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

Status Report

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Submitted August 10, 2010 to:
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Table of Contents

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.

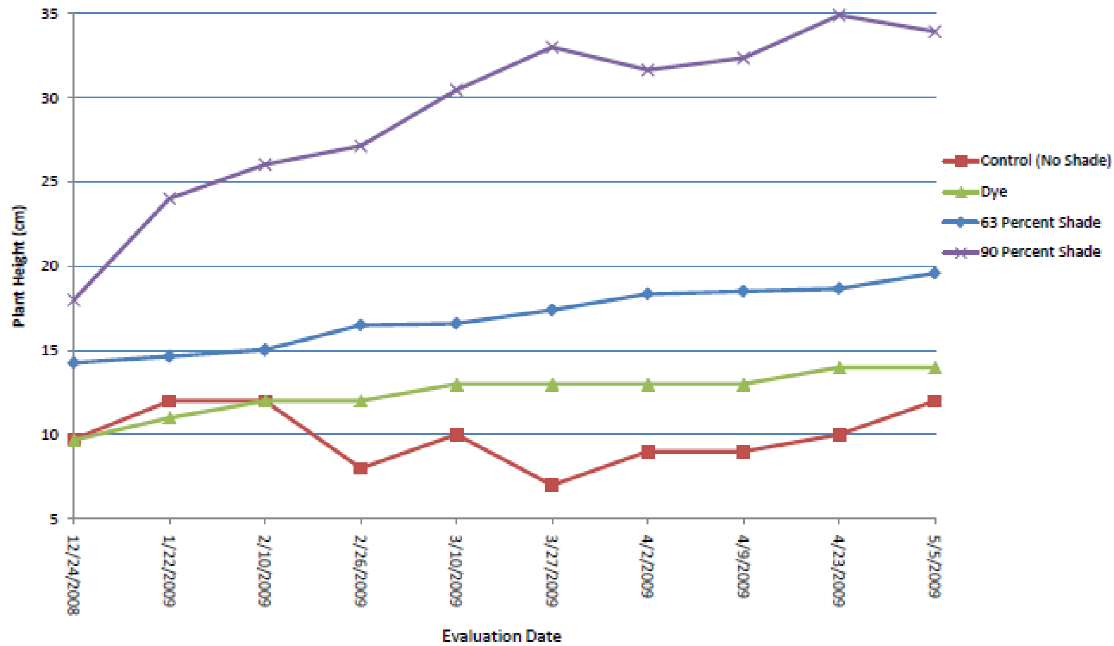

Figure 1. Plant height of *Vallisnaeria americana* as effected by various shade treatments. USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

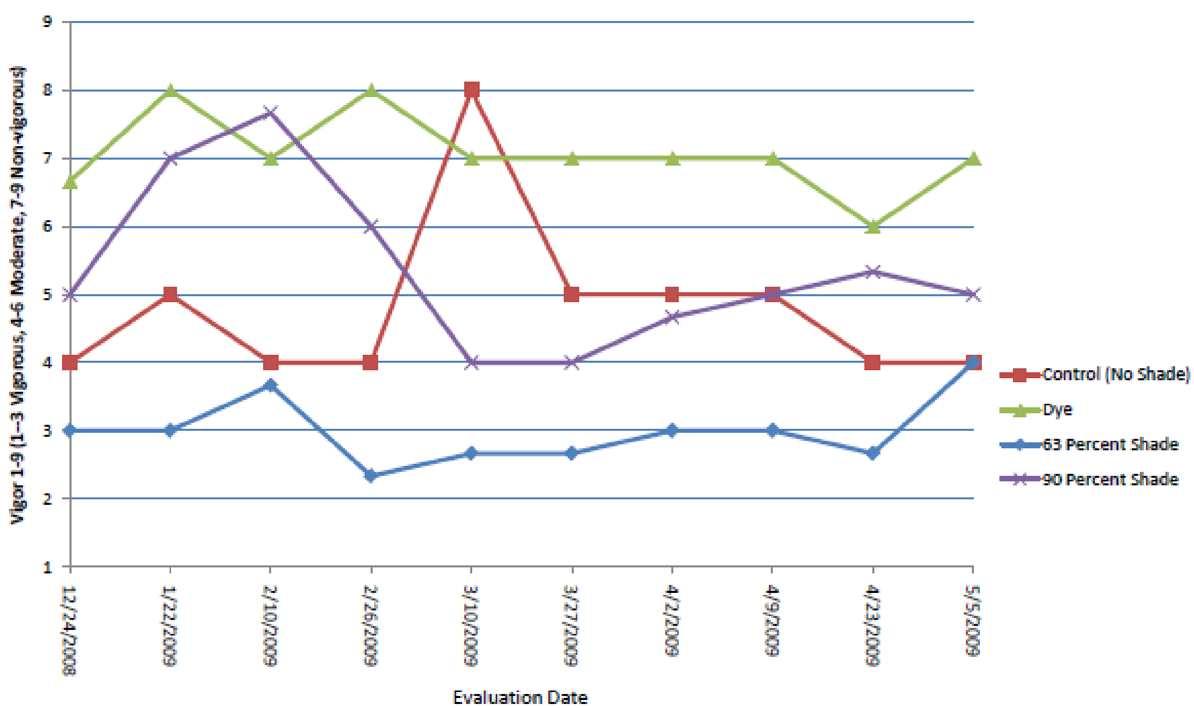

Figure 2. Plant vigor of *Vallisneria americana* as effected by various shade treatments. USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

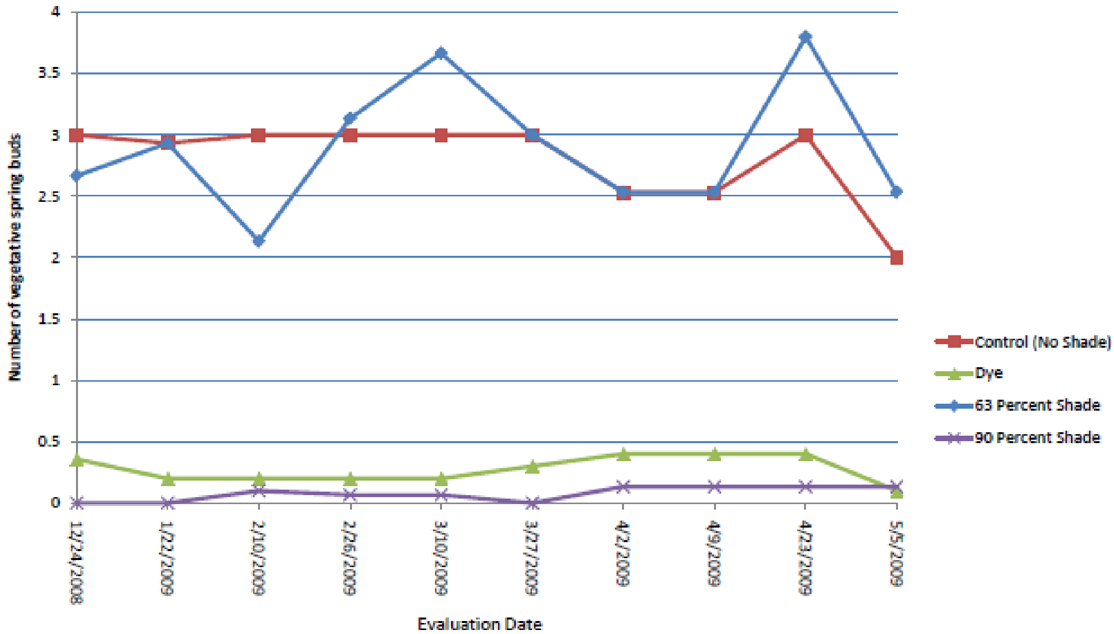

Figure 3. Number of spring buds produced by *Vallisneria americana* s effected by various shade treatments, USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

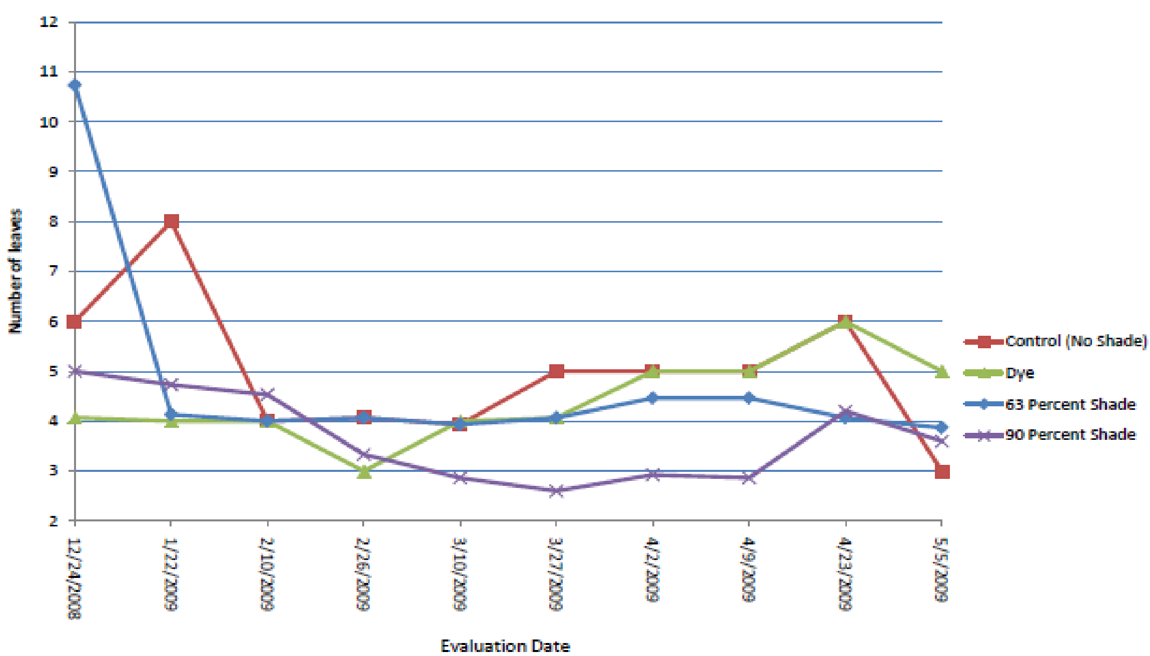

Figure 4. Number of actively growing leaves of *Vallisneria americana* as effected by various shade levels, USDA-NRCS Golden Meadow Plant Materials Center, December 2008 to May 2009

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

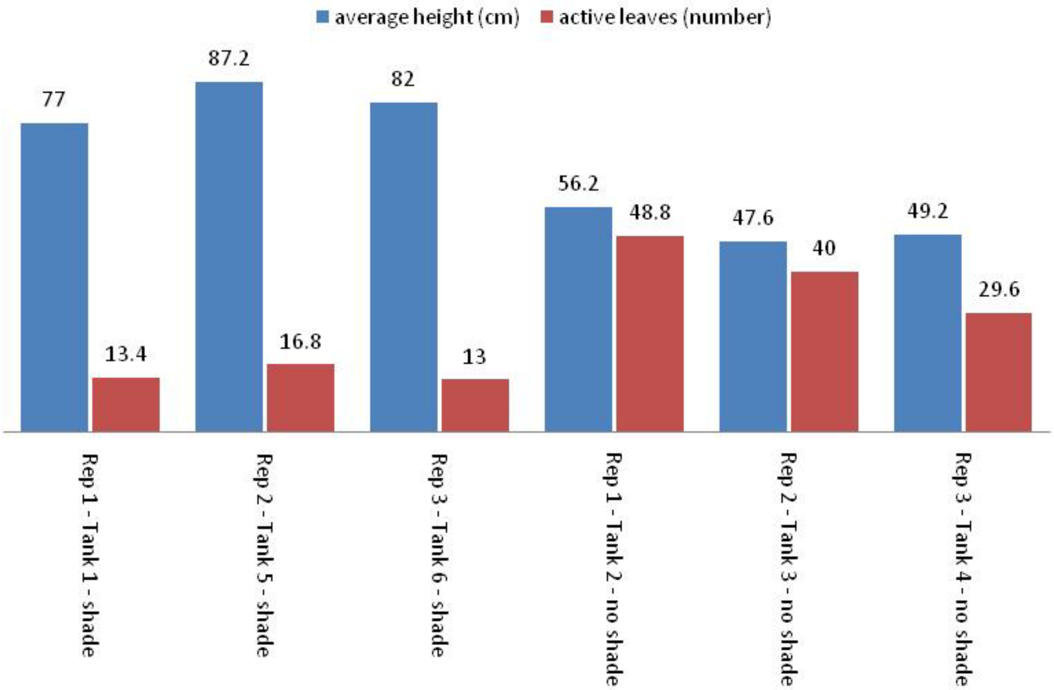

Figure 1. Number of actively growing leaves and average leaf height of *Vallisneria americana* grown in plastic pots as effected by 63% shade and no shade, USDA-NRCS Golden Meadow Plant Materials Center, February 2010 to July 2010

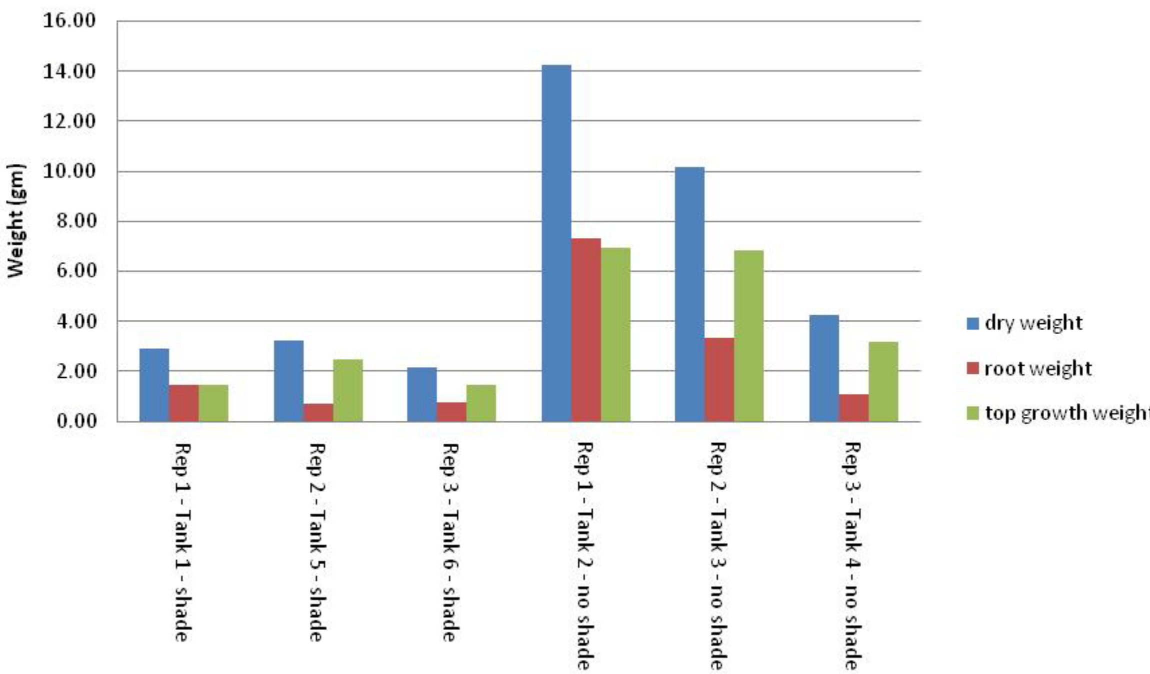

Figure 2. Average biomass weight of *Vallisneria americana* grown in plastic pots as effected by 63% shade and no shade, USDA-NRCS Golden Meadow Plant Materials Center, February 2010 to July 2010

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.

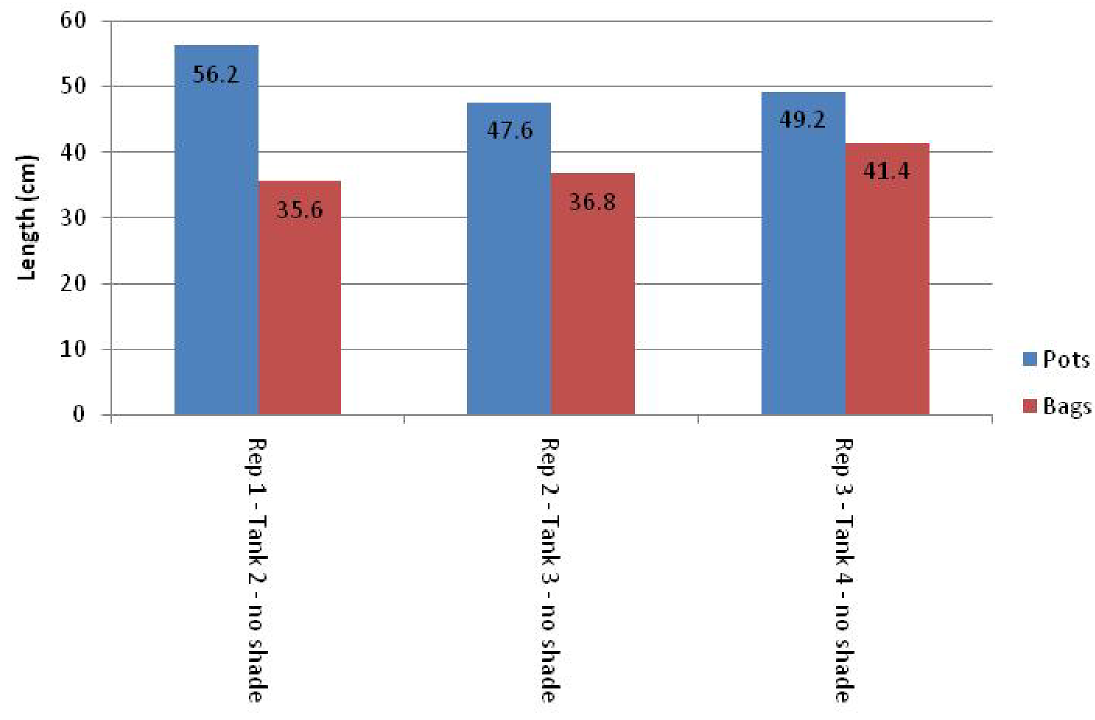

Figure 1. Comparison of average leaf length of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

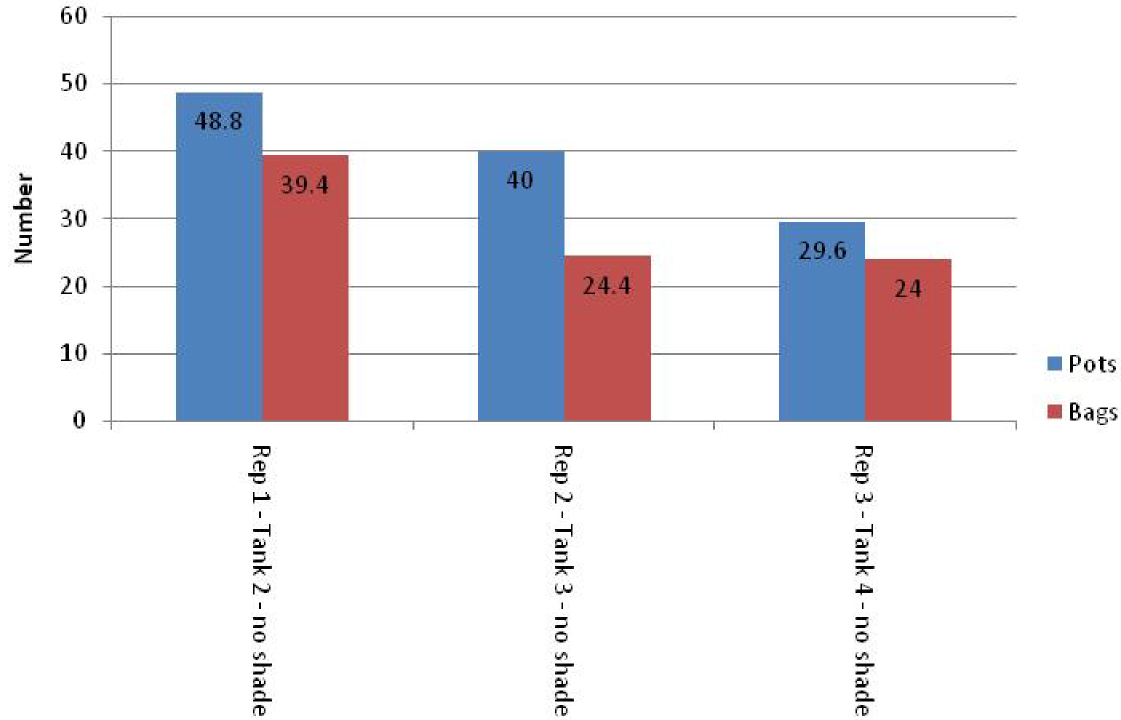

Figure 2. Comparison of the number of actively growing leaves of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

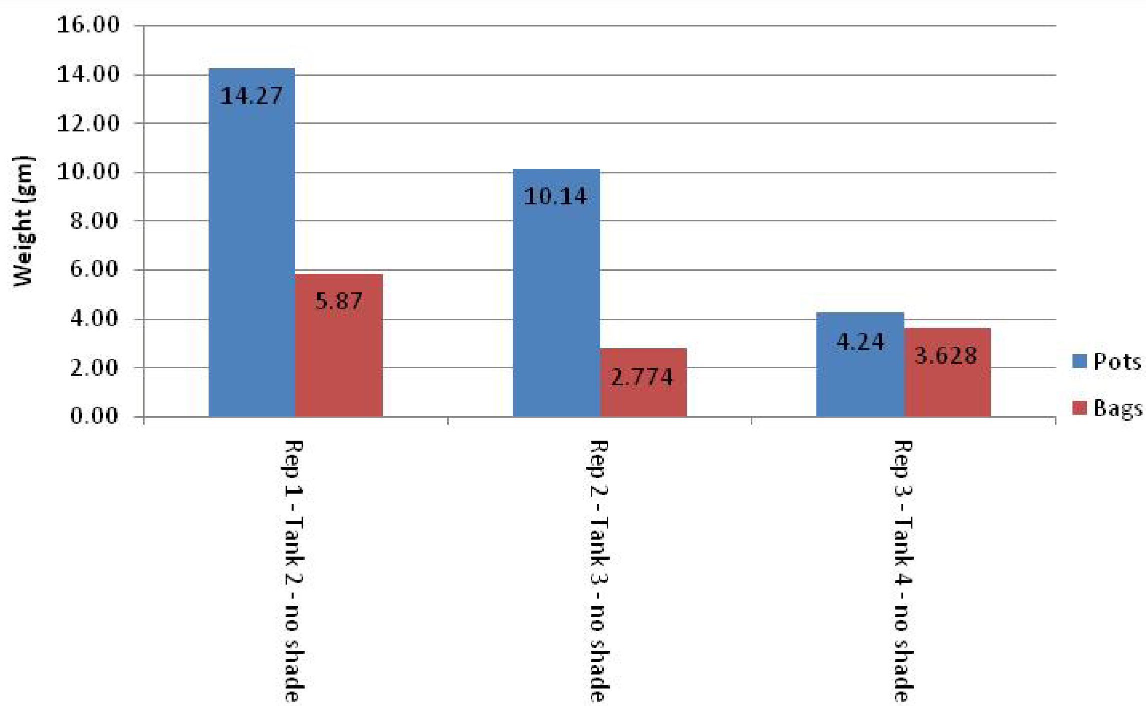

Figure 3. Cpmparison of total plant biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

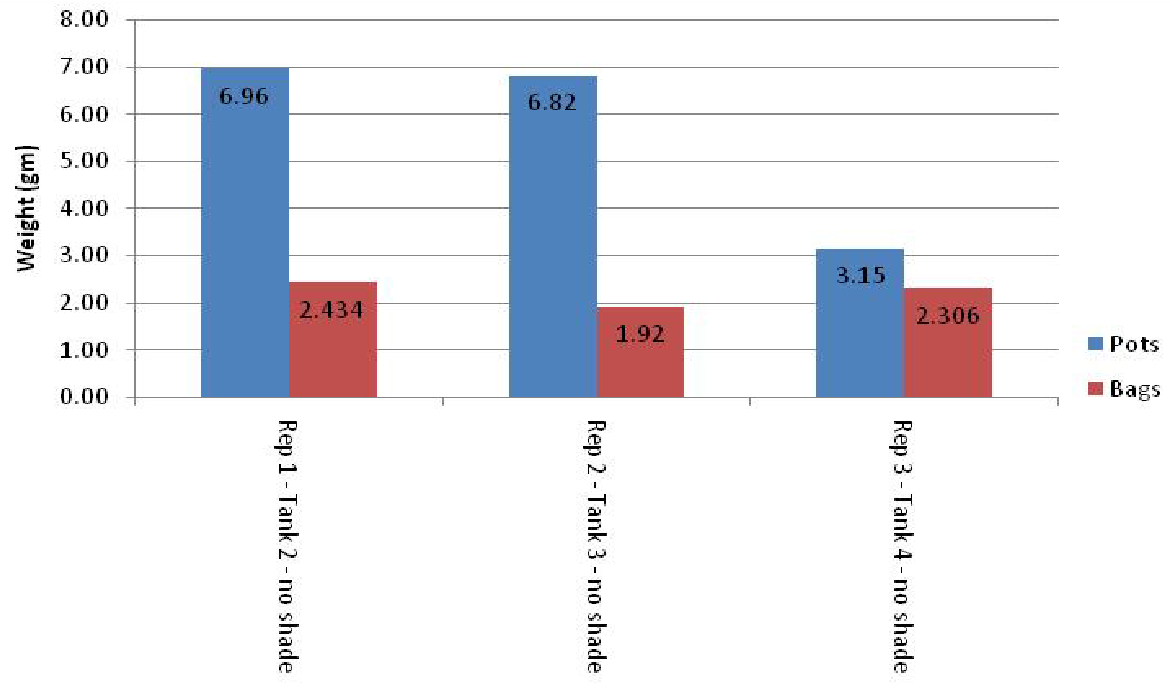

Figure 4. Comparison of top growth biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

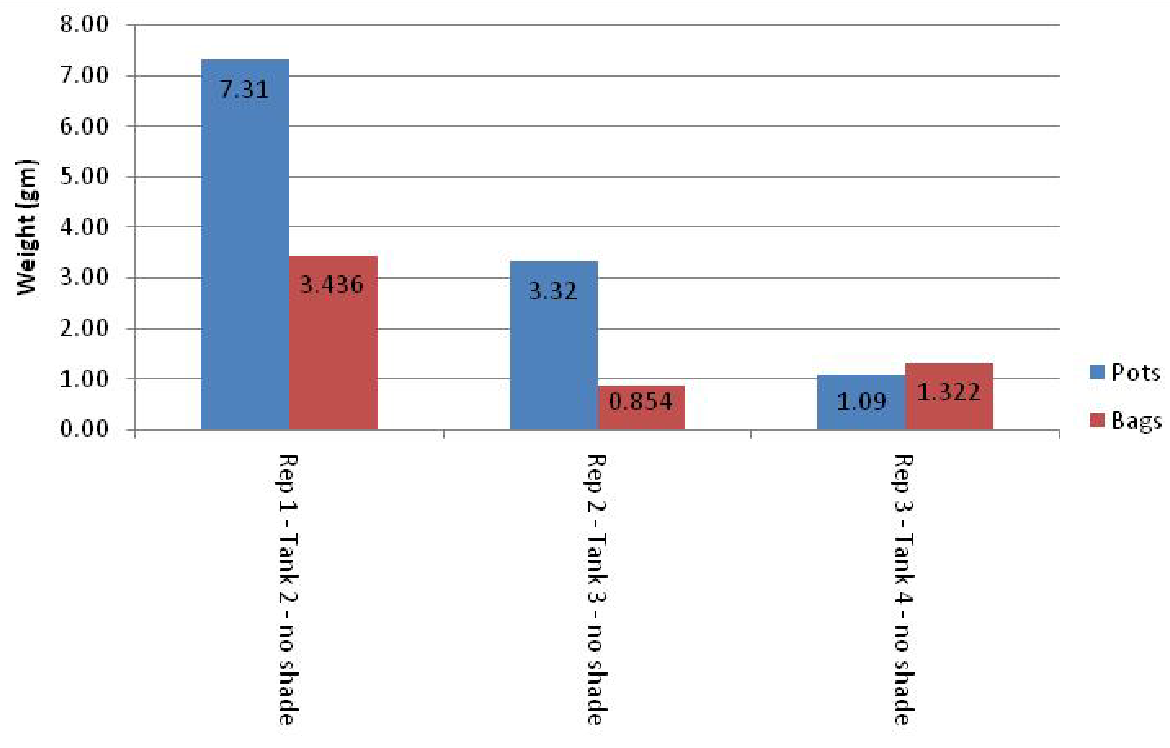

Figure 5. Comparison of root biomass of *Vallisneria americana* grown in plastic pots and biodegradable bags, USDA-NRCS Golden Meadow plant Materials Center, February 2010 to July 2010

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.

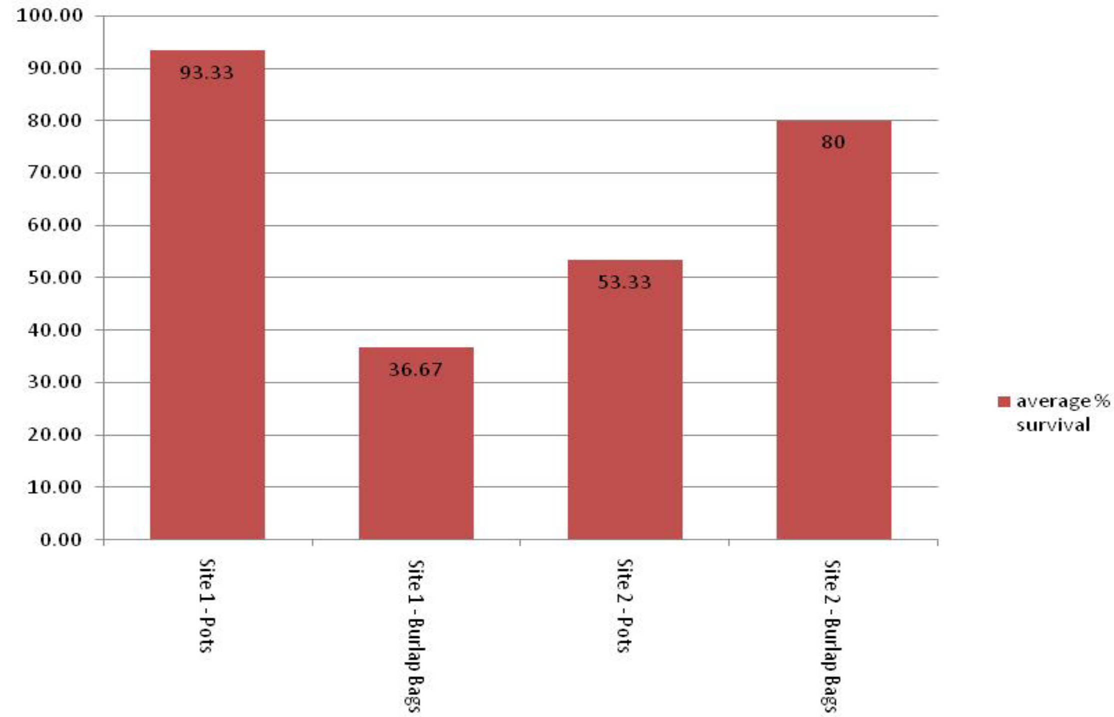

Figure 1. Average field survival of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

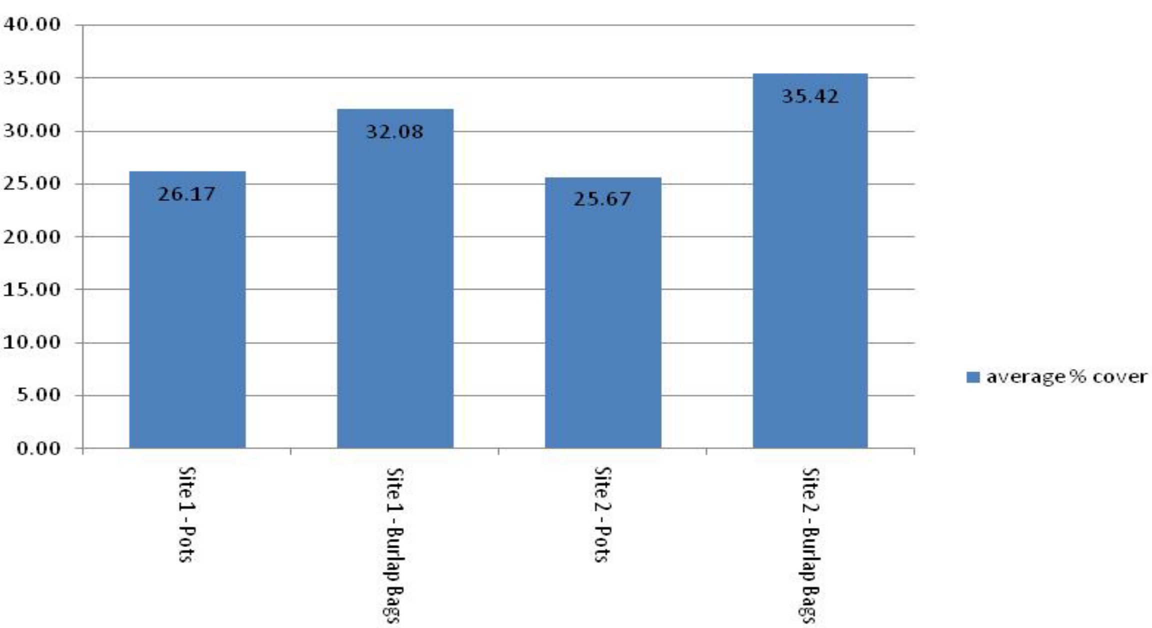

Figure 2. Average % cover of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

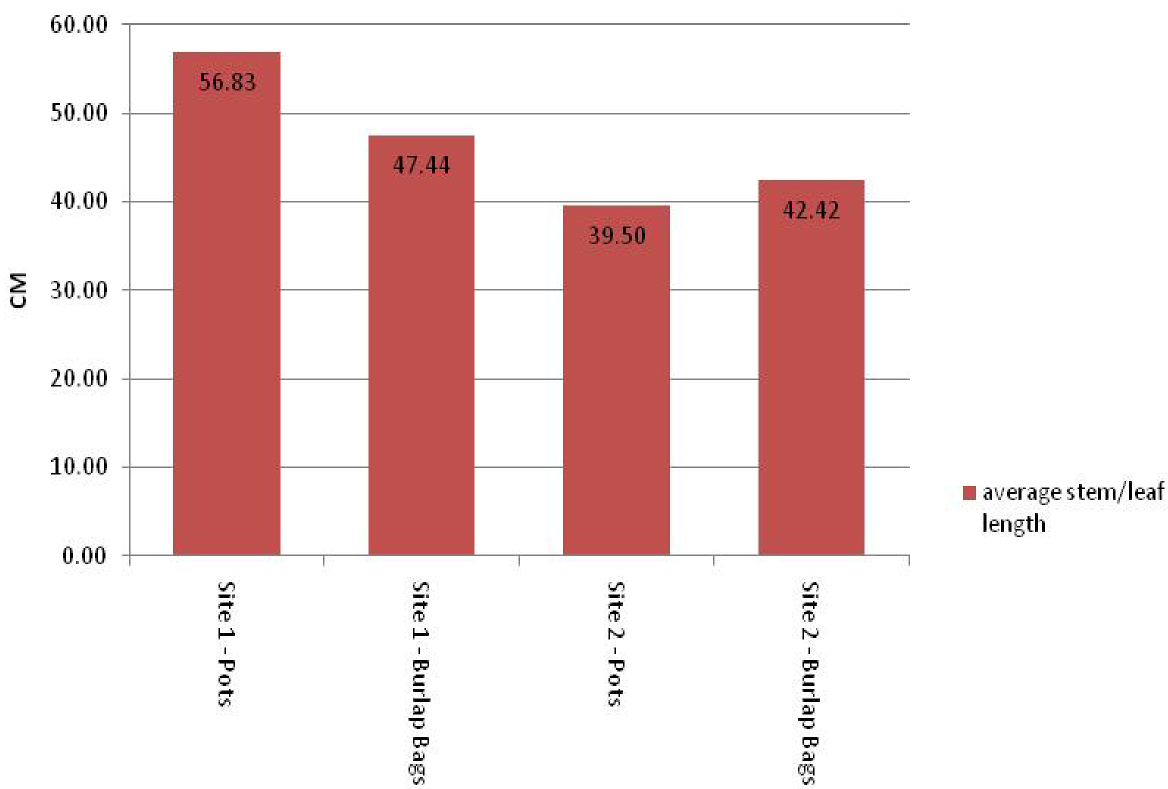

Figure 3. Average stem length of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010

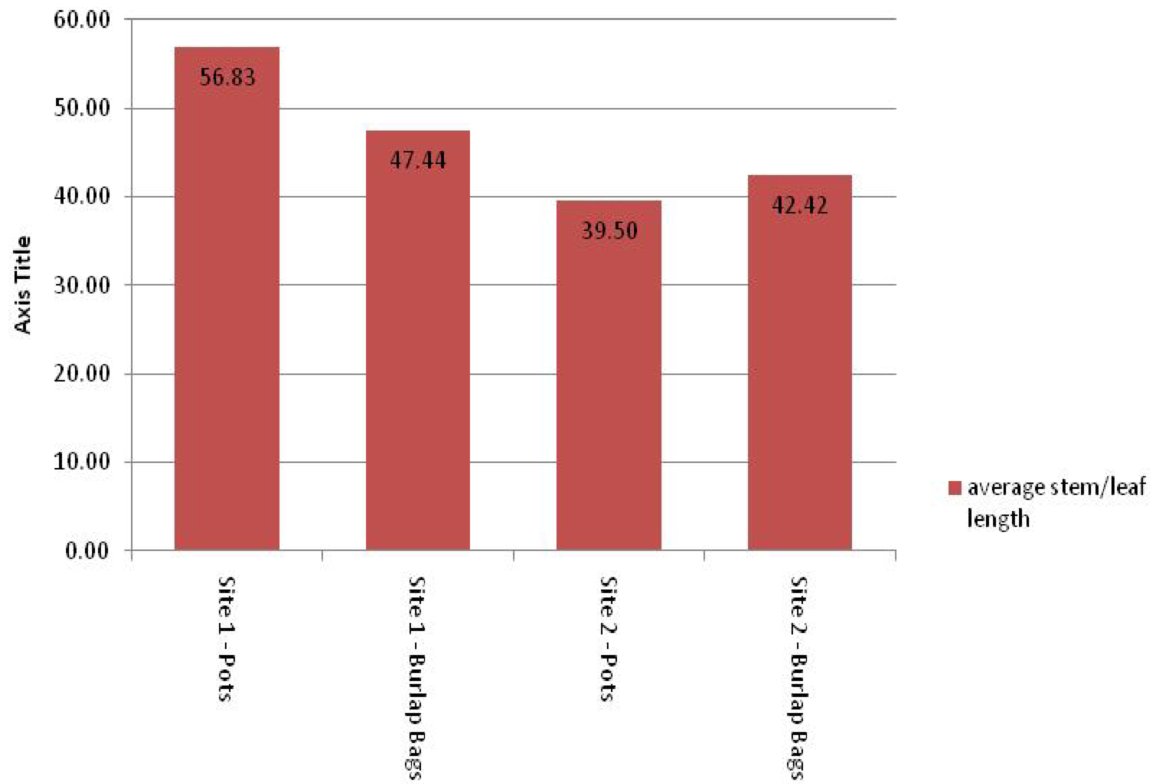

Figure 3. Average stem length of *Vallisneria americana*, USDA-NRCS Golden Meadow Plant Materials Center, July 2010