental substrates, such as erosion control fiber mats, to improve planting success as described by Boustany (2003). The various techniques will be evaluated and standard operating procedures will be developed for transfer to managers and practitioners of restoration.

A.7 Data Quality Objectives

The goal of this project is to establish criteria and methodology for restoration of SAV in Louisiana. The project will consist of two main areas of interests: 1) development of nursery propagation techniques and 2) development of field planting techniques. The nursery propagation will include the identification of important species for restoration and development of explicit methodology for growing the plants. The development of field planting techniques will evaluate various methodologies for planting SAV to determine which methods are most successful, cost effective, and applicable in coastal Louisiana.

A.7.1 Project Constraints

This is a three year project with two identifiable phases. Phase I objectives include the development of nursery propagation techniques in which species will be identified, collected and propagated to determine the optimal growth conditions, type of container system to grow the plants in, growth media, proper handling and care of the plants, and development of standard operating procedures for transfer to the commercial growing trade. Phase II objectives include the development of field planting techniques in which various field planting techniques will be performed and evaluated and standard operating procedures will be developed for transfer to managers and practitioners of restoration.

The major constraints on the project will be determining optimal growth conditions in the greenhouse with respect to light and water quality conditions and in the field the selection of suitable sites for test planting. In the greenhouse, performance evaluation will be based upon growth under varying light conditions. In the field, performance will be evaluated by measurements of survival (absence/presence), cover (expansion) and plant heights. Also, field evaluations will include observations on water quality (e.g. salinity, turbidity), substrate (e.g. mucky vs sandy bottom), and physical environment (e.g. exposure to fetch and tidal action, depth, position relative to nearest bank, etc) that will help to understand success or failure of the plantings. Field sites will be selected that have existing colonies of the test species to best eliminate the unsuitability of the habitat to support growth. The quality of data necessary to determine success or failure will answer two fundamental questions: 1) does the plant grow under the given conditions and 2) does it spread. In the greenhouse, limiting factors are expected to be light, water depth, evaluation of root development, and time for plants to become established (binded) to substrate. In the field, quantitative measures will involve measures of survival, spreading (% cover) and plant height.

Preliminary reports and potential plant materials recommendations will be formalized within the scope of this project. Further evaluation and analysis of plant performance, adaptation, and persistence will need to continue beyond the scope of this project. Potential source and selected plant releases that are performance proven may be made available for commercial plant increase and use in coastal restoration programs. Sampling design will consist of gathering plant performance data from each field plot established. Data collections will consist of recording plant survival, height, percent cover.

A.7.2 Project Objectives

1. To identify native submersed aquatic species suitable for use in coastal restoration
2. Evaluate and document performance of species in nursery propagation
3. Develop propagation and nursery management practices relative to these species
4. Develop recommendations that can be used for the successful use and establishment of these SAV species in using various field planting techniques

These objectives will be achieved by documenting procedures used both in the propagation and field plants   Detailed annual performance documentation collected from the greenhouse propagation and study plots will be provided including evaluations of propagation methods and success of plots using various field planting techniques.

A.8 Special Training Requirements/Certifications

A prerequisite for the use of equipment is prior training, experience, and skills acquired by Plant Materials Center personnel involved in this project. No special certifications are required. NRCS personnel responsible for collecting and recording plant performance data have received undergraduate training in plant sciences.

A.9 Documentation and Records

The following information and records will be included in quarterly and annual progress reports to the BTNEP Project Coordinator for distribution to the appropriate staff:

1. Progress and evaluation of SAV species tested in greenhouse propagation.
2. Progress and evaluation of greenhouse propagation techniques.
3. Progress and evaluation of field planting techniques.
4. Technology transfer documents provided to BTNEP and upon concurrence jointly (NRCS and BTNEP) released to the public.
5. A final report will summarize the accomplishments of this project and provide recommendations for the use and application of SAV materials for coastal habitat restoration
6. Consideration of any plant materials releases resulting from this study will be a partnership between NRCS Golden Meadow Plant Materials Center and BTNEP.
7. Metadata will be included from all data collected in the field.
8. Software format used will be compatible with BTNEP data format.

B.1 Sampling Process Design (Experimental Design)

Collection: At least two species of SAV, including *Vallisneria americana* Michx.and *Ruppia maritime* L., will be collected from various representative collection sites located within the Barataria/Terrebonne Basins. Plants will be collected as close to planned restoration sites to minimize any potential ecotypic differences that could occur from area to area. Soil will be gently washed away from the plants upon collection and placed in insulated containers partially filled with water from the collection site. Plants will then be transported to the greenhouse facilities at the Natural Resources Conservation Service Golden Meadow Plant Materials Center. At the greenhouse facilities, the plants will be sorted out by collection site and placed into holding containers in preparation for propagation.

Propagation: Development of propagation will include determination of the optimal growth conditions, type of container system to grow the plants in, growth media, proper handling and care of the plants, and development of standard operating procedures for transfer to the commercial growing trade. Additionally, the propagation will occur in two phases: 1) development of stock plants for restoration; 2) propagation of plants in artificial substrates in preparation for field plantings.

For developing stock materials, a series of containers will be set up in a randomized block design to facilitate analysis of various growth conditions on growth success. The varying growth conditions will include different levels of shading (0%, 25%, 50%, 75%) to determine optimal light conditions. Each growing tray will be aerated and circulated to avoid stagnation and insure optimal equilibration within the containers. The containers will be monitored weekly for temperature, pH, and dissolved oxygen.

Notations will be made on water clarity, algae growth, and vegetation density (% cover). In order to evaluate growth per treatment, monthly plant sample will be harvested to make growth measurements, including above- and belowground biomass and stem lengths.

Field Deployment: Once the optimal growth conditions are determined for propagation of stock material, preparations will be made for transfer of material to the field to test various restoration methods. Some plants will be grown out in six inch plastic pots to be transplanted as plant plugs and some will be prepared in fibrous matting material to test an alternative method. In the case of the potted plants, the plants will be grown out in the pots using the preferred media and light conditions, and when matured, the plugs will be removed from the pots in the field and planted into a hole in the conventional method of planting. Plants that are pre-rooted in the fibrous matting material will be transported along with the mats to the field site and anchored to the native substrate adjacent to plug plantings. Several sizes of mats and arrangement of out plantings will be evaluated for the plant's ability to spread from the media into the native substrate.

The matted plants will be woven into a biodegradable fibrous matting (exact material not yet determined). The mats will be laid out in the growing containers to allow for the plants to root and mature in the substrate. A thin layer (5 - 10 cm) of growth media will be placed in the bottom of the trough (under the mat) to promote root development. The troughs will be continuously aerated and circulated. Once plants are determined to be established, the mats will be transported to the study sites for planting.

In preparation for transport, the mats will be rolled up and wrapped in a plastic non-porous bagging to prevent desiccation in transport. The mats will then be immediately transported to the field site for planting with minimal delay (ca. 2-3 hours) to minimize trauma to the plants. Once in the field, the bagging will be removed and the mats will be set on bottom of the restoration site and anchored with biodegradable staking. The pre-selected sites will consist of one in the Barataria Basin and one in the Terrebonne Basin.

B.2 Sampling Methods

The two field sites (one in Barataria Basin and one in Terrebonne Basin) will be monitored on a monthly basis to note environmental conditions and general qualitative growth conditions. Environmental information will include notations on weather conditions and water quality conditions including dissolved oxygen, temperature, pH, water depth, and water clarity (secchi depth). Quantitative growth measures will be taken during the first year, 6 weeks following plantings and at the end of the years growing season (September). In the second year, growth measurements will be performed three times during the active growing season – May 1, June 15 and September 1. The growth parameters will include determination of absence or presence of planting, percent cover within a 1 m grid of the individual planting unit, and stem lengths for each planting unit (see sample field data sheet – Figure 3).

Analysis of Variance (ANOVA) will be used to determine the differences in growth success using various propagation scenarios in the greenhouse and, in the field, comparisons will be made to evaluate planting methods.

Greenhouse design will consists of 2 species (*Vallisneria americana*, *Ruppia maritima*), 4 light levels (0%, 25%, 50%, 75% shade) and 3 reps for a total of 24 units.

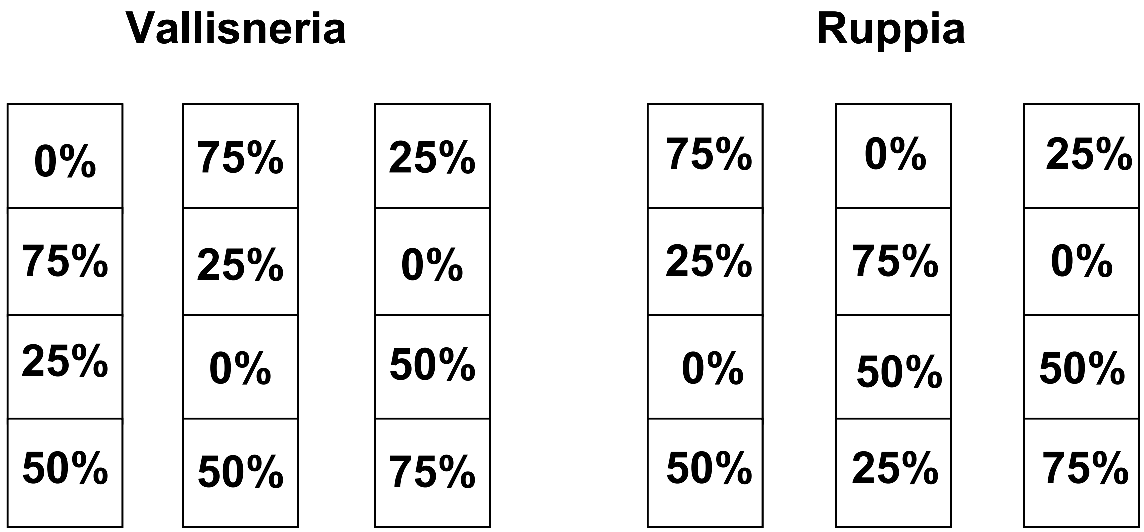
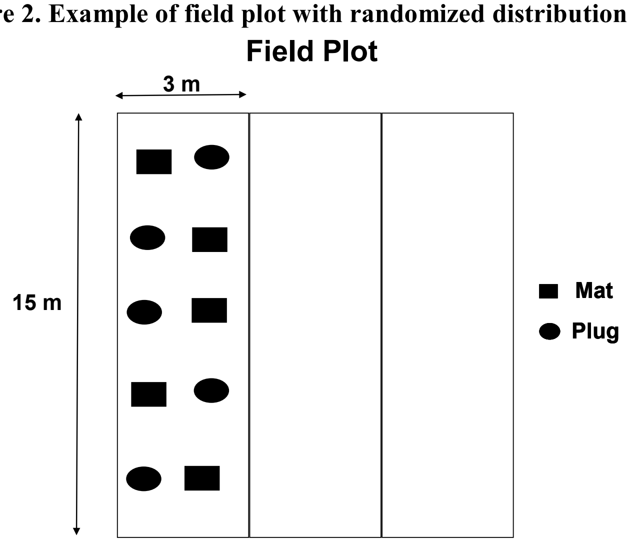


Figure 1. Example greenhouse propagation layout with two species and four different light regimes.

The field analysis will consist of 2 species plots per basin with 3 reps each for a total of 12 units. Within each species plot, one of each type of planting unit (mats vs plugs) will be placed in a randomized block design extending from the shoreline out in 3 m increments for a total of 15 m. Therefore, there will be a total of 2 plantings per planting block, 5 blocks per rep and 3 reps per site for a total of 30 plantings (2 x 5 x 3 = 30) per site. Two sites have been chosen for field plots, one in Lafourche and one in Terrebonne Parish (see Figures 4 and 5), bringing the total number of plantings to 60.

Figure 2. Example of field plot with randomized distribution of planting units.



Field Data Sheets			
Date:			
Site:	**Sample**		
Species	**Val/Rup**		
Notes:			
Weather conditions, field conditions, problems with plots, etc.			

Environmental			
Temperature C			
pH			
Salinity (ppt)			
Water Depth (m)			
Secchi Depth (m)			

Vegetation		Prop Method 1	Prop Method 2
Block 1 (0-3 m)	Absence/Presence	y/n	
	% Cover	%	
	Plant height	cm	
Block 2 (3-6 m)	Absence/Presence		
	% Cover		
	Plant height		
Block 3 (6-9 m)	Absence/Presence		
	% Cover		
	Plant height		
Block 4 (9-12 m)	Absence/Presence		
	% Cover		
	Plant height		
Block 5 (12-15 m)	Absence/Presence		
	% Cover		
	Plant height		

Figure 3. Sample field data sheet including both environmental and vegetation data information.

Field Evaluation Planting Location Quad Maps

B.2.1 Barataria Study Area, Lafourche Parish (Proposed)

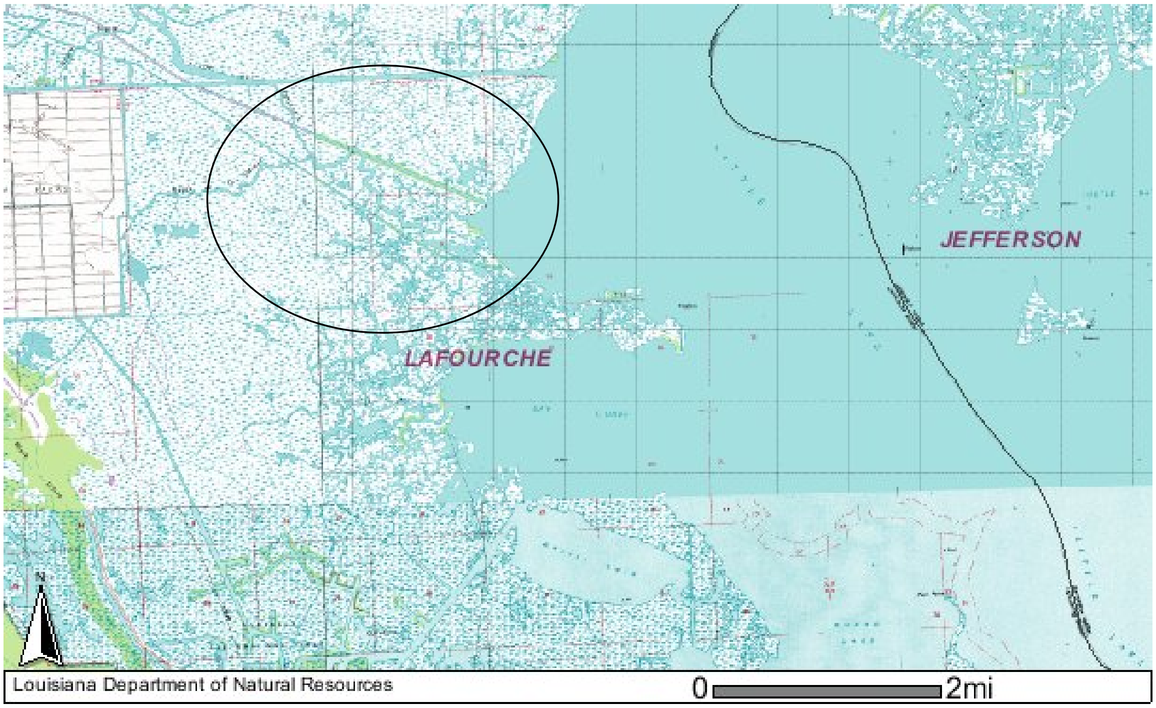


Figure 4. Vicinity of proposed field sample plots in Barataria Basin.

B.2.2 Terrebonne Study Area (Terrebonne Parish, Louisiana)

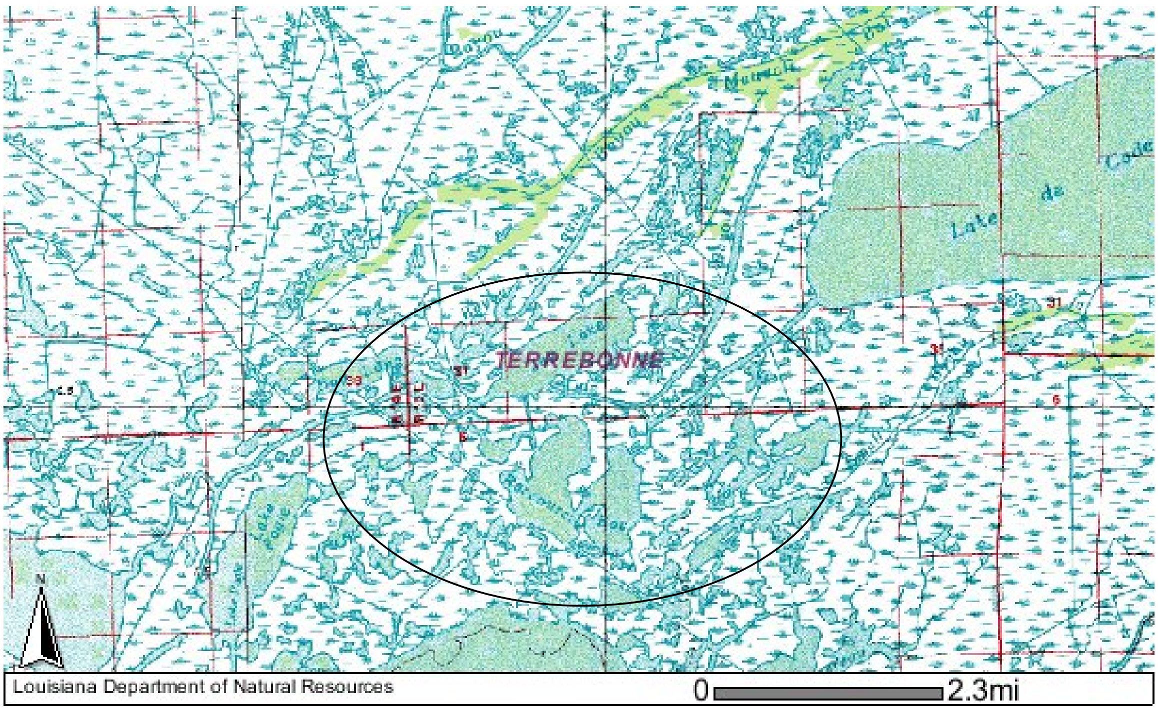


Figure 5. Vicinity of proposed field sample plots in Terrebonne Basin.

B.3 Sample Handling and Custody

Field data will be recorded monthly on data sheets that include: 1) project name, 2) planting date, 3) evaluation date, 4) site location, 5) habitat type, 6) plot/species identification, and 7) evaluation criteria. Field data are entered into electronic format using MS Excel. Entered data are checked and verified against field data collections sheets. Data and name of person collecting the field data and name of the person entering the data on MS Excel spreadsheets are recorded with data documentation preserved in daily system backups. Field data are submitted to the PI for technical review. Printouts are then filed in NRCS project document folders. Foundation plant material releases that may result from this study are the joint custody of the NRCS and BTNEP.

B.4 Analytical Methods

Propagation techniques will be based on the ease of propagation and vigor of the plants involved. Since these techniques will be passed to commercial growers, success will be determined by techniques credible to the growers. Out-planting techniques will be likewise evaluated by successful establishment with a mind to the end user of the technology. Field trials will be randomized and replicated for statistical evaluation.

B.5 Quality Control

For developing stock materials, a series of containers will be set up in a randomized block design to facilitate analysis of various growth conditions on growth success. Two replicate plantings will be performed at each (Barataria and Terrebonne) field site. Data will be analyzed using appropriate statistical analysis. All possible care will be taken to insure consistency in procedures both in the greenhouse propagation and field analysis.

Quality control activities will include:

- routine calibration of all relevant instruments
- cross-check all data
- use of documented standard personnel (visual) techniques to ensure objective measurements

All measurements will be validated in the field to prevent problems and will be cross-checked in the office at data entry. At present, until we have reason to suspect data outliers, all data will be accepted in the analysis because of the broad range of growth conditions expected.

B.6 Instrument/Equipment Testing, Inspection, and Maintenance

All equipment will be calibrated and maintained according to manufacturer's recommendations.

B.10. Data Management

Field and laboratory data collected through the duration of this project will be maintained by the individual investigators. These data will be stored in electronic and hard copy form. Each investigator is responsible for detecting and correcting errors in their section of the project. If corrections are made to hard copy data or records, the original data will be preserved, marked through once, and the changes initialed. All investigators have access to computer hardware and software for data analysis and backup. The principal investigator and quality assurance officer will maintain hard copy and/or electronic backups of field log books, laboratory worksheets and reports.

Data will be maintained at NRCS-PMC (daily) and backed up by NRCS IT services (weekly). Richard Neill will be responsible for the overall administration of the project and data collection. Ron Boustany will serve as technical advisor to the project. Both will be responsible for maintaining quality control through all aspects of the project data gathering. All NRCS employees are required to complete annual computer security and awareness training.

C.1. Assessment and Response Actions

The PI is responsible for ensuring that all data collection personnel have adhered to procedures. This will include checks in the field. Corrective action will be implemented as needed. Assessments of data and analysis will be reported to NRCS headquarters chain of command, signed, and forwarded to BTNEP. All reports will be sent to the attention of Dean Blanchard as signature authority for approval and copies will be forwarded to project and QAPP officers and other appropriate personnel.

C.2 Reports to Management

Progress reports will be prepared and submitted to BTNEP quarterly and annually during the duration of the study. A final project report will be submitted when the project is complete and the resulting data is analyzed. The project reports will be prepared by NRCS PMC (Golden Meadow) in collaboration with Ron Boustany with intentions to publish technical reports concerning collection, propagation, production, and field planting of vegetation. The ultimate goal will be to promulgate recommendations for propagation and field planting for the commercial trade.

D.1 Data Review, Verification, and Validation

Data acquired from this project will be reviewed by the NRCS QA Officer to verify that data quality objectives were achieved. Data to be evaluated include compliance of the QAPP, field evaluations and completeness. Citeria to be evaluated include compliance and qualification of the QAPP, field and greenhouse evaluations and completeness.

D.2 Validation and Verification Methods

All field data will be reviewed for unusual and inconsistent results. If such results are found, the data will be traced through field data worksheets in an attempt to locate the cause of error. If a legitimate cause is found and cannot be corrected, the data will be discarded. If no error can be located then the data will be retained.

Validation results from this study will be used to provide plant materials recommendations for use in coastal environments and potentially provide for the commercial release of tested and proven plant materials for conservation use.

Day to day operation discrepancies will be resolved by BTNEP project officer and NRCS project manager. Administrative issues will be refered to Kerry St. Pe (BTNEP) and Don Gohmert (NRCS).

Raw data will be made available to NRCS headquarters and BTNEP headquarters. The use of raw data will be reserved for scientific publication. Analysis of data will be published in NRCS technical bulletins and other means necessary as seen fit by NRCS and BTNEP.

D.3 Reconciliation with User Requirements

Upon completion of this project, BTNEP and EPA project managers will review the data collected to determine if the required objectives were achieved. Results of this work will be used to further incorporate the use of SAV in coastal restoration and conservation.

Hard copies and electronic copies of data and publications will be provided to the BTNEP as part of quarterly, annual, and final reports. Electronic copies of information will be provided in a format compatible with BTNEP requirements and on duplicate CD ROM discs.

References

Boustany. R.G. 2003. A prevegetated mat technique for the restoration of submersed aquatic vegetation. Ecological Restoration 21:2.

Zieman, J.C. And R.T. Zieman. 1989. The ecology of the seagrass meadows of the west coast of Florida: a community profile. U.S. Fish and Wildl. Serv. Biol. Rep. 85(7.25). 155 pp.