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Greenhouse Production of Native Aquatic Plants

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Summary. Wetland restoration is critical for improving ecosystem services, but many aquatic plant nurseries do not have facilities like those typically used for large-scale plant production. We questioned if we could grow littoral aquatic plant species in a variety of substrates and irrigation methods similar to those used for traditional greenhouse production. Plants were grown in pots with drainage holes that were filled with potting substrate, topsoil, coarse builders' sand, or a 50/50 mix of topsoil and builders' sand. These substrates were amended with 2 g of 15N–3.9P–10K controlled-release fertilizer per liter of substrate and were watered using either overhead irrigation or subirrigation. Plants were grown for 16 weeks, then scored for quality and height before a destructive harvest. Blue-eyed grass (*Sisyrinchium angustifolium)* and arrow arum (*Peltandra virginica*) performed best when subirrigated and cultured in potting substrate or sand. Golden club (*Orontium aquaticum*) and lemon bacopa (*Bacopa caroliniana*) grew best when plants were cultured in potting substrate and maintained under subirrigation. These experiments provide a framework for using existing greenhouses to produce these littoral species and give guidance to growers who wish to produce plants for the restoration market.

Projects that focus on restoration, mitigation, and enhancement of aquatic and wetland regions provide valuable ecosystem services and habitat for native flora and fauna (Brix, 1994). These projects call for a mixture of plant types and sizes to create the diverse architecture needed to provide good habitat for native animals (Ma et al., 2010; Tews et al., 2004). There is strong demand for the native littoral zone (shoreline or shallow water) plants required to execute these projects, but many wetland nurseries are unable to produce sufficient quantities of "right-sized" plant material due to inadequate facilities and infrastructure. This problem can be viewed as an opportunity and may be addressed by determining how to cultivate these species using the greenhouse techniques that are employed to culture landscape plants. In these experiments we focused on four littoral zone species: arrow arum, blue-eyed grass, golden club, and lemon bacopa. All four species are perennials native to the United States and are easily propagated via division [arrow arum (Supplemental Fig. 1), blue-eyed grass (Supplemental Fig. 2), golden club (Supplemental Fig. 3)] or cuttings [lemon bacopa (Supplemental Fig. 4)]. Our objective was to determine optimum greenhouse cultivation conditions for these littoral plants by examining their growth and performance in a variety of substrates and irrigation methods. These experiments were designed to provide guidelines for growers who are interested in capturing part of the growing niche market for littoral zone plants without costly infrastructure upgrades.

Materials and methods

Golden club and arrow arum experiments were started on 29 Sept. 2014 and 12 Jan. 2015, respectively, from nursery-grown starter plants with foliage that was ≈24 cm tall. The blue-eyed grass experiment was started on 2 Nov. 2014 using single plants (derived from division of stock plants) with five to eight leaves that were ≈20 cm long. The lemon bacopa experiment was started on 20 Nov. 2015 from well-rooted cuttings that were ≈25 cm in height. A total of 128 plants of each species were transplanted into traditional 3.05-L (7–7/8 inches diameter) azalea pots with drainage holes and filled with either potting substrate [45% Canadian sphagnum peat, 25% bark, 35% vermiculite (Fafard 4M; Sun Gro Horticulture, Anderson, SC)], topsoil [regionally formulated mix of organic and mineral components comprising 35% to 50% organic matter (Timberline Top Soil; Oldcastle Lawn and Garden, Atlanta, GA)], sand [grain diameter 0.25–0.5 mm (Multi-Purpose Sand; Sakrete, Charlotte, NC)], or 50/50 (v/v) mix of topsoil and sand (hereafter "mix"). Pots were filled to a depth of about 8 cm, and 6 g of 15N–3.9P–10K controlled-release fertilizer formulated for 6-month release in Florida (Osmocote Plus; ICL Specialty Fertilizers, Dublin, OH) was placed in a layer on top of the substrate. The use of 2 g of fertilizer per liter of substrate provided 0.91 g/pot

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.0254	mil(s)	mm	39.3701
28.3495	oz	g	0.0353
7.4892	oz/gal	$g \cdot L^{-1}$	0.1335
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

N and was selected based on previous work that revealed an intermediate fertilizer rate of 2 $g{\cdot}L^{-1}$ was optimal or near optimal for littoral and aquatic species (Gettys and Moore, 2018; Hasandras et al., 2018). Pots were then filled to their final depth with the same substrate before transplanting. Plants were randomly selected for placement into either overhead irrigation or subirrigation treatments. Plants receiving overhead irrigation were grown on greenhouse benches in a completely randomized design with respect to substrate type and irrigated twice per day (10:00 AM and 4:00 PM) with the equivalent of 0.5 inch of water per irrigation. Subirrigated treatments were arranged in a completely randomized design in flood trays constructed from 2 × 6-inch lumber and lined with 6-mil clear polyethylene sheeting. Flood trays were maintained at a constant depth of 2 inches using a float system that automatically released additional water when depth dropped below 1.5 inches. Four replicates of each species were prepared for each treatment combination (substrate × irrigation). Plants were grown in an open-sided greenhouse under ambient air temperatures and relative humidity at the University of Florida Fort Lauderdale Research and Education Center in Davie, FL. Experiments were conducted from Oct. 2014 to May 2015; average daytime high temperatures ranged from 75 to 89 °F, average nighttime low temperatures ranged from 59 to 78 °F, and average relative humidity was 76%. Weather data were collected by the Florida Automated Weather Network station located less than 100 ft from the greenhouse.

Plants were grown for 16 weeks and then assigned a numerical value of 1 through 10 to describe visual quality (1 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, premium color and form, very marketable). Plant height was measured, and above-ground shoots and below-ground roots were harvested to determine dry weights. Shoots were shaken to remove any soil particles or other debris, then placed in paper bags in a forced-air oven maintained at 65 °C. Roots were washed over aluminum screening [mesh size 18 × 16, aperture 0.0445 × 0.0515 inch (Insect Screen; ADFORS Saint-Gobain, Grand Island, NY)] attached to a frame constructed from 2 × 6-inch lumber and air-dried for about 4 h. Air-dried washed roots were examined and any remaining debris was removed by hand before the root material was transferred to paper bags and placed in the forced-air oven at 65 °C for a minimum of 1 week.

Data analysis. Statistical analyses were similar to those reported in Gettys and Moore (2018) using generalized linear model and Proc Mixed (SAS version 9.4; SAS Institute, Cary, NC). We focused our analysis on plant response to substrate, irrigation, and their interaction. Data were analyzed for each species separately. The ultimate goal of these analyses was to determine the importance of interactions between the main effects because strong interactions indicate that optimal growing conditions are best characterized by specific combinations of the effects.

In addition, visual quality, plant height, and dry weights (shoot, root, and total) were ranked from "best" to "worst" within each species. Overall rankings were calculated as the means of these five values, which were used to identify the substrate and irrigation combination that resulted in best growth of each littoral plant evaluated in these experiments. Overall rankings within each species were analyzed using analysis of variance in SAS (version 9.4) with post-hoc analysis using Tukey's test and honestly significant difference separation of means.

Results and discussion

Irrigation main effect was significant ($P < 0.01$) for every response variable measured on all species except blue-eyed grass, where only plant height was affected (Table 1). Overhead irrigated blue-eyed grass height was greater than for subirrigated plants while other growth and quality variables were similar. Growth and quality of arrow arum, golden club, and lemon bacopa, averaged across the four substrates, was greater in subirrigated containers than overhead irrigated containers.

Substrate main effect was significant ($P < 0.01$) for all response variables measured on all species except visual quality of lemon bacopa, which was greatest with topsoil (Table 1). Growth and quality of arrow arum, blue-eyed grass, and golden club averaged across irrigation was greater in containers with potting substrate or sand.

As mentioned before, interaction means (irrigation and substrate) provided the most informative assessment of the response variables; the following discusses results for each species in detail.

Arrow arum. Overhead irrigation did not affect plant performance in any of the substrates evaluated [$P > 0.05$ (Table 1)]. Growth (measured as shoot, root, and total dry biomass) was consistently highest in subirrigated plants grown in potting substrate or sand and lowest in overhead irrigated plants grown in the mix or sand. For example, shoots, roots, and total dry weights of plants grown in sand with subirrigation were 5.1, 7.8, and 12.9 g, respectively, whereas plants grown in the same substrate with overhead irrigation produced shoots, roots, and total biomass that weighed 0.2, 1.9, and 2.1 g, respectively (Table 1).

Blue-eyed grass. Visual quality was the only response variable evaluated that differed among substrates exposed to overhead irrigation [$P = 0.037$ (Table 1)]. Quality ratings were higher for overhead irrigated blue-eyed grass plants grown in potting substrate or topsoil than overhead irrigated sand or mix. However, best quality was achieved with subirrigated plants grown in sand or potting substrate ($P < 0.001$). Blue-eyed grass plants were larger in subirrigated substrates than in overhead irrigated substrates. For example, subirrigated plants grown in sand attained greater shoot dry weights ($P < 0.001$), root dry weights ($P < 0.01$), and total dry weights ($P < 0.001$) than overhead irrigated plants grown in sand (Table 1).

Golden club. Overhead irrigation did not affect plant performance in any of the substrates evaluated [$P > 0.05$ (Table 1)]; in contrast, subirrigation did influence plant performance. Subirrigated golden club plants grew best in potting substrate; plants cultured in this way had the greatest visual quality ($P < 0.01$), height ($P < 0.01$), shoot dry weight ($P < 0.01$), root dry weight ($P < 0.001$), and total dry weight ($P < 0.001$) (Table 1). Growth was consistently higher in subirrigated plants grown in potting substrate or sand.

Lemon bacopa. Irrigation type did not impact plant performance in any of the substrates evaluated [$P > 0.05$ (Table 1)], but growth measurements were consistently higher in

Table 1. Interactive effect of substrate type × irrigation scheme on quality, height, and dry weight means of four littoral plants after 16 weeks of culture in substrate amended with 2 g·L^{-1} (0.27 oz/gal) of controlled-release fertilizer. Values are the mean of four replications and are followed by the value's ranking within the eight treatment combinations in parentheses.

Substrate[z]	Irrigation[y]	Quality [1–10[x] (rank)[w]]	Ht [cm[v] (rank)]	Shoot wt [g[v] (rank)]	Root wt [g (rank)]	Total wt [g (rank)]	Overall ranking[w]
		mean ± SD					
Arrow arum							
Potting	Sub	9.5 + 0.6 (2)	34.0 + 0.7 (1)	6.5 + 2.1 (1)	8.9 + 0.8 (1)	15.4 + 1.9 (1)	1.2[a]
Top	Sub	5.5 + 0.6 (4.5*)	24.0 + 1.9 (4)	1.1 + 0.5 (4)	2.8 + 2.1 (4)	3.9 + 2.5 (4)	4.1[cd]
Sand	Sub	9.8 + 0.5 (1)	32.9 + 2.0 (2)	5.1 + 1.2 (2)	7.8 + 3.8 (2)	12.9 + 2.6 (2)	1.8[ab]
Mix	Sub	7.8 + 1.3 (3)	26.0 + 4.1 (3)	1.5 + 0.7 (3)	4.4 + 1.9 (3)	5.9 + 2.6 (3)	3.0[bc]
Potting	Over	4.3 + 1.3 (7)	18.6 + 7.0 (5)	0.6 + 0.8 (5)	2.6 + 2.8 (5)	3.2 + 3.6 (5)	5.4[de]
Top	Over	4.5 + 0.6 (6)	16.9 + 1.0 (7)	0.2 + 0.1 (6.5*)	2.0 + 0.8 (6)	2.2 + 0.8 (6)	6.3[ef]
Sand	Over	3.8 + 1.5 (8)	13.5 + 6.5 (8)	0.2 + 0.2 (6.5*)	1.9 + 0.9 (7)	2.1 + 0.8 (7.5*)	7.4[f]
Mix	Over	5.5 + 1.0 (4.5*)	17.1 + 3.3 (6)	0.4 + 0.2 (8)	1.7 + 0.7 (8)	2.1 + 0.9 (7.5*)	6.8[ef]
Blue-eyed grass							
Potting	Sub	8.8 + 0.5 (1)	26.3 + 5.1 (2.5*)	5.4 + 1.9 (3)	3.2 + 1.0 (2)	8.7 + 2.9 (3)	2.3[a]
Top	Sub	3.8 + 1.3 (8)	15.6 + 3.5 (7)	0.5 + 0.3 (8)	0.5 + 0.4 (7.5*)	1.1 + 0.7 (7.5*)	7.6[d]
Sand	Sub	7.3 + 0.5 (2)	22.5 + 2.0 (6)	6.1 + 2.9 (1)	3.7 + 1.7 (1)	9.8 + 4.6 (1)	2.2[a]
Mix	Sub	4.0 + 1.4 (7)	14.5 + 3.0 (8)	0.6 + 0.8 (7)	0.5 + 0.6 (7.5*)	1.1 + 1.4 (7.5*)	7.4[cd]
Potting	Over	6.8 + 0.5 (3.5*)	23.4 + 1.1 (4)	4.7 + 1.9 (4)	2.3 + 1.0 (4)	7.0 + 2.8 (4)	3.9[ab]
Top	Over	6.8 + 0.5 (3.5*)	27.6 + 3.4 (1)	6.0 + 2.1 (2)	2.9 + 1.2 (3)	8.9 + 3.3 (2)	2.3[a]
Sand	Over	5.5 + 1.0 (6)	22.8 + 3.0 (5)	3.6 + 2.1 (5)	2.0 + 1.6 (5)	5.6 + 3.7 (5)	5.2[bc]
Mix	Over	5.8 + 0.5 (5)	26.3 + 3.5 (2.5*)	2.5 + 0.5 (6)	1.1 + 0.4 (6)	3.6 + 0.9 (6)	5.1[b]
Golden club							
Potting	Sub	9.3 + 1.5 (1)	18.6 + 4.7 (1)	1.6 + 0.5 (1)	4.9 + 1.1 (1)	6.6 + 1.5 (1)	1[a]
Top	Sub	6.3 + 0.5 (3)	11.0 + 3.1 (5)	0.6 + 0.1 (3)	2.5 + 0.4 (2)	3.1 + 0.5 (2)	3[bc]
Sand	Sub	7.0 + 4.1 (2)	12.8 + 9.0 (2)	0.9 + 0.8 (2)	1.9 + 1.6 (3)	2.8 + 2.4 (3)	2.4[ab]
Mix	Sub	4.8 + 2.2 (4)	8.4 + 3.4 (4)	0.4 + 0.4 (4)	1.3 + 0.8 (4)	1.7 + 1.2 (4)	4[c]
Potting	Over	2.8 + 1.0 (8)	4.0 + 2.4 (8)	0.1 + 0.1 (8)	1.1 + 0.7 (7)	1.3 + 0.8 (7.5)	7.7[d]
Top	Over	3.0 + 0.8 (7)	7.3 + 1.3 (6)	0.2 + 0.1 (7)	1.2 + 0.5 (5.5)	1.4 + 0.5 (6)	6.3[de]
Sand	Over	3.8 + 1.0 (5)	6.4 + 2.5 (7)	0.3 + 0.3 (5.5)	1.2 + 0.8 (5.5)	1.5 + 1.1 (5)	5.6[d]
Mix	Over	3.5 + 0.6 (6)	7.6 + 2.1 (5)	0.3 + 0.1 (5.5)	1.0 + 0.2 (8)	1.3 + 0.2 (7.5)	6.4[de]
Lemon bacopa							
Potting	Sub	6.0 + 0.8 (2.5*)	16.6 + 2.5 (3)	2.7 + 0.7 (2)	0.6 + 0.2 (2)	3.3 + 0.8 (2)	2.3[ab]
Top	Sub	8.0 + 0.8 (1)	18.4 + 2.8 (2)	2.8 + 0.8 (1)	0.7 + 0.2 (1)	3.6 + 1.0 (1)	1.2[a]
Sand	Sub	5.0 + 2.2 (4)	22.0 + 8.2 (1)	2.0 + 1.2 (3.5*)	0.4 + 0.2 (4)	2.4 + 1.4 (4)	3.3[b]
Mix	Sub	6.0 + 2.2 (2.5*)	13.9 + 4.3 (4)	2.0 + 1.3 (3.5*)	0.5 + 0.3 (3)	2.5 + 1.6 (3)	3.2[b]
Potting	Over	2.8 + 1.0 (8)	7.1 + 2.2 (8)	0.5 + 0.3 (6*)	0.1 + 0.1 (7.5*)	0.6 + 0.3 (7)	7.3[d]
Top	Over	3.5 + 0.6 (7)	8.8 + 1.7 (6)	0.4 + 0.1 (8)	0.1 + 0.1 (7.5*)	0.5 + 0.1 (8)	7.3[d]
Sand	Over	4.0 + 1.4 (5.5*)	10.3 + 3.0 (5)	0.5 + 0.2 (6*)	0.2 + 0.1 (5.5*)	0.7 + 0.3 (5.5*)	5.5[cd]
Mix	Over	4.0 + 1.7 (5.5*)	7.8 + 3.6 (7)	0.5 + 0.4 (6*)	0.2 + 0.2 (5.5*)	0.7 + 0.6 (5.5*)	5.9[c]

[z]Potting = 45% peat, 25% bark, 35% vermiculite (Fafard 4M Mix; Sun Gro Horticulture, Anderson SC); topsoil = regionally formulated mix of organic and mineral components comprising 35% to 50% organic matter (Timberline Top Soil; Oldcastle Lawn and Garden, Atlanta, GA); sand = sand (Multi-Purpose Sand; Sakrete, Charlotte, NC); mix = 50/50 (by volume) mix of topsoil and sand.

[y]Sub = subirrigation via flood trays maintained at a depth of 4 cm; over = 0.5 inch of overhead irrigation twice daily (1 inch/d); 1 inch = 2.54 cm.

[x]1 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, premium color and form, and very marketable.

[w]1 = best, 8 = worst, * = tied ranking; overall ranking = mean of rankings of the five characters evaluated for each treatment combination. Means followed by the same letters are not different at $\alpha = 0.05$ according to Tukey's honestly significant difference test.

[v]1 cm = 0.3937 inch, 1 g = 0.0353 oz.

subirrigated plants than in plants grown under overhead irrigation. For example, subirrigated lemon bacopa plants cultured in topsoil had the highest visual quality rating and mean dry weights, whereas overhead-irrigated plants grown in topsoil had the lowest overall ranking and dry weights (Table 1).

The goal of these experiments was to determine whether we could use traditional greenhouse production techniques to grow littoral aquatic plant species to meet market needs for these plants without costly infrastructure upgrades. With the exception of visual quality of blue-eyed grass, overhead irrigation did not affect plant performance in any of the substrates evaluated, and there was no difference in growth of arrow arum, blue-eyed grass, golden club, or lemon bacopa among the substrates when plants were watered using overhead irrigation. These results suggest that when traditional overhead irrigation is used to produce these plants in a greenhouse setting, any of the substrates evaluated in these experiments should support good growth.

All four species grew better when subirrigated than when watered overhead, and substrate choice did influence performance when plants were subirrigated. For example, growth measurements of subirrigated arrow arum, blue-eyed grass, and golden club were greatest in potting substrate or sand, whereas growth and quality of subirrigated lemon bacopa were best when plants were cultured in topsoil or potting substrate. These results are similar to those of Gettys and Moore (2018), who reported high-quality values (6.3 or higher on the same 10-point scale used in these experiments) when broadleaf sagittaria (*Sagittaria latifolia*), cardinal flower (*Lobelia cardinalis*), and skyflower (*Hydrolea corymbosa*) were grown in subirrigated potting soil or sand.

The finding that these species performed best under subirrigated conditions was not unexpected because early research on the culture of littoral and obligate wetland species such as pickerelweed (*Pontederia cordata*), water snowflake (*Nymphoides indica*), and broadleaf sagittaria revealed that best growth was achieved in flooded sand (Sutton, 1991, 1994, 1995). Also, growth of southern naiad (*Najas guadalupensis*), a submersed aquatic species, was greater in 100% sand substrates than in 100% peat substrates (Hasandras et al., 2018). Because sand substrates tend to have lower water holding capacity (Poole et al., 1981), they are best used in aquatic plant production under flooded or subirrigated settings. Peat-based substrates commonly used in the greenhouse industry tend to have high water holding and cation exchange capacities, which also increases nutrient-holding capacity (Poole et al., 1981). In our study, all four species performed well when grown in subirrigated potting substrate comprising 45% Canadian sphagnum peat, 25% bark, and, 35% vermiculite. In fact, this treatment was responsible for the highest (arrow arum, golden club) or second-highest (blue-eyed grass, lemon bacopa) overall rankings attained in these experiments (Table 1).

The benefits of employing culture conditions that use peat-rich potting substrate and subirrigation are not limited to littoral zone plants. For example, subirrigated pentas (*Pentas lanceolata*), crossandra (*Crossandra infundibuliformis*), and philodendron (*Philodendron* 'Hope') were larger when grown in a peat/perlite/vermiculite substrate [75% to 85% Canadian sphagnum peat, 15% to 25% perlite and vermiculite (Pro-Mix BX; Premier Tech Horticulture, Quakertown, PA)] vs. plants that were cultured in a bark/vermiculite/peat substrate [40% to 50% composted pine bark, 20% to 35% vermiculite, 12% to 22% Canadian sphagnum peat (Metro-Mix 500; Scotts Co., Marysville, OH)] (Klock-Moore and Broschat, 2001). Most subirrigation substrates tend to be fine textured with abundant micropores (Biernbaum, 1993) because coarse-textured soils with 40% or more bark often do not saturate efficiently due to a lack of sufficient small pore spaces to facilitate capillary water uptake (Biernbaum, 1993; Newman, 1999).

These experiments reveal that good quality and growth of these littoral zone plants can be accomplished using standard commercially available containers, substrates, controlled-release fertilizer, and inexpensive flood trays that are easily constructed to provide subirrigation. This confirms previous reports that some wetland species, including swamp rosemallow (*Hibiscus grandiflorus*), pickerelweed, pond apple (*Annona glabra*), skyflower, cardinal flower, broadleaf sagittaria, and swamp lily (*Crinum americanum*), are easily cultured under greenhouse conditions (Gettys and Moore, 2018; Gettys and Sutton, 1999, 2001; Gettys et al., 2001, 2013). Although there was no "one size fits all" method for optimal culture of all wetland species, greenhouse production of these perennials should be fairly straightforward without significant modifications or changes to existing infrastructure. It would be wise for growers to evaluate production methods on a species-by-species trial before gearing up for large-scale greenhouse production of wetland plants.

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