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Effects of substrate nutrients on growth of three submersed aquatic plants

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ABSTRACT

Nutrient studies with submersed plants were conducted on select invasive species in the 1970s and 1980s, but little research has been reported in this area in recent years. Additionally, there is a dearth of substrate and nutrient experiments that focus on native submersed plants, but these studies are needed to identify locations where these species may successfully be introduced as a component of aquatic restoration projects. These experiments were designed to obtain a better understanding of the effects of substrate nutrients on growth of submersed plants. There was no difference in growth of Illinois pondweed (*Potamogeton illinoensis* Morong) and hydrilla [*Hydrilla verticillata* (L.f.) Royle] during an 11-wk study that evaluated the effects of controlled-release fertilizer with and without micronutrients. Other experiments compared the growth of Illinois pondweed, hydrilla, and southern naiad [*Najas guadalupensis* (Spreng.) Magnus] when plants were cultured with water-soluble fertilizers mixed in sand to simulate controlled-release fertilizers. Those studies revealed that optimum growth was achieved by Illinois pondweed at 25 to 150 mg nitrogen per kilogram of sand, by southern naiad at 75 mg nitrogen per kilogram of sand, and by hydrilla at 150 mg nitrogen per kilogram of sand. Growth of all three species decreased at substrate nitrogen concentrations of 450 to 500 mg nitrogen per kilogram of sand. Substrate phosphorus concentrations that produced optimum growth of southern naiad and Illinois pondweed were $\geq$ 150 mg phosphorus per kilogram of sand, while maximum hydrilla growth was attained at 200 to 350 mg phosphorus per kilogram of sand. Illinois pondweed was not affected by the addition of potassium to the substrates until $\geq$ 500 mg potassium per kilogram of sand. At low nitrogen concentrations, Illinois pondweed growth was greatest with urea as the nitrogen source, followed by ammonium and nitrate. Highest biomass was produced when plants were fertilized with ammonium at total nitrogen concentrations of 100 and 400 mg nitrogen per kilogram of sand, compared to the other nitrogen sources.

Key words: controlled-release fertilizer, *Hydrilla verticillata*, Illinois pondweed, *Najas guadalupensis*, nitrogen, phosphorus, *Potamogeton illinoensis*, potassium, sand, southern naiad

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INTRODUCTION

Nutrient uptake from the substrate by submersed plants was reported as ambiguous by Sculthorpe (1967) and later by Sutton (1985). In general, it is thought that rooted submersed aquatic macrophytes obtain some nutrients from the substrate, where nutrient concentrations are usually much greater than in the overlying water. However, when nutrient concentrations in the water column are greater than in the substrate, plants can utilize nutrients from the water column via foliar uptake (Wetzel 1976). There is debate in the literature about which nutrients are taken up from substrate, water, or both, and the mechanisms responsible for nutrient uptake can vary among species. Nutrient requirements of submersed aquatic plants have been evaluated only on a few species, and there is a dearth of information regarding the nutrient requirements of Illinois pondweed and southern naiad, which are common submersed species in southern waterways.

Sutton (1985) reported that hydrilla [*Hydrilla verticillata* (L.f.) Royle] growth increased in response to increased substrate phosphorus, but the species obtained other nutrients (nitrogen, potassium, and micronutrients) from the water column. Barko (1982) also concluded that water is the primary source of potassium for hydrilla growth. In contrast, Barko and Smart (1981) reported that optimum growth of eelgrass (*Vallisneria americana* Michx.) required the presence of nitrogen and phosphorus in the root zone, and Zaki et al. (2015) stated that submersed macrophytes rely on substrates for nitrogen, phosphorus, and micronutrients. Nitrogen requirements of Eurasian watermilfoil (*Myriophyllum spicatum* L.) are met by a combination of root uptake from the substrate and absorption from the water column through stem and leaf tissue (Best and Mantai 1978, Nichols and Keeney 1976). Smith and Adams (1986) reported that Eurasian watermilfoil absorbs most of its phosphorus from the substrate, but can also obtain phosphorus through foliar uptake directly from the water column. However, Best and Mantai (1978) reported contradictory findings and stated that Eurasian watermilfoil absorbs phosphorus only from the substrate, not the water. In a survey of 10 temperate lakes, Anderson and Kalff (1986) reported that submersed aquatic plant growth was positively correlated with potassium content in the substrate. The roots of submersed plants are greatly reduced compared to the roots of terrestrial plants and their role in nutrient uptake from lake substrates is not well-understood (Sculthorpe 1967). This uncertainty creates a need for additional research to evaluate separate nitrogen, phosphorus, and potassium fertilizers to determine which substrate-borne nutrients limit growth of submersed aquatic plants.

It is common practice to add a controlled-release fertilizer to substrates for submersed plant studies (Mudge 2013). Controlled-release fertilizers usually comprise a nutrient core encased in a coating that controls the release of the nutrients. In contrast, water-soluble fertilizers quickly release nutrients, making them immediately available to support plant growth (Liu et al. 2014). There are no commercially available controlled-release fertilizers that contain only phosphorus. However, there are separate nitrogen, phosphorus, and potassium fertilizers available without controlled-release coatings. These products may be useful for determining the growth-limiting effects of these nutrients, but little information is available on their release rates from natural or artificial substrates into the overlying water column. If these water-soluble salts leach out of the substrate over a short period of time (hours to days), then long-term studies may not be possible with these individual nitrogen, phosphorus, and potassium sources.

Sutton (1993) evaluated hydrilla growth in 20-cm-deep columns with controlled-release and water-soluble fertilizers placed in a layer 7.6 cm below the surface of the sand. Hydrilla biomass did not differ among treatments after 8 wk of growth, suggesting that water-soluble fertilizer placed in sand can provide nutrients over time (similar to coated controlled-release fertilizers) to support submersed plant growth. Depth of fertilizer placement can also affect hydrilla growth (Sutton 1985). Controlled-release fertilizer was thoroughly mixed in sand or applied in layers at 3 cm, 9 cm (middle), or 18 cm (bottom) below the sand surface in 18-cm-deep pots. Hydrilla growth was equal in two out of three repeated studies when fertilizer was either mixed or placed 3 cm below the sand surface.

In a study comparing nitrogen sources (nitrate, ammonium, and urea), waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] growth was greatest 4 wk after nitrogen (as ammonium carbonate) was applied (Shiralipour et al. 1981). Foliar uptake of ammonium by Eurasian watermilfoil was several times more rapid than uptake of nitrate when both forms of nitrogen were present in the water (Nichols and Keeney 1976).

There were four objectives in these studies. The first objective was to determine the release rate of nitrogen, phosphorus, and potassium from noncoated, water-soluble fertilizers incorporated into a sand substrate. The second goal was to evaluate the growth of Illinois pondweed and hydrilla planted in sand amended with controlled-release fertilizer with and without micronutrients. The third objective was to compare the effects of nitrogen, phosphorus, and potassium concentrations in substrates on growth of three submersed aquatic plants, and the fourth goal was to compare the effects of nitrate, ammonium, and urea on growth of Illinois pondweed.

MATERIALS AND METHODS

Nutrient release from sand substrate

Because controlled-release fertilizers containing only phosphorus are not available, we hypothesized that the incorporation of water-soluble fertilizer containing only nitrogen, phosphorus, or potassium into the sand could slow the movement of these ions into the water column, thus providing longer-term release of nutrients to the root zone, as suggested by Sutton (1993). Water-soluble fertilizers containing only nitrogen, phosphorus, or potassium used in these studies were Hi-Yield®[1] 16–0–0 sodium nitrate ($NaNO_3$), Hi-Yield® 0-18-0 superphosphate [$Ca(H_2PO_4)_2$] and muriate of potash[2] 0–0–62 potassium chloride (KCl). The sand used in these studies was washed masonry sand with the following particle size distribution (USDA and Soil Conservation Service 1987): 6.8% very coarse, 30.8% coarse, 44.8% medium, 17.4% fine, and 0.2% very fine. Analyses by the University of Florida IFAS Analytical Services Laboratories (Gainesville, FL) revealed that the sand used in these experiments had 3.9 mg kg^{-1} total phosphorus and a pH of 7.6; the sand contained no organic matter or nitrogen.

These experiments were conducted under greenhouse conditions at the University of Florida IFAS Center for Aquatic and Invasive Plants in Gainesville, FL. The release of inorganic salts from nitrogen, phosphorus, and potassium fertilizers over 126 d was determined in 95-L mesocosms that were filled with well water and covered with black plastic to exclude light. Each fertilizer type (nitrogen, phosphorus, or potassium) was tested individually by adding 70 g of product to the containers. Factors evaluated in these experiments were nutrient placement (fertilizer in sand vs. in the water column) and water movement (aeration vs. static). Fertilizer-only treatments were established by uniformly distributing 70 g of fertilizer across the surface of the mesocosm. In treatments that required sand plus fertilizer, plastic containers (40 by 30 by 15 cm deep; volume 11.3 L) were filled to a depth of 10 cm with well-blended mixture of sand and fertilizer. A 5-cm-deep cap of unfertilized sand was then added to cover the fertilized sand. A sand-only treatment (containers described above and filled to a depth of 15 cm) was included as a control to verify that no salts were released from the unfertilized sand. In addition, a "blank" (no container of sand) treatment consisting of a mesocosm with well water only was evaluated. Water movement in treatments calling for aeration was accomplished by using standard aquarium aerators and air stones set to deliver 120 mL of air per minute. Four replicates were prepared for each combination (fertilizer type by nutrient placement by water movement) of treatments.

Conductivity readings were performed at regular intervals after fertilizer treatment to determine nutrient release characteristics. Conductivity (µS/cm) was measured with a Mettler-Toledo AG®[3] conductivity meter over the course of the 126-d study. The time required for nutrient release was recorded and these data were subjected to analysis of variance (ANOVA) and nonlinear regression [exponential decay; $y=(b0)e–(b1)x$], using SAS software.[4] Regression model components were used to calculate ET_{10}, ET_{50}, and ET_{90} values—the time required for 10, 50, and 90% of the nutrients to be released into the water, respectively—for each treatment, along with 95% confidence intervals.

Substrate micronutrients

The influence of micronutrients in sand on growth of Illinois pondweed and hydrilla was compared in ponds in Palm Beach County, FL, by measuring plant response to fertilizer containing nitrogen, phosphorus, potassium, and micronutrients vs. fertilizer containing only nitrogen, phosphorus, and potassium. Fertilizers were incorporated into South Florida sand and placed into containers as described in the previous trial. The South Florida sand had the following particle size distribution (USDA and Soil Conservation Service 1987): 6.7% very coarse, 5.6% coarse, 23.4% medium, 60.1% fine, 4.1% very fine sand and 0.1% silt/clay. The sand had 2.2 mg kg^{-1} total phosphorus and a pH of 8.8, but contained no organic matter or nitrogen. The fertilizers were uniformly mixed into the lower 10 cm of sand and an additional 5-cm-deep layer of unfertilized sand was placed over the fertilized sand. Treatments without micronutrients were amended with 18N-6P_2O_5-12K_2O Osmocote®[5] Classic at 40, 120, and 200 mg phosphorus per kilogram of sand, whereas treatments with micronutrients were amended with 15N–9P_2O_5–12K_2O Osmocote® Plus (which includes micronutrients) at the same phosphorus levels.

Five 15-cm-long apical tips of Illinois pondweed or hydrilla were planted in each container, with four replicates of each treatment placed in a completely randomized design. Planted containers were placed on groundcloth in a 0.25-ha South Florida Water Management District (SFWMD) pond in February 2015. The pond used for these nutrient studies is located in Stormwater Treatment Area (STA) 1 West and is described in detail by Guardo et al. (1995). This flow-through pond, which has a hard limerock substrate, received water typically containing 100 to 150 ppb total phosphorus and 500 to 1,000 ppb total nitrogen. The pond was drained several weeks prior to setting up the experiment to facilitate desiccation of existing submersed vegetation, and herbicide treatments were applied to emergent species to minimize interference with these studies. Plants were allowed to grow for 11 wk after planting and water depth was maintained at 1 m with water exchange 2 to 3 times per week. At the end of the culture period, plant material was harvested by removing all shoots from each pan and washing them with water to remove sand and debris. Harvested material was dried in a forced-air oven at 80°C until a constant weight was achieved and then weighed. Treatments means were separated with the use of ANOVA and Tukey's honestly significant differences (HSD) in SAS.

Substrate nitrogen and phosphorus

The influence of nitrogen and phosphorus in the root zone on growth of Illinois pondweed, southern naiad and hydrilla was also studied in the 0.25-hectare SFWMD ponds described above. Plants were grown in containers similar to those used in the nutrient release and micronutrient studies, and fertilizer treatments were incorporated into South Florida sand using the methods described above. Each container was planted with 5 (Illinois pondweed or hydrilla) or 10 (southern naiad) 15-cm-long apical tips. Six replicates were prepared for each treatment and all were placed in a completely randomized design. These 10-wk-long experiments were conducted during Spring 2016.

Illinois pondweed and southern naiad were grown with seven concentrations of nitrogen provided by sodium nitrate (16–0–0) at 0, 25, 75, 150, 250, 450, and 700 mg nitrogen per kilogram of sand. Preliminary hydrilla growth studies performed in Fall 2014 revealed that hydrilla utilized nitrogen and phosphorus at higher concentrations than did Illinois pondweed or southern naiad (data not shown). Consequently, nitrogen concentrations in the hydrilla studies were increased to 0, 50, 150, 300, 500, 1,000, and 1,500 mg nitrogen per kilogram of sand. Superphosphate (0–18–0) was included in all treatments at a uniform concentration of 75 mg phosphorus per kilogram of sand, but potassium and micronutrients were not added to the sand substrate.

Similar studies were conducted on the growth of these three submersed species with seven concentrations of phosphorus in Spring 2016. Phosphorus treatments were 0, 15, 45, 150, 300, 500, and 800 mg phosphorus per kilogram of sand (Illinois pondweed and southern naiad) or 0, 45, 100, 200, 350, 700, and 1,000 mg phosphorus per kilogram of sand (hydrilla). Sodium nitrate was added to all treatments at a constant concentration of 160 mg nitrogen per kilogram of sand.

As in the previous trial, harvested material was cleaned, dried in a forced-air oven at 80°C until a constant weight was achieved, and then weighed. Treatment means were separated with the use of ANOVA and Tukey's honestly significant differences (HSD) in SAS.

Substrate potassium

The effect of substrate potassium on growth of Illinois pondweed was evaluated in Gainesville, FL, with the use of the masonry sand described in the nutrient release experiments. Treatments were 0, 250, 500, 1,000, 2,000, and 4,000 mg potassium per kilogram of sand, with potassium supplied as KCl. Nitrogen and phosphorus were provided by sodium nitrate and superphosphate, respectively, at 75 mg nitrogen per kilogram of sand and 125 mg phosphorus per kilogram of sand. Fertilizer treatments were incorporated into the lower 10 cm of sand and capped with a 3-cm-deep layer of unfertilized sand. Three 15-cm-long apical shoots of Illinois pondweed were planted in each 3-L (19.5-cm diameter by 13 cm deep) plastic container without holes. Each treatment was replicated six times and all pots were placed into a 2.5-m diameter mesocosm in a completely randomized design. Water depth was maintained at 0.75 m and mesocosm water was replaced with a complete well-water exchange every 2 wk. After 10 wk of growth, plants were cleaned, dried, and weighed, and data were analyzed as described in previous experiments.

Nitrogen source

During Summer 2016, growth of Illinois pondweed in response to three sources of substrate nitrogen (16–0–0 sodium nitrate, ammonium sulfate, and Howard's[6] urea) was evaluated. Nitrogen was provided at low (15 mg nitrogen per kilogram of sand), medium (100 mg nitrogen per kilogram of sand), and high (400 mg nitrogen per kilogram of sand) concentrations, and all source by rate combinations were replicated six times. Phosphorus (as superphosphate) was added to all treatments at 200 mg phosphorus per kilogram of sand. Treatments were prepared in 3-L containers without holes as described in the potassium experiments. Containers were placed in a 15 × 3–m rectangular mesocosm filled with well water maintained at a depth of 0.6 m. After 8 weeks of growth, plants were cleaned, dried, and weighed, and data were analyzed as described in previous experiments.

RESULTS AND DISCUSSION

Nutrient release from sand substrate

The goal of this study was to determine whether incorporating water-soluble nitrogen, phosphorus, or potassium fertilizer into a bottom sand layer that was covered with a 5-cm-deep layer of unfertilized sand slowed the movement of these ions into the water column. Visual observations suggested that the addition of nitrogen (as sodium nitrate) spurred bacterial growth during this 18-wk study. Ferrophagous bacteria present in the well water used in these experiments (Larry Tolbert, UF/IFAS, pers. comm.) likely consumed some of the nitrate ions and resulted in lowered water conductivity readings. Therefore, the nitrogen-release results in this study were highly variable and it was not possible to draw conclusions regarding nitrogen release over time. Similarly, phosphorus additions (as superphosphate) caused visible precipitates to form in the water column, likely because of calcium and magnesium ions in the well water. This also lowered water conductivity over time. When phosphorus is added to a calcareous system, it undergoes a series of chemical reactions with calcium, which decreases phosphorus solubility through phosphorus fixation (Zekri et al. 2016). These complications caused inconsistent results in both the nitrogen and phosphorus release studies; therefore, it was not possible to determine nutrient release over time accurately based on these studies (data not shown).

Potassium fertilizers are less affected by soil or water quality (Buckman and Brady 1960) than either nitrogen or phosphorus fertilizers. Potassium did not cause bacteria growth, form precipitates, or undergo other chemical or biological reactions that could have affected the conductivity of the overlying water. Thus, potassium release data were much more consistent and allowed more accurate evaluation of ion release. Potassium release was greatly reduced and ET_{10}, ET_{50}, and ET_{90} values were higher when KCl was incorporated into the lower layer of sand and overlaid with a 5-cm-deep cap of unfertilized sand compared to release when KCl was added to the water without sand (Table 1). For example, under static conditions the ET_{90} for KCl alone (without sand) was 76 d, whereas KCl incorporated into the sand required nearly twice as long—134 days—to release 90% of the applied potassium (Table 1). In aerated treatments, KCl alone achieved 90% release of potassium into the water only 1 d after application, but when KCl was incorporated in sand, 90% release of applied potassium required 145 days. Aeration greatly reduced the ET_{90} of KCl alone (from 76 to 1 d), but did not affect potassium release from KCl incorporated into the sand. These results suggest that water flow in pond studies is unlikely to affect potassium release from experimental containers if potassium is incorporated into the substrate.

Table 1. Estimated time (days) for release of KCl into the water column of a 95-L mesocosm under static or aerated conditions. The KCl-alone treatments utilized KCl placed directly in the water column, whereas the KCl -in-sand treatments had KCl incorporated into the bottom 10 cm of masonry sand in plastic containers and covered with a 5-cm layer of unfertilized sand. ET_{10}, ET_{50}, and ET_{90}: estimated time (days) to release 10, 50, and 90%, respectively, of potassium. Values labeled with asterisks are significantly different as determined by 95% confidence intervals calculated with the use of components of nonlinear regression.

	KCl alone		KCl in sand	
	Static	Aerated	Static	Aerated
ET_{10}	12 (10–13)	1 (0–1)	23 (20–26)*	20 (17–22)*
ET_{50}	35 (32–38)	1 (0–1)	82 (54–110)*	53 (50–56)*
ET_{90}	76 (69–83)	1 (0–1)	134 (120–148)*	145 (128–162)*

The studies above were conducted to determine if noncoated, water-soluble fertilizers could be useful in aquatic plant research. Sutton (1985, 1993) reported that optimum growth occurred when controlled-release fertilizer was mixed in sand rather than being buried under 9 or 18 cm of sand, and he noted that there was no difference in hydrilla growth when plants were grown for 8 wk with controlled-release fertilizers or water-soluble fertilizers. These data support Sutton's findings and illustrate the effectiveness of incorporating fertilizer into sand to slow the release of noncoated water-soluble potassium from the root zone into the water column. The solubilities of sodium nitrate, superphosphate, and potassium chloride are 850, 18, and 360 g^{-1} L, respectively. If potassium mimics the release of nitrogen and phosphorus, and if nitrogen and phosphorus release is slowed by incorporation similar to potassium release, it is possible that these water-soluble fertilizers may provide the nutrients needed while conducting aquatic plant research, provided they are incorporated into the substrate and the duration of experiments is 18 wk or less. However, further research is needed to clarify the release of nitrogen and phosphorus fertilizers from sand substrates.

Substrate micronutrients

The goal of these experiments was to evaluate the growth of Illinois pondweed and hydrilla in sand substrates amended with controlled-release fertilizer containing or lacking micronutrients. When phosphorus concentration was held constant, the addition of micronutrients had no effect on either species over the 11-wk culture period (Figure 1). These results are consistent with those described by Sutton (1985), who suggested that hydrilla growth was influenced only by substrate phosphorus and not by substrate nitrogen or potassium. Sufficient quantities of micronutrients were present (either in the substrate or in

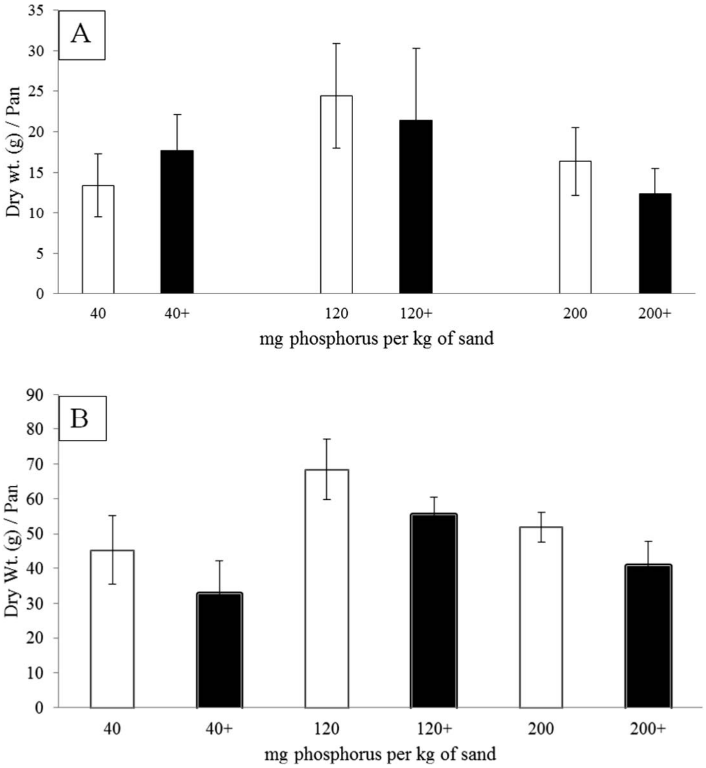

Figure 1. Mean dry weight (± 1 SD) of Illinois pondweed (A) and hydrilla (B) 10 wk after plants were fertilized with varying concentrations of phosphorus supplied as Osmocote® Plus (with micronutrients; black bars) or Osmocote® Classic (without micronutrients; white bars).

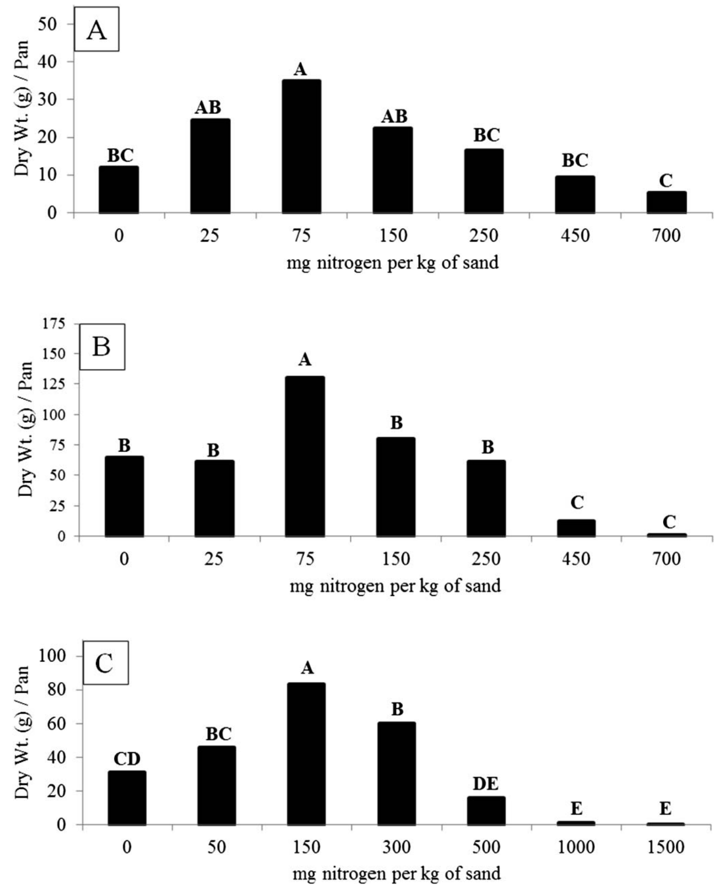

Figure 2. Mean dry weight of Illinois pondweed (A), southern naiad (B), and hydrilla (C) after 12 wk of growth in sand amended with varying concentrations of nitrogen. All treatments were supplemented with 75 mg phosphorus per kilogram of sand. Treatments labeled with the same letter are not significantly different within a species as determined by Tukey's honestly significant test at $P = 0.05$.

the water column) to foster normal growth of Illinois pondweed and hydrilla, so micronutrients were not included in the following studies to evaluate the effects of substrate nitrogen, phosphorus, and potassium on submersed plant growth.

Substrate nitrogen and phosphorus

These experiments were designed to test the effects of substrate nitrogen and phosphorus on growth of Illinois pondweed, southern naiad, and hydrilla in artificial ponds. Illinois pondweed produced the greatest amount of aboveground biomass when fertilized with 25 to 150 mg nitrogen per kilogram of sand. Hydrilla biomass increased with increasing nitrogen concentration until achieving maximum growth at 150 mg nitrogen per kilogram of sand (Figure 2). In contrast, southern naiad performed best when fertilized with 75 mg nitrogen per kilogram of sand. Nitrogen rates higher than the maxima listed above reduced growth of all three species. These data suggest that hydrilla growth is greatest at substrate nitrogen concentrations that are higher than those required for optimum southern naiad growth.

In these 12-wk nitrogen studies, southern naiad produced the most biomass, followed by hydrilla and then Illinois pondweed. Maximum biomass was 2 to 3 times greater than the sand-only control for all three species, but plants did grow in the absence of substrate nitrogen. This suggests these species may be able to partly fulfill their nitrogen requirements via absorption from the water column when grown in nitrogen-deficient substrate, similar to the phenomenon previously reported for Eurasian watermilfoil by Best and Mantai (1978) and Nichols and Keeney (1976).

All three species produced less biomass in the phosphorus studies compared to the nitrogen experiments. The reason for reduced growth in the Spring 2016 phosphorus study is not known, but may have been due to algal growth or reduced light penetration in the ponds, which could have hindered biomass production. The most aboveground biomass was produced by Illinois pondweed and southern naiad cultured with 150 to 800 mg phosphorus per kilogram of sand (Figure 3), and the addition of phosphorus above 150 mg phosphorus per kilogram of sand did not affect growth. Maximum dry weights of both species were similar (11 to 14 g per container). Hydrilla attained the highest aboveground biomass (25 to 35 g per container—two to three times that of Illinois pondweed and southern naiad) when cultured with 200 to 350 mg phosphorus per kilogram of sand, and concentrations greater than 700 mg phosphorus per kilogram of sand decreased hydrilla growth. Substrate phosphorus concentrations for optimum hydrilla

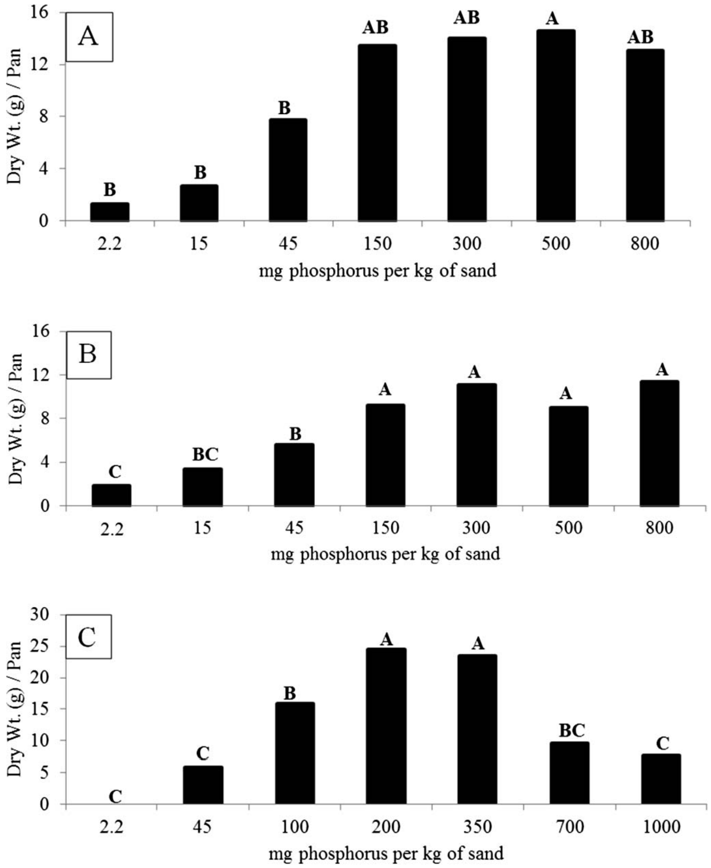

Figure 3. Mean dry weight of Illinois pondweed (A), southern naiad (B), and hydrilla (C) after 12 wk of growth in sand amended with varying concentrations of phosphorus. All treatments were supplemented with 160 mg of nitrogen per kilogram of sand. Treatments labeled with the same letter are not significantly different within a species as determined by the Tukey's HSD test at $P = 0.05$.

growth were greater in this study than those reported by Sutton (1985), but the highest phosphorus concentration evaluated by Sutton was 62 mg phosphorus per kilogram of sand.

Hydrilla growth was limited by the no-phosphorus-added control treatments. Although the South Florida sand used in these studies contained 2.2 mg phosphorus per kilogram of sand, no biomass was produced by hydrilla in this treatment. This is consistent with Sutton's observations (1985) that hydrilla grown with nitrogen, phosphorus, and potassium was only affected by substrate phosphorus concentration. Illinois pondweed and southern naiad produced biomass when grown in control substrates without added phosphorus, although growth was minimal. However, this suggests these species may tolerate very low substrate phosphorus concentrations or may be able to absorb phosphorus from the water column. Greatest biomass produced by Illinois pondweed, southern naiad, and hydrilla was approximately 15, 5, and 30 times greater, respectively, than the biomass produced by the same species subjected to the control treatment. All three species had a more profound response to substrate phosphorus than to substrate nitrogen (5 to 30 times greater biomass vs. 2 to 3 times greater biomass). This

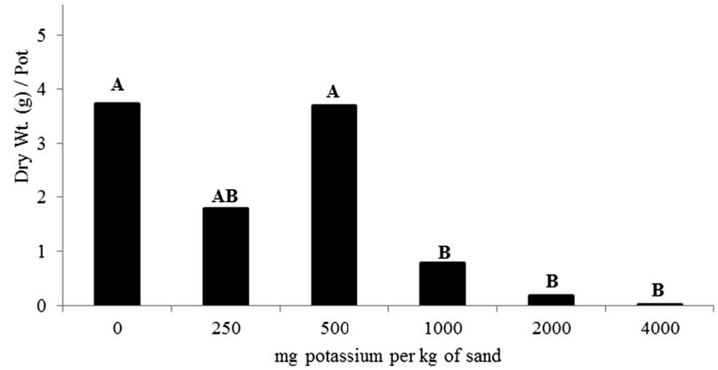

Figure 4. Mean dry weight of Illinois pondweed after 10 wk of growth in sand amended with varying concentrations of potassium. Treatments labeled with the same letter are not significantly different within a species as determined by the Tukey's HSD test at $P = 0.05$.

supports Sutton's 1985 findings that substrate phosphorus is more critical than substrate nitrogen, at least for the species examined in these studies. These experiments reveal that adding nitrogen and phosphorus to the substrate increased growth of the species tested. However, growth was reduced in all species cultured with nitrogen above a specific concentration and hydrilla growth was reduced when substrate phosphorus was greater than 700 mg phosphorus per kilogram of sand. Hydrilla was unable to grow in substrate without phosphorus, whereas all three species were able to produce limited growth in substrate without nitrogen.

Substrate potassium

Illinois pondweed growth was not affected by substrate potassium (Figure 4) when concentrations were 0 to 500 mg potassium per kilogram of sand. However, growth was depressed when potassium concentrations were 1,000 mg potassium per kilogram of sand or greater and growth was minimal at 4,000 mg potassium per kilogram of sand. Leaf tip browning and necrosis was evident at high substrate potassium concentrations and it is possible that root burning [as described by Jones (2003)] occurred, which may have caused decreased growth. Future studies to evaluate the effects of high salt (nutrient) concentrations on plant growth should include harvesting belowground roots in addition to aboveground shoots to determine if root systems are damaged by high nutrient concentrations. Illinois pondweed grew well in these studies even in substrates that were not amended with potassium, which lends support to previous reports that submersed macrophytes likely use potassium in the water column rather than potassium in the substrate (Sutton 1985, Zaki et al. 2015).

Nitrogen source

The objective of this experiment was to evaluate the effects of three sources of substrate nitrogen (all at 15, 100, and 400 mg nitrogen per kilogram of sand) on Illinois pondweed growth (Figure 5). When plants were cultured at the lowest nitrogen concentration (15 g nitrogen per

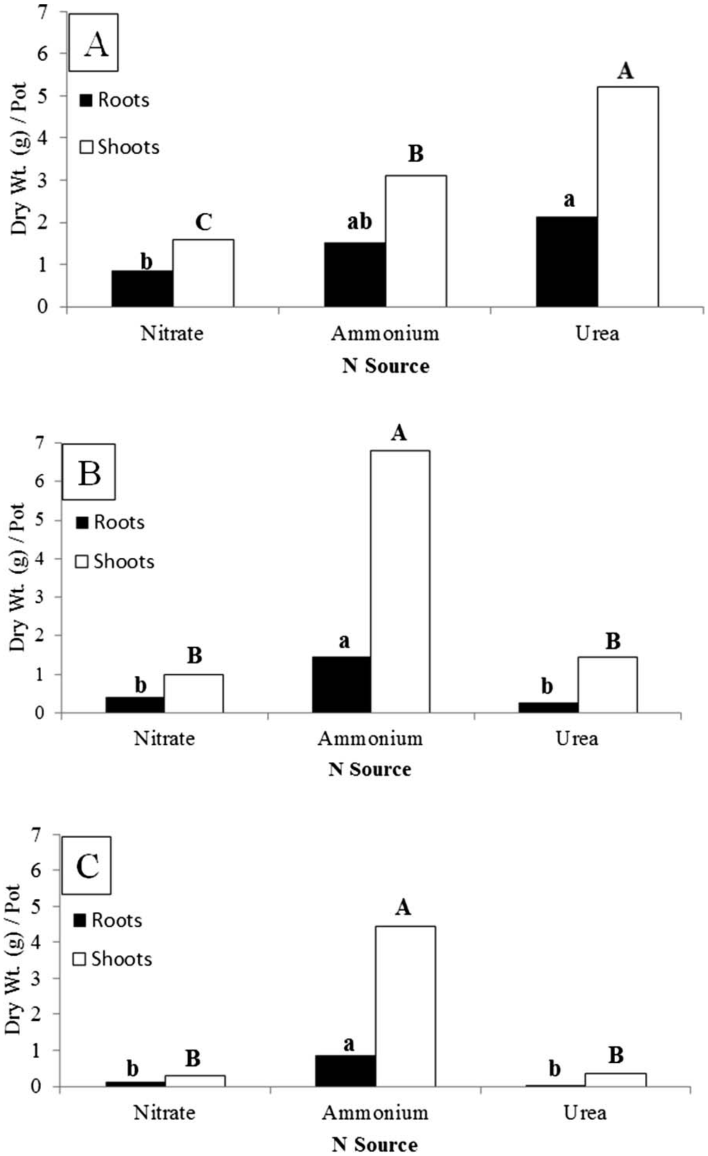

Figure 5. Dry weight of Illinois pondweed roots and shoots after 10 wk of growth in sand amended with nitrate, ammonium, or urea at concentrations of 15 (A), 100 (B), and 400 (C) mg nitrogen per kilogram of sand. All treatments were supplemented with 200 mg phosphorus per kilogram of sand. Treatments labeled with the same letter are not significantly different within a species as determined by the Tukey's HSD test at $P = 0.05$.

kilogram of sand), shoot growth was highest in plants grown with urea and lowest in plants grown with nitrate. In contrast, root and shoot biomass was greatest in the ammonium treatments when plants were cultured under medium and high substrate nitrogen concentrations. The reason for greater shoot growth in the 15 mg nitrogen per kilogram of sand amended with urea is unknown, but urea is rapidly converted to the ammonium ion by soil bacteria (Buckman and Brady 1960). If urea is converted to ammonium in aquatic substrates, this may have contributed to increased growth in the low-nitrogen-concentration urea treatments. However, this does not explain why ammonium in the low nitrogen treatments did not produce more growth. When plants were cultured under medium and high substrate nitrogen concentrations, root and shoot biomass of plants grown with ammonium was approximately two and four times greater than biomass produced by plants grown with nitrate or urea. Optimum growth of Illinois pondweed in the pond study (Figure 2) occurred at 75 mg nitrogen per kilogram of sand and growth was significantly reduced at 400 mg nitrogen per kilogram of sand. Similarly, growth was also reduced with the three sources of nitrogen at the highest nitrogen concentrations (Figure 5). In addition, Nichols and Keeney (1976) reported that foliar uptake by Eurasian watermilfoil of ammonium was much more rapid than the species' uptake of nitrate. The increased growth of Illinois pondweed in ammonium treatments was similar to waterhyacinth, which produced greatest growth 4 wk after being provided with ammonium compared to nitrate (Shiralipour et al. 1981). This study and the limited literature available on nitrogen uptake by aquatic plants suggests that ammonium content of water and soils is more a more important parameter affecting growth than nitrate nitrogen and needs more study.

The nutrient content of soils varies widely throughout the different regions of the world. Buckman and Brady (1960) reported that a typical humid-region agricultural soil comprised soil organic matter contents of 4% or 40,000 mg kg^{-1}, total nitrogen of 1,500 mg kg^{-1}, 440 mg phosphorus kg^{-1} and 11,000 mg potassium kg^{-1}. Total nitrogen and phosphorus values of soils are no indication of the availability of these nutrients for plant uptake because of the complexity of the chemical reactions that occur in soils. Nevertheless, it is interesting to compare the nitrogen, phosphorus, and potassium values of typical agricultural soils to those in our studies designed to determine nutrient levels needed to optimize growth of submersed plants. Assuming the highly soluble salts used in this study represent total nitrogen, phosphorus, and potassium values, our results revealed that submersed plants grow well with 75–150 mg nitrogen per kilogram of sand, 100 to 800 mg phosphorus per kilogram of sand, and less than 500 mg potassium per kilogram of sand. These values represent about 10% of the nitrogen, an approximately equal amount of phosphorus, and much less potassium than is found in the average agricultural soil.

An important result of this research is the finding that sand amended with water-soluble fertilizers containing only nitrogen, phosphorus, or potassium can be used to study nutrient requirements of submersed plants. Direct comparisons of submersed plant growth in substrates containing controlled-release fertilizers to identical nitrogen, phosphorus, and potassium concentrations can now be accomplished with water-soluble fertilizers incorporated into sand. Growth of hydrilla and Illinois pondweed in the micronutrients study (Figure 1) using controlled-release fertilizer was essentially the same as growth in the nitrogen study (Figure 2), which utilized only water-soluble fertilizers. This was originally proposed by Sutton (1993) and additional research on the release of nitrogen and phosphorus from this model system is needed. Although Illinois pondweed, hydrilla, and southern naiad commonly occur in discrete locations or beds in the same lakes in Florida, there is no information published to explain this phenomenon (Gosselin 2016). Because water conditions are presumably similar in separate beds within the same lake, it seems likely that substrate characteristics (e.g., soil type, particle size, nutrient content) influence where these species ultimately become established. We hope that the studies outlined in this article will stimulate additional research into the edaphic factors that determine growth and occurrence of different submersed species within the same body of water.

SOURCES OF MATERIALS

[1]Everris, Dublin, OH 43041.

[2]Growers Fertilizer Corporation, Lake Alfred, FL 33850.

[3]Mettler-Toledo Group, Schwerzenback 8603, Switzerland.

[4]SAS Software Version 9.3, SAS Institute, Cary, NC 27513.

[5]Everris, Dublin, OH 43041.

[6]Howard Fertilizer and Chemical Company, Inc., Orlando, FL 32809.

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