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Inter-Relationships Between *Salvinia rotundifolia* and *Spirodela polyrhiza* at Various Interaction Stages[1]

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ABSTRACT

The biomass yield and nutrient storage of small-leaf floating aquatic macrophytes, *Salvinia rotundifolia* Willd., and *Spirodela polyrhiza* (L.) Schleid., were measured in monoculture and in mixed cultures. The carrying-capacity for each species was investigated in monoculture, while reciprocal replacement-series was used for the intermixed combinations at various interaction stages. The growth rate of *Salvinia* was influenced by the composition of the culture medium. Relatively high growth rates (5.8 to 11.4 g dw m^{-2} d^{-1}) were maintained throughout the experimental period in 10 to 50% modified Hoagland nutrient medium whereas, for the wastewaters evaluated, growth rates were highest for *Salvinia* cultured in primary sewage effluent. In mixed cultures, the growth rates for *Salvinia* were higher than for *Spirodela*. In mixed cultures of equal initial density, *Salvinia* produced 2-fold higher biomass yield than *Spirodela*. The standing-crop of *Salvinia* was not affected by the presence of *Spirodela* and was the same either in monoculture or in mixed culture. In mixed culture, more N and P were accumulated (by about 1.5-fold) in *Spirodela* than in *Salvinia*, whereas N and P contents were similar when these species were grown in monoculture.

Key words: growth rate, competition, replacement series, nitrogen, phosphorus, tissue nutrients.

INTRODUCTION

The true fern salvinia (*Salvinia rotundifolia* Willd., Division pteridophyta, Salviniaceae family) and the giant duckweed (*Spirodela polyrhiza* (L.) Schleid., Lemnaceae family) are small-leaf, floating, aquatic macrophytes, commonly found growing together in many freshwater habitats of Florida and in other subtropical and tropical regions of the world (Hillman, 1961; Landolt, 1982; DeBusk and Reddy, 1987). The ability of *Spirodela polyrhiza* plants to absorb and remove nutrients from polluted waters has been documented (Bitcover and Sieling, 1951; Muhonen et al., 1983; Oron et al., 1984; 1986; Reddy and DeBusk, 1985; Wolverton and McCaleb, 1987), but few studies are available on the growth and nutrient-uptake capability of *Salvinia*. Previous studies have shown that *Salvinia* in monoculture can remove nitrogen (N) and phosphorus (P) under a wide range of environmental conditions (Reddy and DeBusk, 1985a, 1987; DeBusk and Reddy, 1987). The productivity and nutrient uptake by these two plant species also have been compared in monoculture (Reddy et al., 1983; Reddy and DeBusk, 1985b, 1987; So, 1987), but little information is available on their growth and their N and P storage capabilities in mixed cultures.

The objectives of this study were to: (i) determine the growth rates of a monoculture of *Salvinia rotundifolia*, and (ii) study its competitiveness and its nutrient storage when cultured with *Spirodela polyrhiza* in nutrient-enriched waters.

MATERIALS AND METHODS

Plants of *Spirodela polyrhiza* and *Salvinia rotundifolia* were collected from the Wekiva River, near Sanford, Florida and acclimatized in weak nutrient medium for a period of 7 days, before their use in the experiments.

Experiment 1. The first experiment was designed to determine the effect of culture medium composition on growth rates of *Salvinia*. This experiment was conducted outdoors at the Central Florida Research and Education Center—IFAS, University of Florida, Sanford, Florida, from July 13, 1982, until August 18, 1982 in tanks (50 cm long, 50 cm wide, and 30 cm deep) each having a water-surface area of about 0.25 m^2. These 75 L tanks were filled with 50 L nutrient medium (modified Hoagland solution); or with one of three types of wastewater; namely, primary or secondary sewage effluent, or agricultural drainage effluent. Primary and secondary sewage effluent were obtained from the Walt Disney World wastewater treatment facility whereas the agricultural drainage effluent was obtained from drainage canals located among the vegetable farms near Zellwood, Florida. Nutrient composition of the culture media are presented in Table 1. Starting plant density of *Salvinia* in each tank was 2000 g fw m^{-2} (71 g dw m^{-2}). Plants were weighed once each week and then harvested back to their original density in order to maintain optimum density for maximum growth (see Reddy and DeBusk, 1985b). The nutrient medium and wastewaters were replaced with fresh medium only once, at the end of the 2nd week of the 5-week growth period. Water loss due to evapotranspiration was adjusted daily to initial volume

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TABLE 1. CHEMICAL COMPOSITION (Mg L^{-1}) OF THE MODIFIED HOAGLAND SOLUTIONS AND WASTEWATERS USED IN THIS STUDY. MINOR ELEMENTS WERE ADDED AT THE SAME CONCENTRATIONS TO ALL LEVELS OF THE MODIFIED HOAGLAND SOLUTIONS (SEE REDDY AND DEBUSK, 1984).

Nutrient	Modified Hoagland solutions					Primary sewage effluent	Secondary sewage effluent	Agricul. drainage effluent
	2%	5%	10%	20%	50%			
NH_4-N	2.0	5.3	10.5	21.0	52.5	15.5	0.0	0.5
NO_3-N	2.0	5.3	10.5	21.0	52.5	0.0	8.5	0.4
P	1.4	3.4	6.4	12.6	31.5	5.6	4.2	1.7
K	4.7	11.8	23.5	47.0	117.5	7.5	6.0	19.5
Ca	4.0	10.0	20.0	40.0	100.0	18.3	14.5	57.5
Mg	1.0	2.5	5.0	10.0	50.0	4.9	4.7	26.0
*Fe	0.4	1.0	2.0	4.0	10.0	0.2	0.1	0.1

*Fe was added as Fe-EDTA to the modified Hoagland solutions.

by adding tap water. Average solar radiation during the experimental period was 3917 kCals m^{-2} d^{-1}, and average minimum and maximum air temperatures were 22 and 32 C, respectively.

Experiment 2. A second experiment was conducted to evaluate the inter-relationships between *Salvinia rotundifolia* and *Spirodela polyrhiza.* Plants were grown inside a greenhouse (minimum and maximum air temperatures of 15 and 35 C, respectively) in one-liter containers with water-surface areas of 95 cm^2. The nutrient medium (10% modified Hoagland solution, [see Table 1 and Hoagland and Arnon (1950)], along with Mn, B, Zn, Cu and Mo added as Nutrispray™—Sunniland, Chase and Co., Sanford, FL, a commercial micronutrient preparation) in each container was analyzed and replaced completely at weekly intervals with fresh medium. The interaction between *Spirodela* and *Salvinia* was investigated using the reciprocal replacement-series analysis of de Wit (1960), with a total density of 20 plants either in monoculture or mixed culture (i.e. 20:0, 15:5, 10:10, 5:15, 0:20) in each container. In addition, carrying-capacity was determined for each species growing in monoculture at densities of 5, 10, 15 and 20 plants per container. Each treatment was replicated four times. In this experiment individual *Spirodela* plants consisted of two fronds with roots, while individual plants of *Salvinia* had one thallus with rhizoids. Individuals of both species had similar dry weights at the beginning of the experiment (1 mg dry weight). During the experimental period, plants of each species were removed from the container weekly, counted and allowed to drain for 5 minutes, and weighed (fresh weight). Plants were then transferred to their previous containers, which were full with fresh nutrient solution. In mixed cultures, attention was paid to careful placement of each species, so that each plant could interact with its accompanying competitor. At the end of the experiment, the number of plants of each species per container was counted and the plants were dried at 70 C to a constant weight. At the beginning and end of the experiment, total N and P in the plant tissue were determined by digestion followed by analysis using an autoanalyzer (A.P.H.A., 1985). pH was measured at mid-day several times throughout the experiment, using a glass electrode.

RESULTS AND DISCUSSION

The growth rate of *Salvinia* was influenced by the composition of culture medium (Table 2). During the first week, growth rates were approximately the same for *Salvinia* cultured in 2, 5, 10 and 20% of modified Hoagland nutrient medium, with further increase in strength of the nutrient medium decreasing the growth rates. In the 5th week, growth rates in the treatments with 2 to 20% Hoagland medium decreased as a result of nutrient depletion in the culture medium, while a steady growth rate was maintained in the treatment with 50% modified Hoagland solution. Among wastewaters evaluated, growth rates of *Salvinia* were lower when cultured in agricultural drainage water than in primary sewage effluent. Relatively high growth rates (5.89 to 11.4 g dw m^{-2} d^{-1}) were maintained throughout the experimental period in 10 to 50% modified Hoagland nutrient medium. Similar results were also reported by Reddy et al. (1983) and by Reddy and DeBusk (1985b), with net productivity of *Salvinia rotundifolia* in the range of 5 to 13g dw m^{-2} d^{-1}.

Biomass yields of *Spirodela* and *Salvinia* were in direct proportion to the number of plants at all densities in both mixed cultures and monoculture, except for *Spirodela* plants grown in mixed cultures, at the end of the growth period (Figure 1 and Tables 3 & 4). The weight per plant of *Spirodela* grown in mixed culture was much lower (by 2-6 fold) than the weight per plant of *Spirodela* grown in

TABLE 2. GROWTH RATES (g dw m^{-2} d^{-1}) OF *SALVINIA ROTUNDIFOLIA* DURING A 5-WEEK GROWTH PERIOD BEGINNING JULY 13, 1982, AS INFLUENCED BY THE GROWTH MEDIUM.

Growth medium	Growth period (weeks)				
	1	2	3	4	5
Modified Hoagland solutions					
2%	10.7	4.9	8.1	4.7	3.6
5%	11.0	5.3	8.9	7.6	5.3
10%	11.4	5.8	10.7	8.9	7.3
20%	10.9	6.6	10.9	8.4	7.0
50%	7.9	7.4	10.4	6.9	7.2
Agricultural drainage water	8.4	4.7	6.9	3.7	1.8
Secondary sewage effluent	9.6	4.9	6.0	5.1	3.6
Primary sewage effluent	9.7	8.1	10.0	7.4	3.4
$LSD_{0.05}$	1.6	0.9	1.3	1.5	1.2

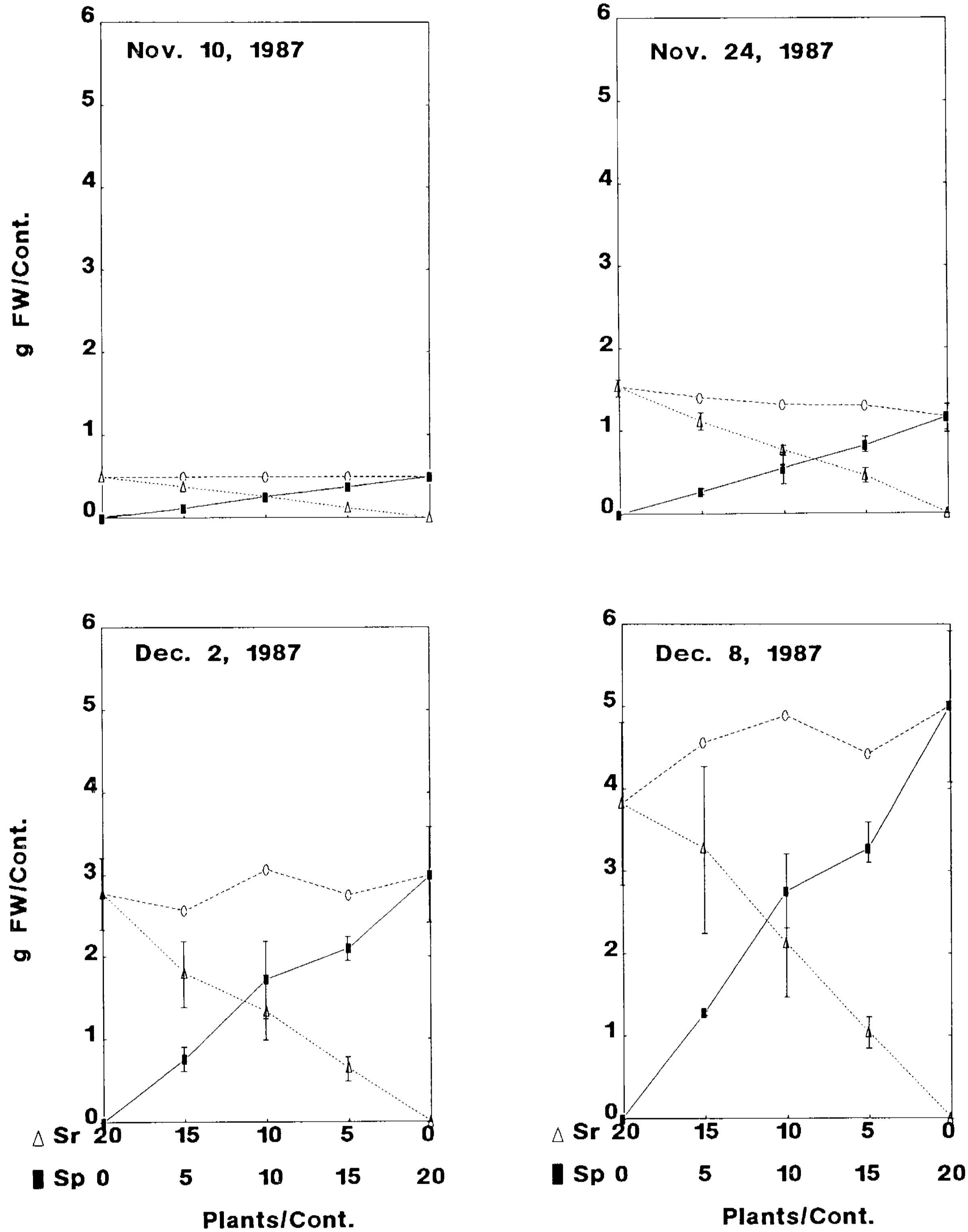

Figure 1. Replacement diagrams for the standing-crop of *Spirodela polyrhiza* (Sp—closed rectangles) and *Salvinia rotundifolia* (Sr—open triangles) which have been grown at the indicated densities throughout the first 4 weeks of the experiments. Total standing-crop on each date is indicated with open circles. Vertical bars represent standard deviations of the means.

monoculture (Table 4). Hence, the numbers of stressed *Spirodela* plants grown in mixed cultures with *Salvinia* remained almost the same, but the weight of each plant decreased significantly.

During the first four weeks (until December 8, 1987), both plant species grew without any interspecific interference. Their biomass in mixed cultures was similar to that in monoculture (Figure 1). By one week later, however (on

TABLE 3. FRESH WEIGHT (g) OF PLANTS PER CONTAINER (95 cm²) FOR *SPIRODELA POLYRHIZA* (Sp) AND *SALVINIA ROTUNDIFOLIA* (Sr) GROWN IN MONOCULTURE AT VARIOUS STAGES OF THEIR INTERACTION. MEANS ± SD OF 4 REPLICATES. EXPERIMENT PERIOD = NOVEMBER 10, 1987 THROUGH DECEMBER 15, 1987.

Initial plant density (%)	Nov. 24	Dec. 2	Dec. 8	Dec. 15
Spirodela polyrhiza				
25 Sp	0.28 ± 0.05	0.74 ± 0.06	1.82 ± 0.23	3.88 ± 0.30
50 Sp	0.59 ± 0.04	1.50 ± 0.30	3.08 ± 0.61	5.65 ± 0.23
75 Sp	0.71 ± 0.07	2.06 ± 0.32	4.12 ± 0.34	5.97 ± 0.30
100 Sp	1.16 ± 0.17	3.01 ± 0.28	5.01 ± 0.92	7.05 ± 0.56
Salvinia rotundifolia				
100 Sr	1.54 ± 0.10	2.78 ± 0.44	3.83 ± 0.99	6.85 ± 1.01
75 Sr	1.34 ± 0.22	2.18 ± 0.32	2.62 ± 0.69	4.70 ± 0.28
50 Sr	1.11 ± 0.11	1.39 ± 0.15	1.80 ± 0.38	2.53 ± 0.36
25 Sr	0.47 ± 0.05	0.66 ± 0.07	1.00 ± 0.01	1.73 ± 0.22

December 15, 1987), the *Spirodela* had become significantly suppressed by the *Salvinia* (Figures 2 and 3a). In mixed cultures of equal initial density, *Salvinia* produced 2-fold higher biomass than did *Spirodela* (Figure 2). Moreover, the standing-crop of *Salvinia* was not affected by the presence of *Spirodela* and was the same in monoculture and in mixed cultures (Figure 3b). As a result of this clear advantage of *Salvinia* over *Spirodela*, the necessity of using additional, improved, competition models (Firbank and Watkinson, 1985; Connolly, 1987) seems less significant in this case.

In spite of the suppression of *Spirodela* by *Salvinia*, these two plant species grow together in many natural ecosys-

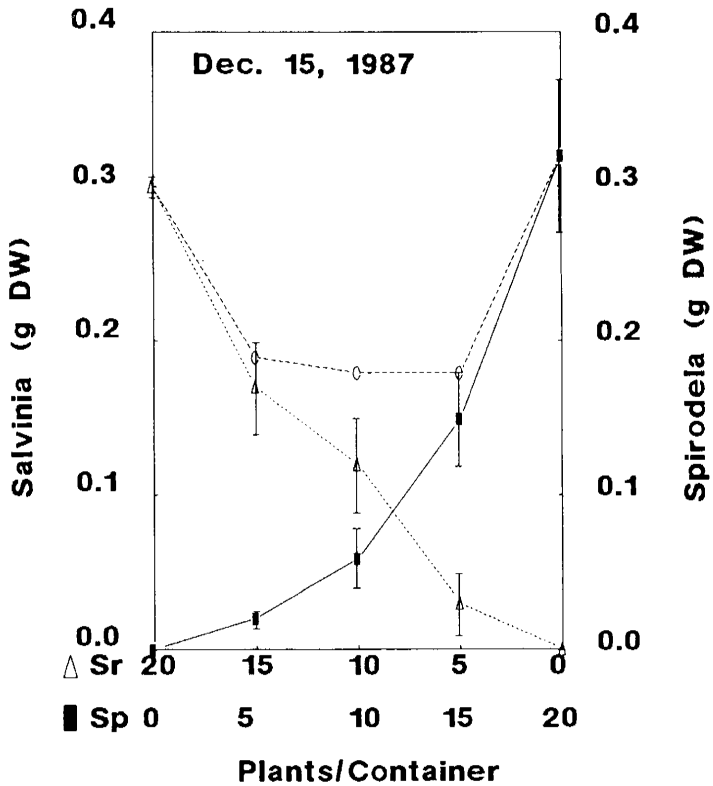

Figure 2. Replacement diagrams for the standing-crop per container of *Spirodela polyrhiza* (Sp—closed rectangles) and *Salvinia rotundifolia* (Sr—open triangles) which were grown at the indicated densities throughout the entire 5 weeks of the experiments. Total standing-crop is indicated with open circles. Virtical bars represent standard deviations of the means.

TABLE 4. NUMBERS OF PLANTS PER CONTAINER (95 cm²) FOR SPIRODELA POLYRHIZA (Sp) AND *SALVINIA ROTUNDIFOLIA* (Sr) GROWN INTERMIXED IN RECIPROCAL REPLACEMENT-SERIES AS WELL AS IN MONOCULTURE, AT VARIOUS STAGES OF THEIR INTERACTION. MEANS ± SD OF 4 REPLICATES. EXPERIMENTAL PERIOD = NOVEMBER 10, 1987 THROUGH DECEMBER 15, 1987.

Initial plant density (%)	No. of Plants/Container						mg DW/ plant
	Nov. 10	Nov. 17	Nov. 24	Dec. 2	Dec. 8	Dec. 15	Dec. 15
Monocultures—*Spirodela polyrhiza*							
25 Sp	5	10 ± 1	12 ± 2	31 ± 4	80 ± 40	202 ± 22	0.5
50 Sp	10	20 ± 2	25 ± 2	71 ± 6	210± 18	304 ± 26	0.7
75 Sp	15	28 ± 2	32 ± 3	103 ± 6	220 ± 13	380 ± 22	0.6
100 Sp	20	39 ± 5	47 ± 4	133 ± 5	300 ± 16	500 ± 28	0.6
Mixed cultures—*Spirodela polyrhiza* + *Salvinia rotundifolia*							
75 Sp	15	29 ± 6	33 ± 3	107 ± 3	157 ± 12	430 ± 14	0.3
25 Sr	5	9 ± 1	16 ± 3	30 ± 6	56 ± 19	160 ± 19	0.2
	20	38	49	137	213	590	
50 Sp	10	20 ± 3	23 ± 3	74 ± 10	147 ± 30	277 ± 42	0.2
50 Sr	10	18 ± 1	28 ± 1	56 ± 7	121 ± 22	309 ± 26	0.4
	20	38	51	113	268	586	
25 Sp	5	10 ± 2	11 ± 2	38 ± 5	67 ± 3	145 ± 11	0.1
75 Sr	15	25 ± 2	40 ± 5	75 ± 10	139 ± 30	375 ± 35	0.5
	20	35	51	113	206	520	
Monocultures—*Salvinia rotundifolia*							
100 Sr	20	34 ± 3	47 ± 6	123 ± 23	325 ± 62	445 ± 75	0.7
75 Sr	15	27 ± 1	44 ± 2	93 ± 10	235 ± 24	380 ± 40	0.5
50 Sr	10	20 ± 4	28 ± 5	61 ± 8	118 ± 15	260 ± 31	0.4
25 Sr	5	10 ± 1	16 ± 2	35 ± 5	70 ± 6	190 ± 18	0.3

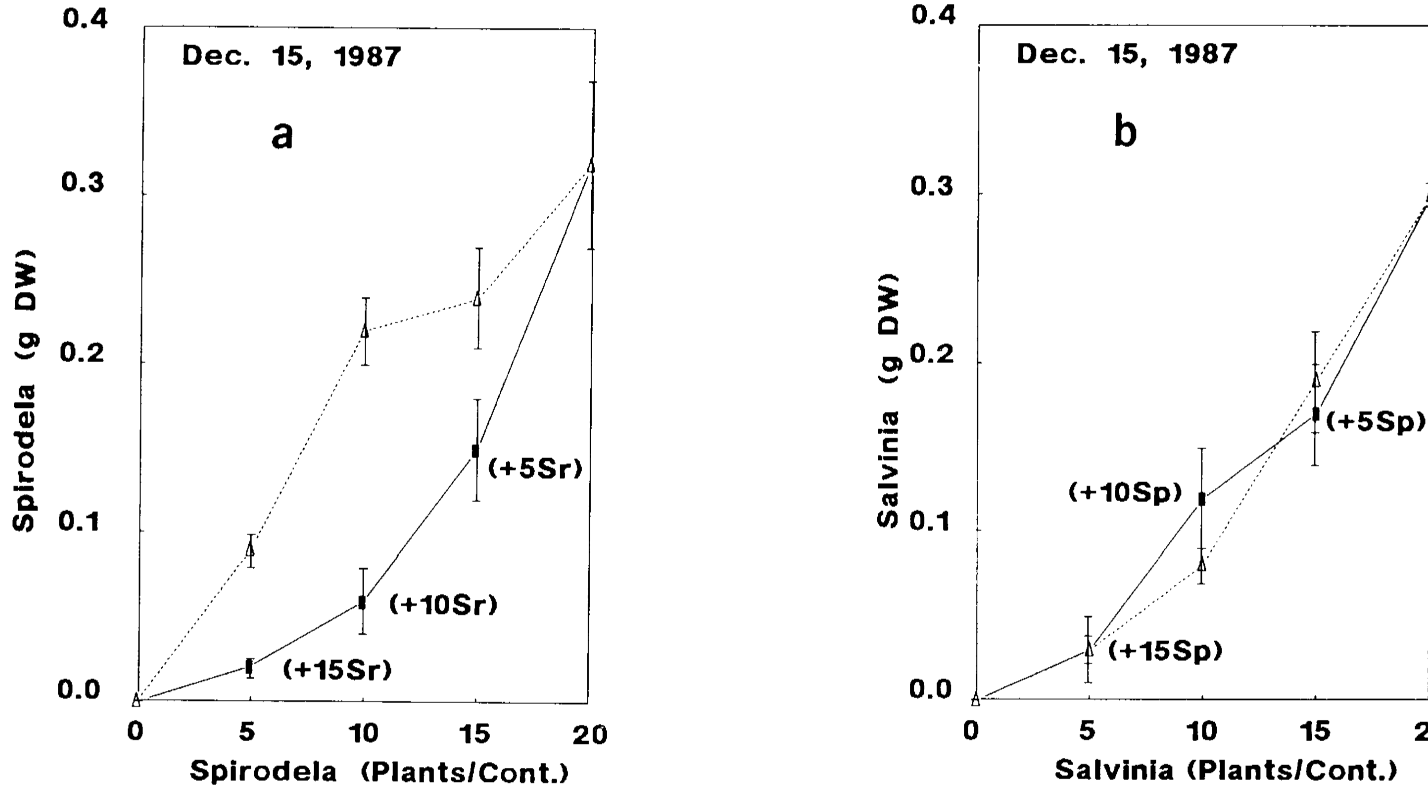

Figure 3. Comparison at the end of the experiments between standing-crop per container of (a) *Spirodela polyrhiza* plants grown in monoculture (broken line—open triangles) compared to plants of the same species grown from the beginning of the experiments with 5, 10 and 15 plants of *Salvinia rotundifolia* (Sr) (closed rectangles—unbroken line) and (b) yields for *Salvinia rotundifolia* plants grown in monoculture (broken line—open triangles) compared to values for plants of the same species grown from the beginning of the experiments with 5, 10 and 15 plants of *Spirodela polyrhiza* (Sp) (closed rectangles—unbroken line). Vertical bars represent standard deviations of the means.

tems. Examples of such aquatic habitats in Florida are the Wekiva River near Sanford, or the littoral zone of Lake Okeechobee. It seems that, in most natural ecosystems, competition between the two species is not only for space (as probably occurred during the present study, since no nutrient shortage in the culture medium was found), but also for nutrients (Reddy and DeBusk, 1985b).

Results obtained for the growth rates of aquatic macrophytes in monoculture may be inadequate to predict the growth rates in mixed cultures (Sutton, 1983). In four studies including the present one (Agami and Waisel, 1985; Agami and Reddy, 1989a,b), conducted recently in order to investigate the inter-relationships between several aquatic plant species, we have obtained different results from each experiment. For instance, submerged macrophytes such as *Najas marina* and *Myriophyllum spicatum*, do not naturally grow together in mixed stands. Competition experiments between them revealed bilateral negative relationships (Agami and Waisel, 1985). However, different results were obtained in the case of plant species that usually grow together in mixed stands; *Najas marina* was not negatively affected when grown in mixed cultures with *Potamogeton lucens* or *Scirpus litoralis* (Agami and Waisel, 1985). In mixed cultures of large-leaf floating aquatic macrophytes such as *Eichhornia crassipes* and *Pistia stratiotes*, the former had significant advantage and produced 5-fold higher biomass yield than *P. stratiotes* (Agami and Reddy, 1989a). On the other hand, *E. crassipes* and *Hydrocotyle umbellata* plants were not negatively affected by each other in mixed cultures and even produced much higher biomass yield than in monoculture during the winter season in Florida (Agami and Reddy, 1989b).

Plant tissue N and P contents of *Spirodela* and *Salvinia* by the end of the second set of experiments are shown in Table 5. Both plant species contained higher levels (by 2 to 3 fold) of N and P than before starting the experiments (Table 5). The concentration of N and P in *Spirodela* grown in mixed cultures was higher than in *Salvinia* (Table 5). However, plant N and P content of both species grown in monoculture was similar. The pH of the culture medium during mid-day in all containers was between 7.2 and 7.8 throughout the course of the second set of the experiments.

Among different species of duckweeds which have been tested (*Lemna gibba*, *L. minor*, *Wolffia arrhiza* and *Spirodela polyrrhiza*), it was found that the "competetive strength" of *S. polyrrhiza* was the highest (Wolek, 1974, 1979, 1984). However, *Spirodela* plants in the present study were suppressed when grown intermixed with *Salvinia*. Nevertheless, the storage of N and P in *Spirodela* plant tissue was higher than for *Salvinia* plants. The capability of duckweeds to assimilate nutrients from the culture medium has been reported in numerous studies (Sutton and Ornes, 1975; Porath and Pollack, 1982; Oron et al., 1984, 1986; Porath and Agami, 1986; Koles et al., 1987). However, the annual biomass yields of small-leaf floating

TABLE 5. PLANT TISSUE N AND P (mg^{-1} DRY WEIGHT) CONCENTRATIONS OF *SPIRODELA POLYRHIZA* (Sp) AND *SALVINIA ROTUNDIFOLIA* (Sr) GROWN INTERMIXED IN RECIPROCAL REPLACEMENT-SERIES AS WELL AS IN MONOCULTURE TO THE END OF THE SECOND EXPERIMENTS. CONTROL PLANTS WERE COLLECTED FROM THE WEKIVA RIVER AND MEASURED BEFORE STARTING THE EXPERIMENTS. MEANS ± SD OF 4 REPLICATES.

Initial plant density (%)	N	P
Monocultures—*Spirodela polyrhiza*		
25 Sp	42.0 ± 1.0	6.8 ± 1.1
50 Sp	42.0 ± 0.8	6.8 ± 0.7
75 Sp	32.7 ± 8.0	5.2 ± 2.0
100 Sp	34.0 ± 5.1	5.2 ± 1.0
Mixed cultures—*Spirodela polyrhiza* + *Salvinia rotundifolia*		
75 Sp	40.0 ± 1.7	6.9 ± 1.1
25 Sr	29.4 ± 5.6	3.8 ± 0.9
50 Sp	41.4 ± 1.6	7.3 ± 2.2
50 Sr	34.4 ± 1.7	4.5 ± 0.2
25 Sp	40.6 ± 3.7	6.6 ± 1.9
75 Sr	31.2 ± 2.2	4.2 ± 0.7
Monoculture—*Salvinia rotundifolia*		
100 Sr	33.4 ± 6.1	5.5 ± 0.5
75 Sr	31.3 ± 4.8	5.5 ± 0.3
50 Sr	36.8 ± 1.5	6.7 ± 0.6
25 Sr	38.5 ± 3.7	7.4 ± 0.4
Control Sp	22.1 ± 0.9	3.3 ± 0.4
Control Sr	19.4 ± 1.0	2.1 ± 0.4

macrophytes such as *Spirodela polyrhiza* and *Salvinia rotundifolia*, as well as *Lemna minor* and *Azolla caroliniana* (Reddy and DeBusk, 1985b), were significantly lower than for large-leaf floating aquatic macrophytes such as *Eichhornia crassipes*, *Pistia stratiotes* and *Hydrocotyle umbellata* (DeBusk et al., 1981; Reddy and DeBusk, 1984). Also, overall N and P removal by these small-leaf floating plants was low compared to that by the above-mentioned large-leaf floating macrophytes (Agami and Reddy, 1989a,b). Nevertheless, these small-leaf floating aquatic macrophytes may be suitable for growth intermixed with larger aquatic macrophytes (floating or/and emergent) in order to improve overall wastewater treatment efficiency.

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