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The Characterization of Axenic Culture Systems Suitable for Plant Propagation and Experimental Studies of the Submersed Aquatic Angiosperm *Potamogeton pectinatus* (Sago Pondweed)

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ABSTRACT: Clonal lines of the submersed aquatic angiosperm *Potamogeton pectinatus* were grown in three culture systems. The first, which used sucrose as a carbon source in a liquid medium, supported vigorous vegetative growth and can be used to propagate large numbers of plants in axenic conditions. In this culture system, plants were responsive to increasing photosynthetically active radiation (PAR) photon flux density (PFD) and were photosynthetically competent. However, their growth was heterotrophic and root development was poor. When these plants were transferred to a second nonaxenic culture system, which used 16-l buckets containing artificial sediments and tap water, growth was autotrophic and plants were morphologically identical to field-harvested *P. pectinatus*. The last culture system which consisted of a sand substrate and inorganic nutrient bathing solution aerated with 135 ml min^{-1} ambient air enhanced to 3.0% CO_2 was axenic and supported autotrophic growth by plants that were also morphologically normal.

Introduction

Submersed aquatic angiosperms occupy important niches in the shallow water environments of freshwater lakes, estuaries, and marine coastal environments (for reviews see Den Hartog 1977; Cattaneo and Kalff 1980; Odum et al. 1984; Thayer et al. 1984). These plants provide habitat for numerous organisms, contribute significant amounts of primary production to the ecosystem, and exert profound effects on abiotic attributes of the aquatic environment such as sediment and water chemistry, sediment stability, and water movement. The submersed aquatic angiosperms in many aquatic systems have undergone drastic changes in species diversity and population size. These changes may have had either detrimental or beneficial effects on the quality of the aquatic environment. For instance, an overabundance of native species in irrigation ponds and drainage ditches may interfere with their intended uses (Bruns et al. 1955; Yeo 1967). In contrast, the decline of submersed aquatic angiosperms in natural ecosystems, like the Chesapeake Bay (Orth and Moore 1984), may profoundly limit the productivity of these ecosystems.

Nutrient enrichment, increased turbidity, and chemical pollution have all been implicated as contributors to observed changes in submersed aquatic

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angiosperm populations (Stevenson and Confer 1978; Kemp et al. 1983). However, definitive studies, based on control of diverse factors which often complicate field studies, are necessary to elucidate and confirm the underlying causes of the observed changes in submersed aquatic angiosperm populations (Van Wijk 1983; Kautsky 1987). Although reliable and reproducible results have been achieved with some systems (Van Wijk et al. 1988; Van Wijk 1989), conventional laboratory studies on field-collected submersed aquatic angiosperms are often hampered by their seasonal availability, marked variability in their growth characteristics, and experimental design problems associated with submersed aquatic angiosperms cultured with other organisms (Seeliger et al. 1984; Smart and Barko 1985). In contrast, axenic systems of plant cultivation have the potential to efficiently propagate large numbers of healthy, easily maintained clonal plants (Madsen 1985; Durako and Moffler 1987). These plants can be used in rigorous experiments to establish growth requirements, to evaluate the effects of environmental stresses on plant performance, and to determine the availability of genetic variation for stress tolerance among the different populations of a particular species.

The submersed aquatic angiosperm *Potamogeton pectinatus* (sago pondweed) is reported to have worldwide distribution in freshwater ponds and streams and brackish coastal waters with salinities of 0 to 15‰ (Yeo 1965, 1966; Van Wijk 1983). In parallel with other submersed aquatic angiosperm species in the Chesapeake Bay, *P. pectinatus* has undergone a general decline since the mid-1960s. Since *P. pectinatus* is an important waterfowl food (Martin and Uhler 1939) and since it provides protective habitat for fish, oysters, crabs, and benthic creatures in shoal areas (Fassett 1960), it is likely that its loss has had an effect on ecosystem composition.

An important objective of our work is to characterize the biology of *P. pectinatus* so that we can effectively manipulate its growth under both laboratory and field conditions. As our initial effort, this paper describes and evaluates two axenic culture systems for *in vitro* manipulation of this plant. In particular, we evaluate whether 1) the first system, which employs a standard culture medium with an organic carbon source (Ailstock 1986), is useful for large-scale plant propagation, and 2) the second system, which provides supplemental inorganic carbon in addition to the basal medium, would be useful for experimental studies. Growth responses of axenically propagated plants on artificial sediments also were measured to assess the behavior of these plants when they are returned to nonaxenic conditions.

Materials and Methods

Experimental Organism

The *P. pectinatus* in these studies exhibited the morphological features similar to those described in Singh (1964, 1965). The apical meristems of horizontal rhizomes give rise to a succession of nodes which produce upright photosynthetic shoots during periods of active growth. Additional rhizomes, 1 to 3 in number, are frequently initiated from the base of these shoots, and thus each *P. pectinatus* plant appears densely branched. Several roots, which form at each node of the rhizome, will branch profusely if buried in sediment. *P. pectinatus* produce fertile seeds and vegetative reproductive structures called turions which form at the rhizome apex. The turions were used as the explant source for establishing *in vitro* clonal cultures.

Culture Initiation

The turion sterilization procedure followed the methodology detailed in Ailstock (1986). Turions were rinsed in tap water, exposed to 10% (v/v) solution of commercial bleach with 0.1% (v/v) Triton X-100 for 5 min, and soaked in 10.0 g l^{-1} of the fungicide Captan (active ingredient: (n-trichloromethylthio)-4-cyclohexene-1,2 dicarboximide) for 24 h. Subsequent work was performed in a laminar flow hood under sterile conditions. Turions with an intact epidermis undamaged in the previous treatments were again treated with 10% bleach solution for 5 min. After dissecting rhizome apices from the turions and removing their largest sheathing leaves, the apices were sterilized in the bleach solution for 5 min and rinsed 3 times in sterile distilled water. Depending on the plant sample, this surface sterilization procedure was sometimes unable to eradicate all bacteria, some of which appear to actually reside within the plant tissue. These endophytic bacteria were isolated from contaminated explants, and the Kirby-Bauer method was employed to determine the bacterial sensitivity to various antibiotics (Ailstock 1986). A 12-h treatment of the dissected apices with 10% (v/v) solution of the antibiotics nitrofurantoin, polymyxin B, kanamycin, or novobiocin (all purchased from Sigma) before their transfer to propagation medium reduced the evident contamination to an acceptable level of 20%. When the explants pretreated with antibiotics were chopped in smaller pieces and cultured on the basal medium enriched with sucrose, it was confirmed that the antibiotics had effectively eradicated the endophytic bacteria from the explants. The antibiotics had no observable effects on subsequent growth of the treated explants.

The explants were placed in 150-ml culture tubes with 20 ml of the basal medium of Murashige Shoot Multiplication Medium B (Huang and Murashige 1976), which was supplemented with 10 g l^{-1} sucrose to serve as the standard propagation medium. The medium pH was adjusted to 5.0 with 1 N HCl and 0.1 N NaOH prior to autoclaving at 18 psi for 20 min. The cultures were maintained in growth chambers at 20°C under constant illumination with cool white fluorescent light at 70 μmol m^{-2} s^{-1}.

Culture Systems

Three culture systems were devised for different purposes: 1) the large-scale propagation of plants in axenic conditions, system I; 2) the evaluation of cultural plant performance in nonaxenic substrates, system II; and 3) the axenic culture of plants for experimental studies, system III. Except where otherwise noted, all systems were irradiated from the top under constant illumination with cool white fluorescent light at 70 μmol m^{-2} s^{-1} at 20°C.

System I

For the purpose of mass propagation to increase plant stock, sterile rhizome fragments including at least one rhizome bud were routinely transferred to sterile 1-l autoclavable jars (Kerr Glass Mfg. Corp., Sand Springs, OK) containing 500 ml of propagation medium. Preliminary experiments indicated that this system supported vigorous plant growth; for example, 2.5 g of plant material developed an average of 106 rhizome buds and 294 photosynthetic shoots with a total weight of 25.3 g in 8 wk in these jars. More uniform explants were selected to minimize variability within subsequent treatments. Explants consisting of a single rhizome bud with two subtending photosynthetic stems were placed in 25 mm × 150 mm culture tubes containing 25 ml of propagation medium. After 4–5 wk, cultures were visually inspected for uniformity, with the few (5–10%) showing exceptional growth then being discarded. The remaining cultures weighing between 1.0 and 2.0 g were used either as experimental material for evaluating the photosynthetic competence of axenic plants grown on the propagation medium or for establishing the other two culture systems, as described below.

System II

The growth of plants from system I axenic cultures was monitored on artificial substrates in nonaxenic conditions by placing the plants in 16-l plastic buckets containing 3 l of substrate and 14 l of tap water. The artificial substrates, selected in the attempt to mimic a range of natural sediments, included commercial grade peat moss, crushed oyster shell (maximum size of 5 mm), and acid-washed sand either singly or in layered combinations with the first substrate listed being positioned on the top.

System III

To devise an axenic culture system for supporting autotrophic growth, a preliminary experiment was conducted to evaluate the importance of a substratum for root development in axenic cultures of *P. pectinatus*. Forty rhizome explants each consisting of a single rhizome bud with two subtending photosynthetic shoots were placed in 1-l jars containing 500 ml of the propagation medium. Twenty were anchored in small jars filled with sterile acid-washed sand. After 4 wk, plants floating in the propagation medium had produced 8 roots per plant while those anchored in sand had formed an average of 26 roots per plant. In all subsequent experiments, 4-week-old plants grown on the propagation medium were anchored in 70 ml (4.7 × 5.4 cm) jars containing 20 ml of the basal, DNR salt (96 mg l^{-1} $NaHCO_3$, 60 mg l^{-1} $CaSO_4 \cdot 2H_2O$, 60 mg l^{-1} $MgSO_4$, 4 mg l^{-1} KCl), or Murashige Minimal Organic (MMO) medium (Huang and Murashige 1976) solidified with 6 g l^{-1} agar. After autoclave sterilization, the cooled agar was covered with 40 ml of sterile acid-washed sand and the small jars were submersed in sterile 1-l jars filled with 750 ml of basal medium, DNR salt solution, or 60 mg l^{-1} $NaHCO_3$ and 15 mg l^{-1} $KHCO_3$. Air mixed with 100% CO_2 to achieve a final CO_2 concentration of 3%, was passed through a 0.2-mm filter to remove particulates and bacteria. A PVC manifold system delivered the filtered gas to individual jars that had been steam sterilized. Flame sterilization was used to aseptically connect the manifold to the jars.

Photosynthetic Measurements

To characterize the growth responses of axenic plants from system I to different photosynthetic active radiation (PAR) photon flux density (PFD), plants were transferred to 1-l jars filled with fresh propagation medium and grown for 8 wk in open-topped boxes constructed of 1-cm plywood painted with flat black latex paint. The boxes were covered with a variable number of neutral-density filters to adjust the PAR PFD rate. To determine the photosynthetic competence of plants grown in system I, plants were placed in 1-l jars with fresh propagation medium and grown for 4 wk. Photosynthetic rates were determined by measuring oxygen evolution. Photosynthetic oxygen evolution was measured by placing the intact plants in 300-ml,

clear Winkler bottles filled with fresh propagation medium which had been purged with N_2 gas to reduce the O_2 concentrations to 2–3 mg l^{-1}. Sufficient $NaHCO_3$ was then added to make a final saturating concentration of 10 g l^{-1} $NaHCO_3$. After a 3-h incubation in water baths at the standard light and temperature conditions mentioned above, oxygen concentration was determined with an Orion (Cambridge, MA) model 97-08-00 air calibrated oxygen electrode coupled to an Orion model 901 microprocessor/ion analyzer.

The suitability of different carbon sources for supporting autotrophic growth was assessed by exposing plants to 1) serial dilutions of sucrose; 2) different levels of filter-sterilized sodium, potassium, and calcium bicarbonate added to autoclaved medium at room temperature; 3) multiple bicarbonate sources in combination with vigorous aeration at ambient CO_2 levels at 135 ml min^{-1} and 4) a mixture of 1% CO_2, 1% O_2, and 98% N_2 at a constant 135 ml h^{-1}. Gases were delivered to the medium through sterile Tygon tubing inserted through foam plugs in the tops of the jars.

Growth Measurements and Data Analysis

Plant growth was evaluated by recording the number and weight of rhizomes, roots, and shoots. Fresh weight was determined by weighing plants which had been blotted with filter paper. Dry weight was obtained from the same plants after they were dried to a constant weight at 55°C. Unless otherwise noted, replicate number within each treatment consisted of 10 plants. Bonferroni (Dunn) *t* tests or one-way analysis of variance with Scheffes' range test were used to determine statistical significance among treatments.

Results and Discussion

Axenic culture systems provide a number of distinct advantages over other types of culture systems for evaluating plant responses under varied environmental conditions. They are particularly useful for evaluating the individual and synergistic roles of substrate microflora, algal epiphytes, sediment composition, and water chemistry on the growth and development of submersed aquatic angiosperms. However, it is important that *in vitro* culture systems developed for conducting such studies support growth which is representative of field populations. The experiments described below evaluate two axenic culture systems in terms of the following criteria: vigorous growth, photosynthetic competency, long-term inorganic carbon gain, normal morphology, and transferability to non-axenic conditions.

System I

Supplemental carbon sources required for plant biomass accumulation are a common feature of many *in vitro* culture systems. Therefore, considerable effort was made to evaluate the carbon requirements of axenic cultures of *P. pectinatus.* Figure 1 shows the accumulated biomass of *P. pectinatus* plants grown in the propagation medium and exposed to light PFD of 0 to 120 μmol m^{-2} s^{-1} for 4 wk. The highest biomass was achieved at a PAR PFD of 120 μmol m^{-2} s^{-1}, which is identical to the PFD reported to saturate the photosynthesis of field-harvested *P. pectinatus,* as measured by net O_2 evolution (Correll and Wu 1982). Despite the presence of sucrose in the culture medium, the plants which were grown in total darkness accumulated little, if any, additional dry weight over the course of the experiment. Nevertheless, cultured plants exposed to constant illumination of 120 μmol m^{-2} s^{-1} PAR had an obligate requirement for exogenous sucrose. Those explants without sucrose exhibited no additional growth in 4 wk, whereas higher sucrose concentrations caused progressive increases in total biomass, with the maximal growth occurring at the highest sucrose concentration (Fig. 2). This unanticipated finding that axenic cultures required both light and sucrose suggests that the light is not acting in this culture system as the energy source for long-term growth but rather as a promoter of sucrose uptake. Although we are not aware of similar examples of light-mediated sucrose uptake, light is known to stimulate the plasma membrane H^+ pump in other plant cells (Hendrich and Schroeder 1989); the resulting H^+ gradient is then able to drive the uptake of small molecules such as sucrose via co-transport mechanisms. The present data suggest that a light-mediated H^+ sucrose cotransport system may also be operating in *P. pectinatus.*

The unusual features of their apparent sucrose requirement prompted further studies of these plants which are reported to carry out net photosynthesis with either HCO_3^- or CO_2 (Sand-Jensen 1983). Photosynthetic competency was confirmed when short-term oxygen evolution, (14–15 mg O_2 gdw^{-1} h^{-1}) was obtained under PAR at 70 μmol m^{-2} s^{-1} on the propagation medium supplemented with $NaHCO_3$ (Table 1). These rates are similar to those reported for field-harvested material (Correll and Wu 1982).

The ability of the plants to carry out oxygen evolution in this culture system led to a preliminary evaluation of different sources of bicarbonate as a carbon source for supporting long-term growth. Initially, the plants were supplied with different concentrations of HCO_3^- as solubilized $KHCO_3$ to

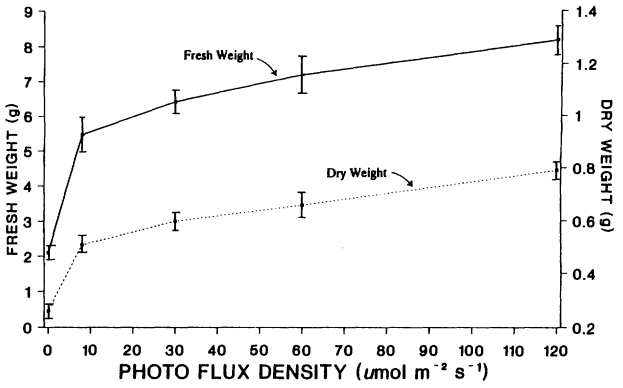

Fig. 1. The effects of photosynthetically active radiation (PAR) photon flux density (PFD) on the fresh and dry weights of 4-week-old *Potamogeton pectinatus* plants cultured for 8 weeks on the propagation medium. Temperature was 20°C. The symbols represent the means of 10 replicates ± the standard error.

avoid the salinity differences associated with variable Na^+ concentrations, but no significant growth was recorded at any $KHCO_3$ concentration in the absence of supplemental sucrose (data not shown). Because $NaHCO_3$ was reported to improve growth of axenic cultures of *Myriophyllum spicatum* at 20 mg l^{-1} (Godmaire and Nalewajko 1986), the effect of this bicarbonate source at concentrations from 10^{-3} to 10^{-2} mg l^{-1} was also tested on *P. pectinatus* cultures. The $NaHCO_3$-supplemented medium was periodically changed in several treatments to ensure that the medium was not being depleted of its bicarbonate. However, the treated cultures exhibited the same slight increase in total biomass as the control culture without added bicarbonate (data not shown). Similar results were obtained with tested concentrations of $CaCO_3$. Finally, it was observed that multiple bicarbonate sources, $NaHCO_3$

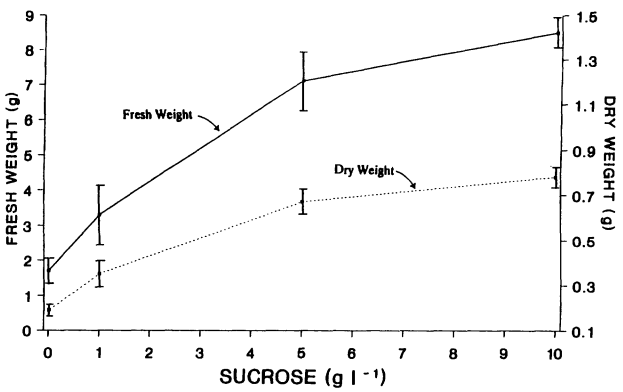

Fig. 2. The effects of sucrose concentration on the fresh and dry weights of 4-week-old *Potamogeton pectinatus* plants cultured for 8 weeks on the basal medium. Temperature was 20°C; photosynthetically active radiation (PAR) photon flux density (PFD) 120 μmol m^{-2} s^{-1}. The symbols represent the means of 10 replicates ± the standard error.

TABLE 1. The effect of 10 g l^{-1} $NaHCO_3$ and 10 g l^{-1} sucrose on short-term oxygen evolution of four-week-old axenic plants of *Potamogeton pectinatus* grown on the basal medium. Oxygen evolution per gram fresh (gfw) and gram dry (gdw) weight per hour was determined after 3 hr incubation on the various media. The data represent the means ± the standard errors among eight replicates. Temperature was 20°C; PAR fluence rate 70 μmol m^{-2} s^{-1}. Letters denote differences in significance in pairwise comparison ($p > 0.05$).

Carbon Supplement	mg O_2 gfw^{-1} h^{-1}	mg O_2 gdw^{-1} h^{-1}
None	0.02 ± 0.01 (a)	0.15 ± 0.04 (a)
10 g l^{-1} sucrose	0.04 ± 0.01 (a)	0.36 ± 0.06 (a)
10 g l^{-1} $NaHCO_3$	1.58 ± 0.14 (b)	14.29 ± 1.31 (b)
10 g l^{-1} sucrose 10 g l^{-1} $NaHCO_3$	1.66 ± 0.14 (b)	14.78 ± 1.14 (b)

and $KHCO_3$, in combination with aeration at 135 ml min^{-1} of ambient CO_2 (i.e., 0.03%) could not support long-term autotrophic growth of the axenic cultures on the propagation medium without sucrose (Table 2). However, when the plants were aerated with a mixture of 1.0% CO_2, 1.0% O_2, and 98.0% N_2 at 135 ml min^{-1}, they doubled both their fresh and dry weights in 2 wk (Table 2). Thus, it appears that cultured plants require much higher than ambient levels of exogenous CO_2 and do not utilize bicarbonate for biomass accumulation in this axenic system. This does not eliminate the possibility that bicarbonate use is a conditioned response requiring long-term exposure.

These results demonstrate that the ability to exhibit net oxygen evolution in short-term experiments (Table 1) should not be taken to indicate that the cultured plants are reliant on autotrophic pathways for long-term growth (i.e., net biomass accumulation) (Table 2). Moreover, the variable responses of cultured submersed aquatic angiosperms suggest that the different species have diverse responses to culture conditions. Thus, it is likely that the right treatment of inorganic carbon to sustain long-term growth will require empirical manipulations for each species (Kadono 1980; Ma-

TABLE 2. The effect of $NaHCO_3$, $KHCO_3$ and aeration on the fresh and dry weight of four-week-old plants grown on the basal medium for two weeks. The initial fresh weight of plants averaged 1.31 g with no significant statistical differences among treatments. The data represent the means ± the standard error among 10 replicates. For additional details see the legend of Table 1.

Carbon Supplement	Aeration 135 ml min^{-1}	Final Fresh Weight (g)
60 mg l^{-1} $NaHCO_3$ 15 mg l^{-1} $KHCO_3$	ambient	1.04 ± 0.13 (a)
600 mg l^{-1} $NaHCO_3$ 150 mg l^{-1} $KHCO_3$	ambient	1.11 ± 0.10 (a)
None	1% CO_2, 1% O_2 98% N_2	3.86 ± 0.22 (b)

TABLE 3. Growth and development of 4-week-old axenic plants of *Potamogeton pectinatus* after 8 weeks growth in 3 l of natural sediment and 14 l of tap water. Each bucket was planted with one plant of each of the four clonal lines. The initial fresh weight of plants in each bucket averaged 5.2 g with no statistical differences among treatments. The data represent the means ± the standard error of eight replicates. Temperature was 22°C; PAR fluence rate at the bucket surface was 120 μmol m^{-2} s^{-1}. Letters denote differences in significance in pairwise comparison ($p > 0.05$).

Sediment	Final Fresh Weight (g)	Final Dry Weight (g)	Number of Turions	Number of Photosynthetic Stems	Number of Roots (>3 cm in Length)
Sand	11.6 ± 0.91 (a)	1.4 ± 0.2 (a)	5.4 ± 2.0 (a, b)	63.8 ± 16.2 (a)	180.2 ± 13.2 (a)
Shell	15.1 ± 1.2 (a, b)	1.7 ± 0.2 (a, b)	9.0 ± 1.4 (a, b, c)	74.4 ± 19.8 (a)	177.8 ± 21.8 (a)
Sand shell	13.6 ± 0.98 (a)	1.7 ± 0.2 (a)	15.2 ± 2.7 (a)	67.4 ± 21.1 (a)	197.6 ± 17.5 (a)
Sand peat	15.6 ± 2.38 (a, b)	1.3 ± 0.2 (a)	0.0 ± 0.0 (a)	57.8 ± 16.5 (a)	184.6 ± 12.9 (a)
Shell peat	29.9 ± 3.4 (c)	3.3 ± 0.4 (c)	11.4 ± 2.6 (b, c)	70.0 ± 21.2 (a)	223.6 ± 30.6 (a)
Sand shell peat	23.0 ± 1.3 (b, c)	2.9 ± 0.2 (b, c)	5.6 ± 2.0 (a, b)	73.2 ± 22.1 (a)	194.2 ± 40.0 (a)

berly and Spence 1983). The remainder of this paper evaluates two questions. Do the sterile plants from this axenic culture system thrive when transferred to nonaxenic conditions similar to those of the native environment? Can axenic conditions be created so that the plant growth resembles that observed in their native environments?

System II

Different environmental conditions have been observed to significantly affect the vegetative morphology of various *Potamogeton* species (Barko et al. 1982; Kautsky 1987; Spencer and Anderson 1987; Van Wijk et al. 1988). Therefore, it was essential to evaluate the morphological responses of plants propagated in axenic culture when they were transferred to environments more representative of field conditions (Bristow and Whitcombe 1971). It was clear from visual inspection that system I promoted luxuriant growth of both rhizomes and shoots but inhibited root expansion of the cultured plants (data not shown). When these plants were placed in nonaxenic substrates, including sand, crushed oyster shell, and peat moss, vegetative growth was variable, but extensive root systems were quickly developed (Table 3). Optimal biomass accumulation was obtained on shell-peat combinations; however, no difference in stem length or number of roots was observed among the substrates tested. Turions were observed on all sediment types with the exception of the sand-peat substrate and a single flower was produced on the sand-shell-peat substrate. The difference in overall growth between treatments seems attributable to the more abundant nutrients and different sediment densities (Barko and Smart 1981, 1986; Smart and Barko 1985) available in some media combinations. The superiority of shell-peat combinations for supporting vegetative growth may be attributable to the enhanced availability of inorganic carbon. In these sediments both bicarbonate from shell degradation and carbon dioxide from microbial respiration could serve as photosynthetic substrates. Additionally, these conditions may also favor more rapid nutrient cycling by soil microflora.

System III

An ideal axenic system would produce cultured plants that exhibit substantial biomass increase and normal morphology in the presence of inorganic carbon. The previous work in this paper has demonstrated that such a system for *P. pectinatus* plants requires that they are transferred from the propagation medium to another system with both substrate and enriched CO_2. Table 4 presents our experiments to test the results of different bathing solutions, substrate solutions, and CO_2 concentration on biomass accumulation in an axenic system, as described in Materials and Methods. While no significant differences were found among the various media used as substrate or as a bathing solution, increased growth was observed on all media supplemented with 135 ml min^{-1} of ambient air enriched to approximately 3.0% CO_2.

The combination of MMO substrate solution and DNR bathing solution aerated with 135 ml min^{-1} ambient air enhanced to 3.0% CO_2 (treatment 8), satisfies the requirement for substantial biomass increase in the presence of inorganic carbon under axenic conditions. To compare vegetative development in the axenic propagation system I (which uses sucrose) and the axenic experimental system III (which uses CO_2), a single clonal line was grown under the conditions of treatment 8 and in the axenic propagation system (Table 5). In the propagation system, plants accumulated greater biomass and they produced more photosynthetic stems. Numerous starch grains were also observed in the rhizomes, stems, and leaves of the plants grown in the presence of sucrose. This suggests possible carbon limitation in the axenic system supporting autotrophic growth or a surplus in the propagation system. In contrast, plants grown in sediment with an inorganic carbon source produced an abundance of well developed roots. Thus, this system produces axenic autotrophic plants useful for ex-

TABLE 4. Growth of 4-week-old axenic plants of *Potamogeton pectinatus* after 6 wk growth in various bathing solutions, substrate solutions with and without CO_2 supplemented aeration. The initial fresh weight of plants averaged 0.94 g with no statistical differences among treatments. For additional details see the legend of Table 1.

Treatment	Bathing Solution	Substrate Solution	Air (ml min^{-1})	Percent CO_2	n	Final Fresh Weight (g)
1	Basal	Basal	0	0	5	1.32 ± 0.32 (a)
2	DNR	Basal	0	0	8	1.63 ± 0.36 (a)
3	DNR	MMO	0	0	8	1.83 ± 0.32 (a, b)
4	DNR	Basal	135	0.03	8	2.23 ± 0.82 (a, b)
5	DNR	MMO	135	0.03	8	2.84 ± 0.64 (a, b)
6	Basal	Basal	135	3.00	6	3.74 ± 2.01 (b)
7	DNR	Basal	135	3.00	8	6.34 ± 1.49 (c)
8	DNR	MMO	135	3.00	8	7.45 ± 0.95 (c)

perimental studies which are designed to mimic natural conditions.

Conclusion

Axenic culture systems developed for conducting studies in physiological ecology should support growth which is rapid, morphologically normal, and physiologically equivalent to that observed in field populations. It is clear from the results that neither system satisfies all of these criteria. The propagation system described here for *P. pectinatus* can be used to produce large numbers of plants independent of constraints of seasonality. These plants are easily established in nonaxenic conditions as rapidly growing plants on the artificial substrates tested. Once established, their vegetative growth seems very similar to field-derived populations. However, the plants in the propagation system are anomalous with respect to their limited root development and their dependence on exogenous sucrose.

In contrast, the *P. pectinatus* plants grown axenically on nutrient substrates in the presence of both bicarbonate and high CO_2 exhibit both normal root development and substantial levels of photosynthetic carbon gain. Thus, system III provides conditions which support normal autotrophic growth of *P. pectinatus* and is useful in a variety of experimental applications. For example, these plants are currently being used to assess the relative phytotoxicity of various aquatic pollutants known to act upon photosynthetic processes (Fleming et al. 1988). Taken collectively, the different culture systems described in this paper provide a valuable alternative for studying the growth and development of submersed aquatic angiosperms in natural and modified environments.

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TABLE 5. Comparison of the growth and development of 4-week-old *Potamogeton pectinatus* plants of Pooles Island clonal line PIB grown for 4 wk in the axenic propagation culture system and in an axenic system employing an inorganic carbon source; treatment 8 of Table 4, consisting of the DNR bathing solution, MMO substrate solution with 135 ml min^{-1} of ambient air enhanced to 3% CO_2. The initial fresh weight of plants averaged 0.7 g with no significant statistical differences among treatments. The data represent the means ± the standard errors among eight replicates. Temperature was 20°C; PAR fluence rate 70 μmol m^{-2} s^{-1}. No flowers or turions were produced during the culture period.

Axenic Culture System (Carbon Source)	Final Fresh Weight (g)	Final Dry Weight (g)	Number of Photosynthetic Stems	Number of Roots (>3 cm)
System I—(Propagation-Sucrose)	13.4 ± 1.4 (b)	1.2 ± 0.1 (b)	145.8 ± 15.6 (b)	2.4 ± 1.4 (a)
System II—(Experimental studies CO_2)	4.4 ± 0.4 (a)	0.3 ± 0.0 (a)	45.8 ± 3.8 (a)	62.5 ± 6.6 (b)

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