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Growth of a Floating Aquatic Weed, Salvinia Under Standard Conditions

by

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ABSTRACT

1. Growth of the floating aquatic weed, *Salvinia*, in sterile culture was exponential for at least 2 weeks under standardized conditions.
2. Increase in light intensity or in CO_2 resulted in increases in growth rate, but did not extend the exponential period of growth.
3. This aquatic plant, like many others, discriminates against calcium relative to strontium.
4. In culture *Salvinia* exhibited luxury consumption of N and P.
5. Because of high C/N ratios, *Salvinia* may not be a favorable source of animal food, but might be useful in nutrient removal schemes.
6. In sterile culture, *S. molesta* produced fewer leaves than *S. minima*, but maintained a significant increase in leaf area and dry weight. This may be correlated with the ability of the first species to rapidly spread over tropical waterways.

INTRODUCTION

Salvinia is an interesting aquatic plant in that it is a fast-growing, free-floating fern. It produces dark green heartshaped floating leaves in pairs (Fig. 1) and often completely covers the surface of small bodies of fresh water. *Salvinia* differs markedly from other aquatic ferns, such as *Marsilea* and *Azolla*, and most other vascular plants in that it has no roots. In place of roots it sends out a very dissected, hairy, submerged leaf (Fig. 1), which is suspected of functioning like a root by absorbing nutrients and acting as a

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stabilizer. It has been grown in non-sterile culture in an inorganic nutrient medium by some workers (CLATWORTHY & HARPER, 1962; BLACKMAN, 1961). They did not investigate the nutritional requirements of the plant. RAO & NARAYANA (1968) studied the effect of minerals and auxins on *Salvinia* in aseptic cultures. GAUDET & KOH (1968) have described the growth and development of *Salvinia* and its response to various growth regulators in sterile culture. In these early experiments 2% sucrose was supplied as a carbon source. This was necessary because of the low light intensities used. In nature *Salvinia* must rely mostly on endogenously produced carbon compounds, thus, a culture medium should be designed and tested which to some extent simulates the nutrient content of the fresh water in which *Salvinia* grows. Also some general data are available regarding such variables as: temperature, light intensity, dissolved gases, pH, and specific conductance to which *Salvinia* is subjected in its natural tropical environment. These data could be used to design a simulated natural environment in controlled environment reach-in type growth chambers. The intent of this paper is to describe: 1) the establishment of such an environment and 2) the growth of *Salvinia* under these standardized conditions.

Many species of *Salvinia* are native to South America and have restricted ranges in that country (DE LA SOTA, 1962) while others have spread throughout the tropics and now threaten important fresh water resources (SCULTHORPE, 1967). Once standard growth conditions are established for sterile culture of this plant, it will be possible to do comparative growth studies among the seven South American species and to determine whether or not some are more compatible or more aggressive than others. In the experiments described below *Salvinia minima* BAKER (=*S. rotundifolia auct. var.* non WILLDENOW) was used since it has the widest range throughout the neotropics. It would seem to be the ideal species to use as a standard to which other species can be compared. A second species, used for comparison, was obtained from the Lake Kariba Fisheries Research Institute in Rhodesia. This species is still taxonomically uncertain but is a member of the *S. auriculata* complex of species which includes *S. auriculata* AUBL., *S. herzogii* DE LA SOTA, *S. biloba* RADDI and the *Salvinia* which has infested Lake Kariba (MITCHELL, 1970, pers. comm.) Because of their "closed" leaf hairs, all members of this complex can be readily distinguished from *S. minima* which has "open" leaf hairs (Fig. 1). The species from Lake Kariba has recently been named *S. molesta* (MITCHELL, 1972, pers. comm.)

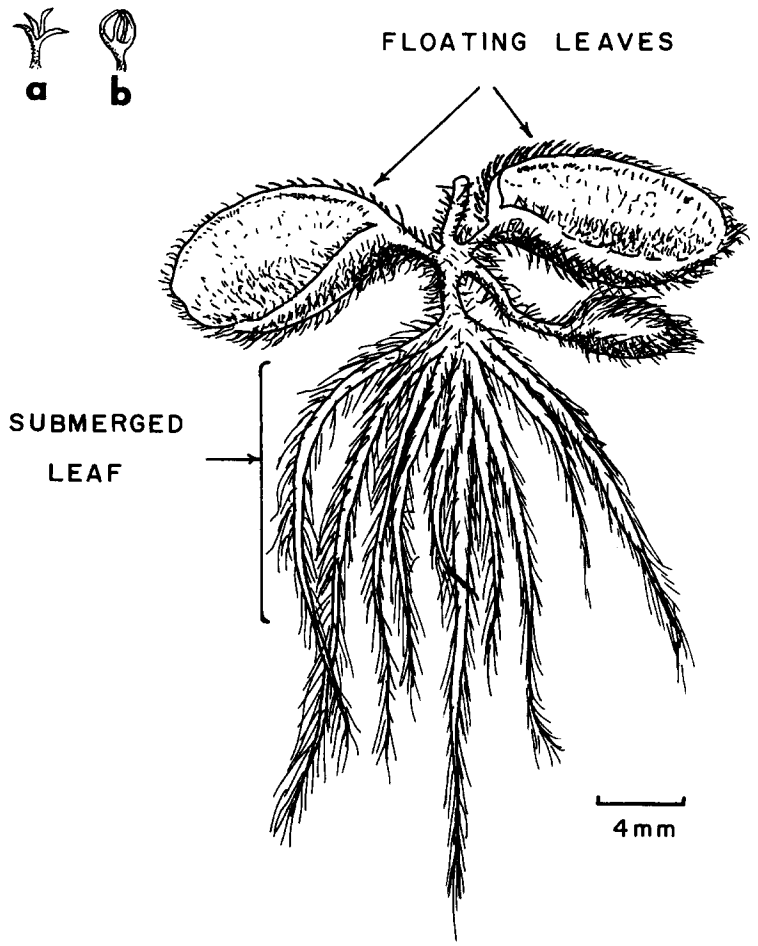

Fig. 1. *Salvinia minima*, a small terminal portion of an adult plant. a) leaf hair from *S. minima;* b) leaf hair from *S. molesta.*

Methods

Sterile Culture

The experimental flasks were inoculated using stock cultures of *Salvinia* which were maintained on a slightly modified White's nutrient medium as previously described (Gaudet & Koh, 1968). The experimental flasks were placed in growth chambers for the stated period of time. Each experiment was repeated at least once and 5 flasks were used for each treatment. The growth chambers (reach-in Model M-2, Environmental Growth Chamber Co.,

Chagrin Falls, Ohio) were programmed to simulate a generalized tropical environment, that is, an approximation of the climate in American, African and Asian equatorial regions (0°—10°N or S latitudes). Data from various sources (RICHARDS, 1957) indicate that in these areas one can expect an average daily photoperiod of approximately 12 hours, a yearly average of 6 hours of full sunlight per day, and a mean annual temperature of 27°C. In these regions higher temperatures with lower relative humidities occur during the day and lower temperatures with higher relative humidities occur at night. Thus, each chamber was programmed for a 12 hour day with "sunrise" beginning at 0800 EST (1/3 lights on), continuing through 0900 (2/3 lights on), and ending at 1000 (all lights on). Daytime temperature was 27 $\pm$ 0.6°C with relative humidity of 70 $\pm$ 4%. "Sunset" began at 1800 (2/3 lights on), continued through 1900 (1/3 lights on), and ended at 2000 (lights off). Nighttime temperature was 23 $\pm$ 0.6°C with a relative humidity of 86 $\pm$ 4%. The light intensity inside the flasks at the level of the plants was 0.68 x 10^5 ergs/cm^2/sec, as measured with a Radensiometer (GAUDET & DAMM, 1968). The chamber light source consisted of 10 incandescent (75 W) and 20 fluorescent lamps (215W, FR 96 T12-Cool White-1500, General Electric). The distribution of spectral energy within the chambers, outside in an unshaded area, and in our greenhouse was determined on a clear day at noon on June 2, 1969 with an Isco model SR Spectroradiometer (Fig. 2). The chamber light differed from sunlight in that 1) there was less far-red, infra-red, and blue light emitted; and 2) the total amount of radiation was considerably less. Two other light intensities were used for comparison to the normal intensity by placing plants closer to or further away from the light source. The total radiation and spectral emission is shown in Fig. 2 for each position. Inside the flasks at plant level the intensity was 0.46 and 1.12 x 10^5 ergs/cm^2/sec at the low and high positions.

Nutrient Medium

Salvinia is usually grown in liquid media (GAUDET & KOH, 1968; BLACKMAN, 1961; and RAO & NARAYANA, 1968) which contain ions far in excess of those levels normally found in fresh water (Table I). For comparison, the range of some ions present in natural water in which various species of *Salvinia* grow is shown in Table II. The data are for: 1) Lake Kariba in Africa (HATTINGH, 1962) in which *S. molesta* is found in abundance, 2) Singapore in South East Asia (JOHNSON, 1967), at the Botanic Garden (Station 29) and on the University grounds (Station 30) where *S. cucullata* ROXB. ex

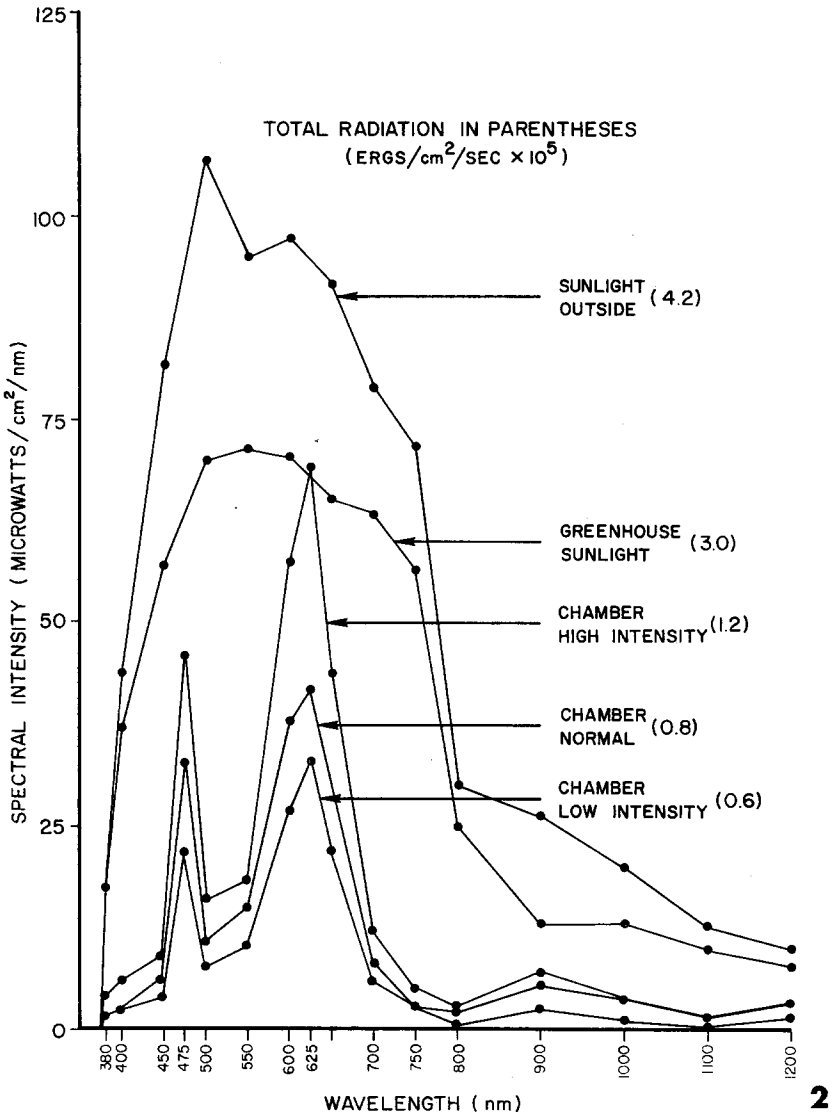

Fig. 2. Spectral emission and total radiation of various light sources.

Bory is prevalent, 3) and the Amazonian region of South America (Sioli, 1968) where both *S. minima* and *S. auriculata* are found. Nitrate, ammonia, organic N and phosphate determinations were done on Station 30 water by the author using techniques listed below.

Salvinia reaches a high growth rate in: 1) tropical ponds and

TABLE I

Summary of nutrients and conditions used for growth of Salvinia in culture.

	NO_3 (Mg/L)	NH_4 (Mg/L)	PO_4 (Mg/L)	PH	# of Days on Medium
Gaudet and Koh, 1968	457	-	15	4.5	28
Blackman, 1966*	215	-	559	5.1	2**
Rao and Narayana, 1968	239	50	16	6.5	30

*Personal communication
**Changed every 2 days

tanks after the monsoon rains when the water contains high levels of nutrients and 2) in rice paddies where the water has often been treated with various amounts of fertilizers, such as, ammonium sulphate, potash, and superphosphate. In order to simulate such enriched water, and to insure that nitrogen and phosphorus were not limiting, a basal nutrient medium was designed to contain levels of ions similar to the natural water supplemented with N,P, and K in the ratio of 5 : 10 : 5 (calculated as NH_3 : P_2O_5 : K_2O). The ionic composition of such a basal medium is shown in Table II. It was made up by adding (mg/l) : Mg SO_4 (16.8); $NaH_2PO_4.H_2O$ (73.7); $(NH_4)_2SO_4$ (356.6); $CaCl_2.2H_2O$ (29.0); KH_2PO_4 (219.2) and $Na_2SiO_3.9H_2O$ (3.4) to glass distilled water. To each liter of this was added 1 ml of a micro-nutrient solution containing (mg/100 ml): H_3BO_3 (60), $ZnSO_4$ (5), $MnCl_2$ (40), $CoCl_2$ (15), and MoO_3 (10); and 6.5 ml of an iron stock solution which contained 0.8 gm disodium EDTA and 3 ml of 10% $FeCl_3.6H_2O$ per liter. The pH after autoclaving (15 lbs/in^2 for 15 min) was adjusted to 6.5 with 0.1N KOH unless otherwise indicated. This last step is quite important since adjusting the pH previous to autoclaving results in a precipitate. Thus, the medium must be kept slightly acid while autoclaving.

After determining initial growth rates using the basal medium, experiments were done to determine the most usable form of nitrogen and the most practical levels of both phosphate and nitrogen. The first standard medium (SMI in Table II) was designed by replacing $(NH_4)_2SO_4$ with urea (106 mg/l) and reducing the Na and K phosphates to 52 and 20 mg/l respectively. The second standard medium (SMII in Table II) was identical to SMI except that: 7.2 mg of KNO_3 was added per liter.

In designing a standard medium, it was not intended to design a medium which produced excessive yields in culture. This can easily be done by growing *Salvinia* in White's medium with sucrose. On such a medium, calculated final yields exceed those obtained

TABLE II

Nutrients present in natural water, in which Salvinia grows, and in various media.

	Kariba, Africa[1]	Kariba, Africa[2]	Amazon R., S. Amer.[3]	Salvinia Ponds, Singapore[5] Sta.29	Sta.30	Range of all Stations	Basal Medium	Standard Medium I (SMI)	Standard Medium II (SM II)
pH	-	7.3	6.6	6.0	6.9	6-7.3	6.5	6.5	6.5
Cl		1.6	3.5	2.9	11.7	1.6-11.7	13.3	13.3	13.3
K	1.0-10.8	1.9	1.5	0.6	0.7	0.6-10.8	64.4	5.4	8.4
Mg	0-42.5	2.7	5.6	1.3	4.9	0.0-42.5	3.4	3.4	3.4
Na	-	4.1	2.5	1.7	2.9	1.7-4.1	12.4	9.2	9.2
Ca	8.8-108.9	12.7	18.4	6.0	11.7	6.0-108.9	7.6	7.6	7.6
SO_4	-	1.1	2.7	2.8	35.6	1.1-35.6	279.0	13.4	13.4
NO_3	0.00-0.07	0.02	0.20	-	0.00[4]	0-0.20	0.0	0.0	4.4
PO_4	0.03-1.21	0.03	0.11	-	0.00[4]	0-1.21	194.5	50.0	50.0
NH_3	-	0.05	-	-	0.05[4]	0-0.05	100.0	0.0	0.0
UREA-N	-	-	-	-	-	-	-	50.0	50.0
Org-N	-	0.13	2.62	-	0.13[4]	0.13-2.62	0.0	0.0	0.0

1. Mitchell, 1920, Ranges, Mg/Liter.
2. Hattingh, 1962, Averages.
3. Sioli, 1968, Maxima.
4. Obtained by Gaudet, 1967, Average of 2 samples.
5. All data except those marked "4" from Johnson, 1967, Averages.

in fertilized greenhouse tanks (Table III). In contrast, a standard medium would be designed to:

1) have an ionic content similar to enriched natural fresh water.
2) prevent any large change in pH during growth.
3) not precipitate after autoclaving.
4) allow exponential growth without deficiency symptoms under high light intensity for a given period of time (e.g. two weeks).

All sterile cultures were grown in 200 ml of medium in 500 ml Erlenmeyer flasks capped with a 100 ml beaker. This allowed for top lighting and air flow through the flask interior.

For the experiments with various sources of CO_2, the culture vessels were sealed and either air, or 5% CO_2 (in air) was passed through a metal tube (after filter sterilization) over the surface of the culture. The flow rate was approximately 3 cm^2/sec. Filter-sterilized bicarbonate was added as $NaHCO_3$ (100 mg/l) after autoclaving.

TABLE III

Total yield after 28 days of growth.

	Dry Weight (Gm/M^2)	Total Nitrogen (Gm/M^2)	Leaf Number ($\#/M^2 \times 10^3$)	Leaf Area (Dm^2/M^2)	Chloro-phyll (Gm/M^2)	MPR
Greenhouse Tanks (± S.E.)	25.05 (1.20)	1.45 (0.07)	26.70 (2.50)	183.37 (8.42)	0.427 (0.091)	1.92 (0.05)
White's Medium (± S.E.)	96.90 3.83	2.81 (0.08)	97.10 (3.42)	388.40 (64.41)	0.559 (0.014)	2.55 (0.01)
Basal Medium (± S.E.)	4.56 (0.25)	0.22 (0.01)	13.11 (1.03)	64.03 (7.66)	0.088 (0.018)	2.44 (0.07)

Growth Measurements

Fresh and dry (24 h at 100°C) weights were taken for each flask with a microbalance. Leaf area was determined as follows: Five paper squares each measuring 25, 50, or 100 mm^2 were xeroxed and images were cut out and weighed individually on a Gram-atic balance. A regression line was calculated and drawn for these standards. Forty-four leaves were then selected at random from plants growing in greenhouse tanks and in the sterile cultures. These were pooled and xeroxed in the center of the image plate to minimize distortion. The xeroxed images were cut out, weighed individually, on a Cahn microbalance and the mean area found from the regression line was 48.8 mm^2 (S.E. $\pm$ 4.2). The average area of a similar set of leaves determined from tracings with a planimeter was 45.9 mm^2. In order to maintain a check on possible fluctuations in the xerox paper, due to differences in batches, in every experiment, standards were taken along with the leaf samples. In all cases the weight of the 25, 50, and 100 mm^2 standards fell within our 95% confidence intervals for means, 2.1 $\pm$ 0.08, 4.5 $\pm$ 0.07, and 9.2 $\pm$ 0.16 mg, respectively. In the experiments below, 15 leaves were taken for areal measurement at 0, 7, 14, 21, or 28 days. Since each experiment was repeated, the data are averages of two trials. The 15 leaves used for area determination were also used to determine the amount of photosynthetic pigments. Where necessary the growth data were compensated for the omission of these 15 leaves.

The yields from greenhouse tanks and culture flasks were converted to a square meter basis for comparison. Otherwise, data are reported as averages per flask. As a standard for comparison the 95% confidence limits were calculated from standard deviations of growth data obtained from 35 cultures grown on basal medium. A specific treatment or modification was not considered significantly different unless two or more growth parameters fell above or below the limits of this standard. In one experiment using minimum concentrations of urea the 95% confidence limits were calculated from standard deviations of growth data obtained from 20 cultures.

Greenhouse Culture of Salvinia

Two fiberglass tanks 70 x 70 x 30 cm were set up on May 6, 1968 in the southern end of an unshaded greenhouse. Each tank was divided into 4 equal quadrants (35 x 35 x 30 cm) with wooden dividers (15 cm wide). One small terminal piece of *Salvinia minima* consisting of 2 sets of leaves, 2 axillary and 1 terminal bud, averaging 129 mg fresh weight, and 5 mg dry weight, was used as an inoculum.

After 0, 7, 14, 21 and 28 days the plants in each quadrant were harvested, and total leaf number was recorded. Fresh and dry weights were taken with a shadowgraph balance rather than a microbalance, otherwise the plants were treated to the same analyses as before. The tank cultures were repeated (June 3 to July 1) so that the data represent averages for four tanks.

The tanks were set up during May through June to take advantage of the long days and warmer temperatures. The greenhouse was equipped with automatic air vents and coolers which supplied cool moist air during very hot days. The average day temperature was 28.8 $\pm$ 8.5°C with a relative humidity of 43.1 $\pm$ 27.5%. During the night the average temperature was 24.3 $\pm$ 5.0°C with a relative humidity of 77.0 $\pm$ 26.0%. The average photoperiod was 14.1 $\pm$ 1.4 h. Light energy was measured with an Isco model SR Spectroradiometer (Fig. 2). The average light intensity at plant level was 3.03 ($\pm$ 0.01) x 10^5 ergs/cm^2/sec. Each tank was filled with tap water and 40 g of all-purpose, water soluble fertilizer (Miracle-Gro, Stern's Nurseries, Geneva, N.Y.) was then added. This fertilizer provided N,P and K at a ratio of 5 : 10 : 5 (calculated as NH_3: P_2O_5 : K_2O) with nitrogen supplied in equal parts by ammonium phosphate and urea. The average total combined nitrogen added was 48.0 mg/l of tank water.

Chemical Determinations

1) Total plant nitrogen – This was determined on 10 mg of dry plant material by micro-kjeldahl digestion.

2) Plant pigments – fresh, floating leaves were ground for 5 minutes in a mortar and pestle with 5 ml of 80% acetone buffered with Mg CO_3 (50 mg/l). The extract was brought up to 10 ml, centrifuged for 5 minutes, decanted and the supernatant was immediately read at 750, 665, 652, and 430 nm on a B&L "Spectronic 20" spectrohotometer. Total chlorophyll was calculated by subtracting the optical density at 750 from that at 652 mm to correct for turbidity, then multiplying by 0.019 (Arnon, 1949) to obtain mg total chlorophyll per leaf. The Margalef Pigment Ratio (MPR) was determined as a ratio of corrected optical densities, i.e., 430 nm/665 nm.

3) Water loss – On the average, 15 ml of water was lost from each flask during 28 days. Calculations are corrected where necessary to account for this.

4) pH – This was routinely determined with a Photovolt pH meter, but for those experiments in which pH changes were significant, a Beckman Expandomatic meter (model SS-2) was used.

5) Other determinations were done using the methods outlined in IBP Handbook No. 8 (GOLTERMAN, 1969). Determinations with handbook reference numbers are: Specific conductance – 3.1.2, Oxygen – 8.1.3, Nitrate – 5.3.1, Urea and ammonium ion – 5.5.1, Phosphate – 5.6.1, and Sulphate – 4.7.1.

6) Total iron was determined using the thiocyanate method (ANON. 1946).

7) Potassium, sodium, magnesium, calcium and trace elements were determined by semiquantitative spectroscopy on a 500 ml aliquot or a 2 g sample of dry plant.

8) C^{14} uptake was determined after the plants were exposed for one hour in growth chamber light to $Na_2C^{14}O_3$ (spec. activ. 5—20 mc/mM) added to the medium. A sample (7 mg dry weight) was rinsed several times in 0.05 N HCl until the rinse showed very little activity (10 ml final rinse produced 47 CPM). The sample was then homogenized in buffer, centrifuged at 12,000 x g and the supernatant was evaporated nearly to dryness, spotted on filter paper and counted in scintillation fluid.

RESULTS AND DISCUSSION

Growth

According to CLATWORTHY & HARPER (1962) floating aquatics such as *Salvinia* or *Lemma* show an exponential increase in dry weight during the first week (= Phase I). After this the relative growth rate (gm/gm/day) decreases due to frond crowding with the result that during the next 6 weeks the dry weight increases arithmetically (= Phase II). Phase I is limited by capital present, Phase II is limited by the amount of light or the rate of supply of nutrients.

The growth of *Salvinia minima* in sterile culture on basal medium in growth chambers (Fig. 3 and Table III) followed a similar pattern during the 4 week period. Growth during the first week was quite rapid followed by 3 weeks of slower growth. CLATWORTHY & HARPER show a decrease in relative growth rate of 85% during the first 4 weeks in non-sterile cultures of *S. natans*. In the experiments reported here similar results were obtained. Also it was noted that the doubling time of the cultures $\left(\frac{\text{Log } 2}{\frac{\log N_1 - \log N_0}{t_1 - t_0}} \right)$ calculated using leaf number (N) increases during the last 3 weeks (Fig. 3A). Clearly the first week in culture in basal medium is the period of maximum growth. It is interesting to note the differences obtained between greenhouse growth in tanks and growth in White's

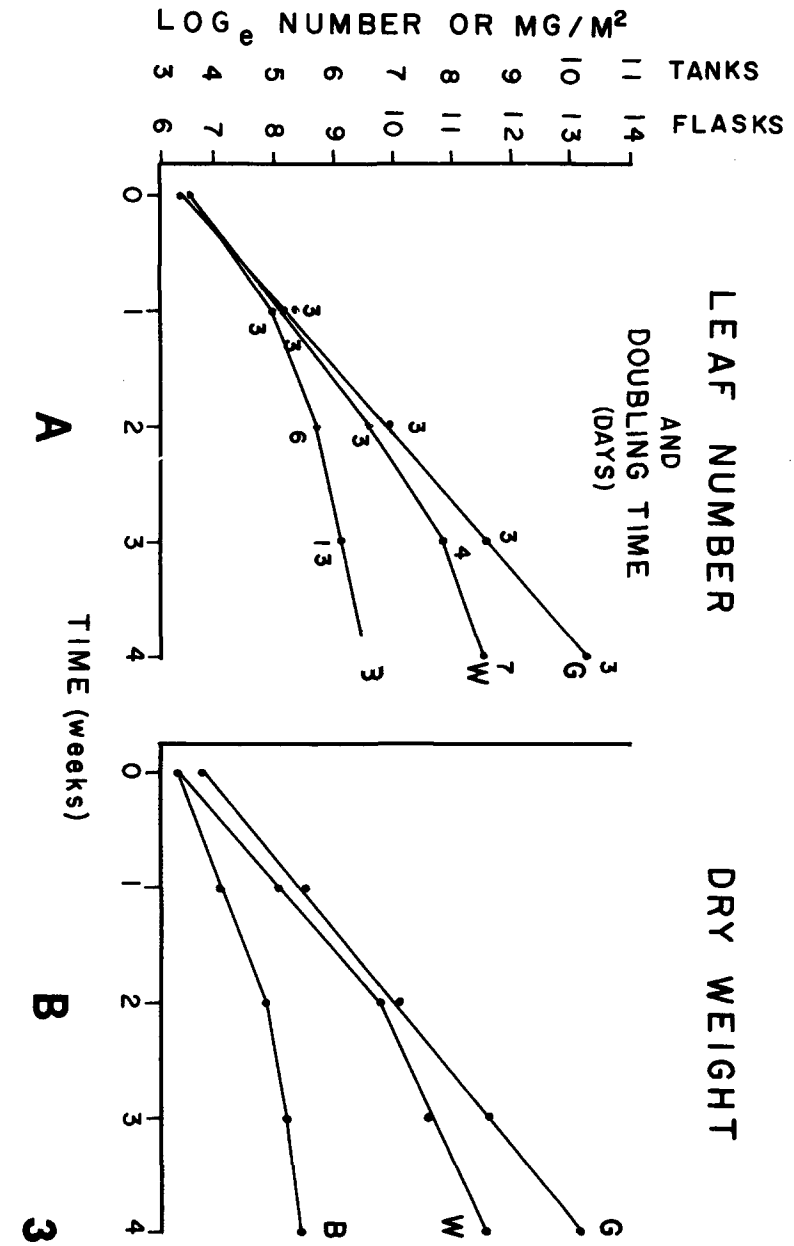

Fig. 3. Growth of *Salvinia minima* in basal medium (B), White's medium (W) and in greenhouse tanks (G).

or basal medium in the chambers. Figure 3A shows leaf number increases in an exponential fashion in all 3 cases during the first week with a doubling time of 3 days. During the next week leaf production of the plants in basal medium falls to less than exponential rates. During the third week the rate of leaf production in White's medium also decreases, but those in the greenhouse tanks maintain an exponential rate throughout the 4 week period. Not surprisingly, area and chlorophyll determinations follow patterns similar to leaf production.

Previous to the development of this basal medium it was noticed that plants in a high nitrate medium (White's medium, 475 mg/liter) remained quite green and sustained a high growth rate in low light intensity (0.04×10^5 ergs/cm^2/sec) with sucrose supplied (2%). But when these same cultures were grown in the growth chambers in which the light intensity is much higher, although appreciable growth continued (Fig. 3), the successive leaves produced after the first week appeared light green to yellow. The MPR after 28 days in White's medium was 2.55 compared to 2.44 for basal medium or 1.92 for the greenhouse tanks. This high MPR occurred because of a greater relative decrease in chlorophyll compared to carotenoids. This is evident when values for White's medium are subtracted from those in the greenhouse, e.g., the difference in O.D. at 665 nm was 0.813 and at 430 nm it was only 0.540. This relative decrease in chlorophyll in White's medium is indicative of either nitrogen or iron deficiency according to RAO & NARAYANA (1968). But an assay of the medium after 12 days of growth in White's medium, after these symptoms had begun, showed that nutrients were still present (PO_4=3.7 mg/l, NO_3= 311 mg/l, and total iron = 0.20 mg/l. It may be possible that nitrate alone does not supply enough nitrogen for growth of *Salvinia* in culture under high light intensity.

Source of Nitrogen in Basal Medium

Several types of nitrogen were compared to a high nitrate medium. The results are summarized in Table IV. All of the nitrogen sources used are adequate for growth and in this respect *Salvinia* is like other floating aquatic plants such as *Lemna* or *Spirodela* which are able to grow on a wide variety of nitrogen sources (JOY, 1969; FERGUSON, 1969). But only in the cases of ammonium ion and urea did the results fall within or above the confidence levels for all six parameters. Because of this, reduced nitrogen was accepted as the best nitrogen source in these cultures. Also reduced nitrogen would seem to be the most natural source since regeneration in fresh water is mostly in reduced forms such as ammonia or organic compounds

TABLE IV

Effect of different nitrogen sources on the total yield after 28 days.

Treatment (Mg/L)	Dry Weight (Mg/Flask)	Total Nitrogen (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR
0	10.3	0.069	18	0.077	0.026	2.99
Nitrate (100)	20.3	0.914	64	0.246	0.329	2.49
Nitrate (475)	22.7	0.953	81	0.276	0.411	2.43
Glycine (100)	21.0	0.909	50	0.220	0.330	2.55
Urea (100)	24.0	0.993	68	0.360	0.460	2.39
Ammonium (100)	23.0	1.122	72	0.281	0.424	2.32
95% Conf. Levels for B.M.	24.8 22.4	1.442 0.692	66 59	0.311 0.281	0.461 0.327	2.28 2.46

(JOHANNES, 1968). Nitrate significantly increased the number of leaves in *Salvinia* and this is also true of *Spirodela* in buffered medium (FERGUSON & BOLLARD, 1969). None of the combinations of nitrate and ammonium salts (Table V) fell within or above the confidence levels for all six parameters. As before, nitrate alone allowed a significant increase in leaf number. It is also obvious that increasing the reduced form of nitrogen increases the chlorophyll content of the leaves. This relationship holds even if nitrate is excluded and only ammonium ion is supplied in varying quantities as in Table VI.

On the basis of the higher pigment content induced, it was felt that reduced forms supplied as either ammonium ion or urea, would be the most appropriate nitrogen source in the medium. But NH_4 has a notorious tendency to create acidic conditions in a plant's environment. In this case NH_4 produced a marked drop in the pH of the medium (Fig. 4). Accompanying this drop in pH was a loss of chlorophyll (Fig. 5) which could be prevented somewhat by correcting the pH of the medium. That is, each week the pH was adjusted back to the original level. Here pH seems to have an important effect on the pigment status probably by its effect on the solubility of iron which in turn would be necessary for chlorophyll

TABLE V

Effect of combinations of nitrate and ammonium on total yield after 28 days.

Treatment (Mg/L)	Dry Weight (Mg/Flask)	Total Nitrogen %	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR
NO_3 (100)	21.0	4.2	87	0.261	0.284	2.70
NH_4 (25) + NO_3 (75)	24.0	4.3	58	0.272	0.365	2.23
NH_4 (50) + NO_3 (50)	25.5	4.7	63	0.279	0.398	2.25
NH_4 (75) + NO_3 (25)	25.5	4.9	58	0.279	0.349	2.28
NH_4 (100)	24.0	5.0	69	0.337	0.465	2.44
95% Conf. Levels for B.M.	24.8 22.4	5.8 3.0	66 59	0.311 0.281	0.461 0.327	2.28 2.46

TABLE VI

Effect of ammonium on total yield after 28 days

Treatment (Mg/L)	Dry Weight (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR
NH_4 (10)	21.0	54	.302	0.319	2.39
NH_4 (20)	22.0	61	.366	0.373	2.32
NH_4 (40)	20.5	54	.302	0.437	2.36
NH_4 (80)	22.3	60	.342	0.440	2.37
NH_4 (100)	24.0	69	.337	0.465	2.44
95% Conf. Levels for B.M.	24.8 22.4	66 59	0.311 0.281	0.461 0.327	2.28 2.46

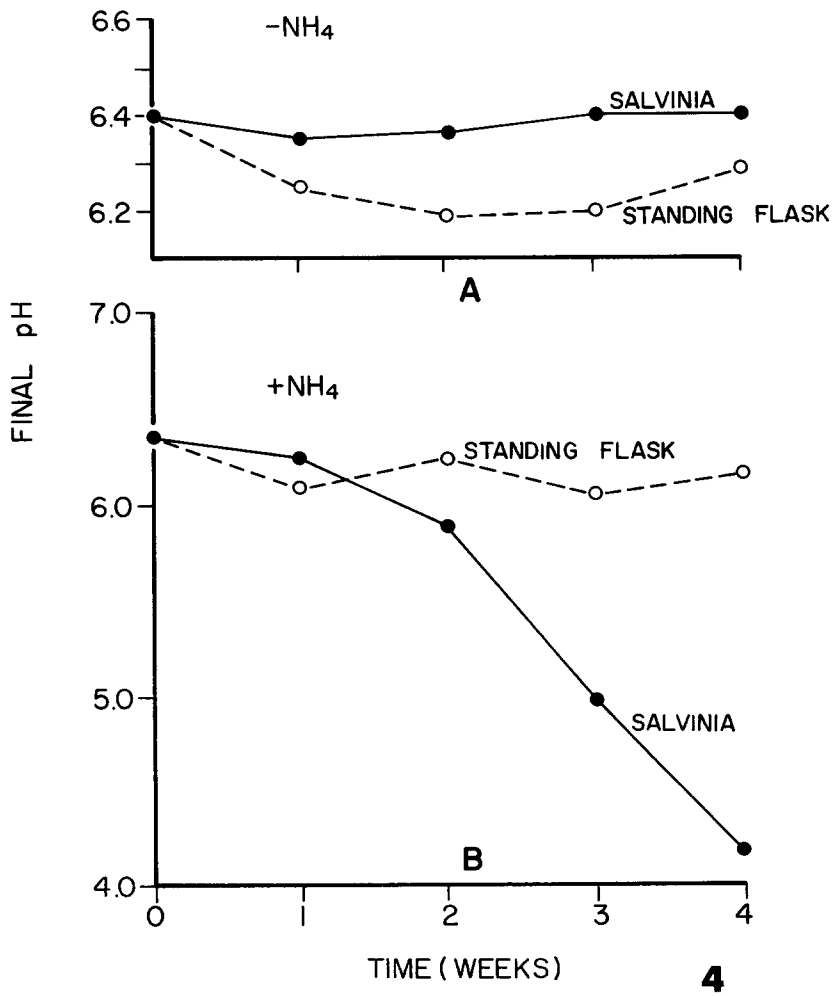

Fig. 4. Effect of ammonium ion on final pH of medium.

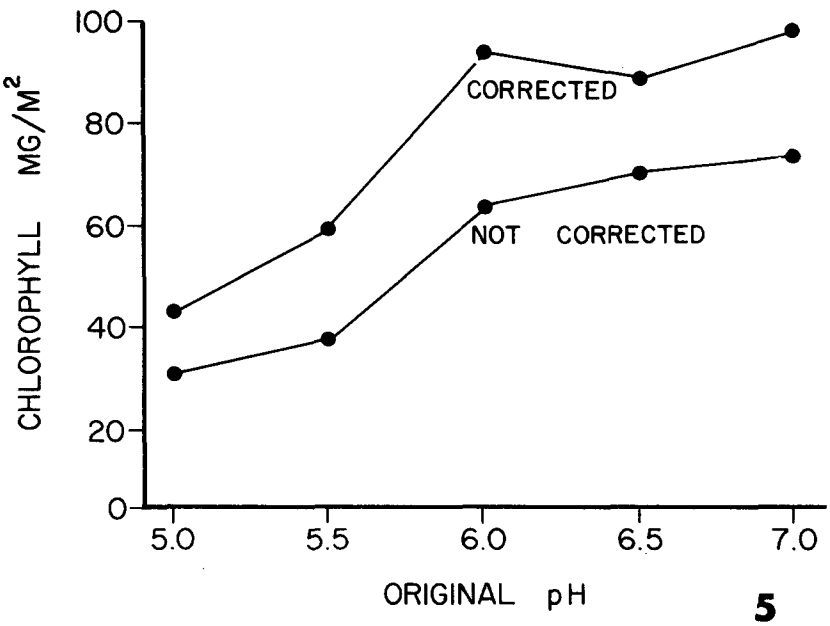

Fig. 5. Effect of pH on chlorophyll level after 28 days.

formation. It was decided to use urea in place of ammonium ion. This would prevent any drop in pH so that the final pH of the medium would remain within a range comparable to that found in nature (ca. 6.0—7.3). In this case a pH of 6.5 was selected (after autoclaving). It was also necessary at this point to determine what the effects, if any, would be of small amounts of ammonium ion. Small amounts of ammonium ion have been shown to increase the number of fronds in another floating aquatic plant, *Spirodela oligorhiza*. BOLLARD & COOK (1968) found that the number of fronds increased when urea was supplied (56 mg N/liter) but only if the pH of the medium was lowered. A small amount of ammonium ion (2 mg N/l) added to their medium lowered the pH sufficiently to allow for an increase in frond number when urea was supplied. The results for a similar experiment are shown in Table VII. In this experiment N-starved plants were used as an inoculum. This was accomplished by using plants which had been transferred to a N-free medium for 4 days and then rinsed with sterile distilled water prior to use.

In this case a slight decrease in pH was noted, but small amounts of ammonium have little effect on growth of *Salvinia* in urea.

TABLE VII

Effect of combinations of urea and ammonium on the total yield after 28 days.

Treatment (Mg N/L)	Dry Weight (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR	Diff. in Final pH from Orig. (6.5)
NH_4 (1)	18.3	43	0.176	0.112	2.52	-0.152
UREA (56) + NH_4 (1)	21.6	59	0.299	0.391	2.22	-0.161
UREA (56)	25.0	67	0.298	0.386	2.24	-0.132
95% Conf. Levels for B.M.	24.8 22.4	66 59	0.311 0.281	0.461 0.327	2.28 2.46	-2.733 -2.291

Nutrients in Standard Medium

Up to this point the experiments were done on basal medium with modification chiefly in the nitrogenous components and in pH. In designing a standard medium, the fourth requirement mentioned earlier was that this medium should supply most of the common ions in sufficient quantities such that growth would not be limited by nutrients. Macronutrients were probably not limiting in the basal medium, since preliminary analyses indicated that there were still excess nutrients after 28 days of growth. In order to adjust the levels of SO_4, Na and K to those present in natural water, the amount of PO_4 was reduced to 50 mg/l and urea was substituted for ammonium ion at 106 mg/l. This last modification reduced the level of N to 50 mg/l which is still more than sufficient for growth. This modified medium is referred to as SMI (Table II). An analysis of SMI after 21 days of growth (Table VIII) shows a decrease in most nutrients. In regard to micronutrients the semiquantitative technique used was not accurate enough to show significant differ-

TABLE VIII

Effect of Salvinia on nutrients in SMI.

Nutrient	Day 0 (Calculated Mg/L)	Day 0 (By Analysis Mg/L)	Day 21 (By Analysis Mg/L)
Chloride	13.3	-	-
Potassium	5.4	5.0	1.5
Magnesium	3.4	2.1	1.4
Sodium	9.2	6.6	6.5
Calcium	7.6	7.1	7.0
Sulphate	13.4	8.0	5.0
Phosphate	50.0	34.0	30.0
Nitrogen (Urea)	50.0	48.0	41.5
Loss on ignition (%)		0.0131	0.0019
Specific conductance (micromhos/cm)		204.5	165.0

ences due to growth, but it is evident (Table IX) that SMI contains sufficient supplies of trace elements to support growth in culture during the period of the experiments. Silicon and boron, in

TABLE IX

Effect of Salvinia on trace elements in SMI.

Trace Element	Day 0 (Calculated Mg/L)	Day 0 (By Analysis Mg/L)	Day 21 (By Analysis Mg/L)
Iron	0.406	0.285	0.290
Silicon	0.250	0.400	0.380
Boron	0.105	0.275	0.310
Zinc	0.010	-	-
Manganese	0.110	0.135	0.150
Cobalt	0.035	0.014	0.017
Molybdenum	0.065	0.041	0.030
Aluminum	-	0.100	0.055
Tin	-	0.010	0.036
Copper	-	0.004	0.003
Titanium	-	0.028	0.013
Nickel	-	0.003	0.004
Strontium	-	0.004	0.004
Chromium	-	0.001	0.001

fact, increased over calculated values probably as impurities from the glassware.

Floating aquatic plants are said to thrive in water of low K + Na: Ca + Mg ratios (SCULTHORPE, 1967). Low ratios would be common, e.g., in Florida in the USA and in the Asian and American tropics (Table II). The ratio for SMI is 1.3. Although this is higher than most Asian and American tropical water, it is still similar to water in some areas, e.g., the average ratio for rivers in Malaya is 1.0 (KOBAYASHI, 1967), also in one pond in India the average ratio was 1.1 and this was a eutrophic and very productive pond with total N and P contents of 33 and 8 mg/l, respectively (MUNAWAR, 1970). In the African tropics, however, a ratio of more than 1 is common. Lake Kariba (Table II) has a low ratio, but of 65 African lakes listed by TALLING & TALLING (1965) more than half have ratios higher than 1. In fact, Lake Naivasha in Kenya has a ratio of 2.5 and in this lake *S. molesta* recently has undergone an "explosive" growth phase reminiscent of its performance in Lake Kariba (GAUDET, 1972, personal observation).

Nitrogen in the Standard Medium

One noticeable effect of varying the nitrogen content in SMI was the change in length of the submerged leaf (Fig. 6). N concentrations lower than 50 mg/l resulted in a dramatic increase in the length of this leaf. MITCHELL (1970, pers. comm.) has noticed this phenomenon in the field where *Salvinia* plants tend to have longer

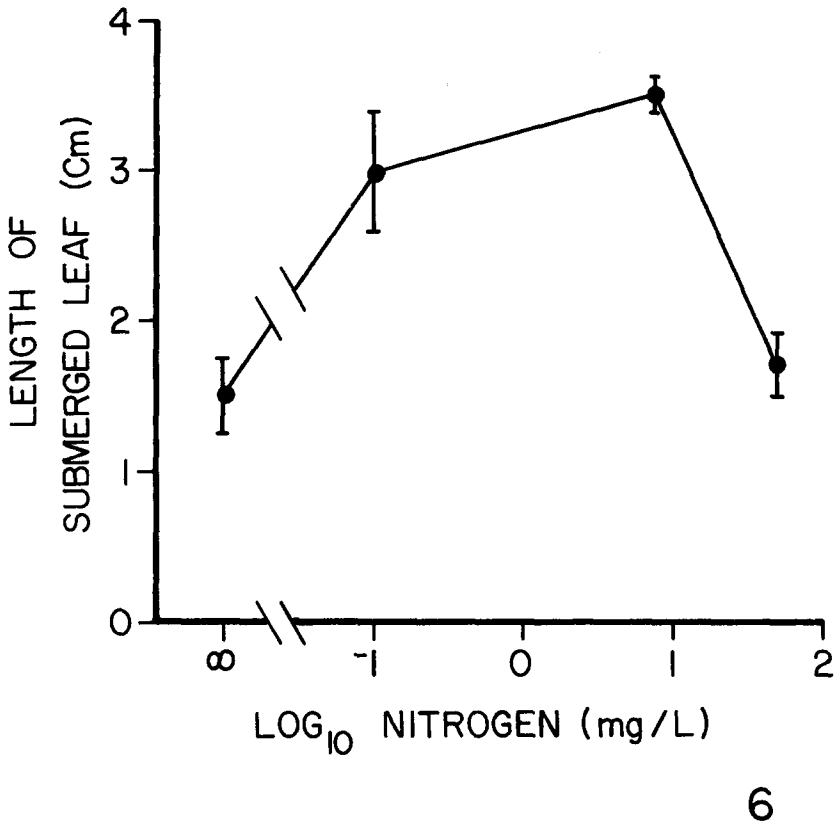

Fig. 6. Effect of nitrogen on the submerged leaves of plants in SMII after 21 days. Each vertical line represents two standard errors.

submerged leaves when growing under low nutrient conditions. The increase in submerged leaf length in low nitrogen medium was accompanied by a decrease in size of the floating leaf such that the ratio of length of submerged to floating leaf is 1.7 in 7.5 mg N/l versus 0.7 in 50 mg N/l.

In order to assay the effect of other forms of reduced N, such as amino acids, it would be useful to know the minimum level of urea-N in SMI. That is, the lowest concentration of urea which does not result in deficiency symptoms such as leaf yellowing (or reduction in chlorophyll level). In deciding on a minimum level any level would be accepted which results in a production of chlorophyll higher than 0.150 mg. Since this amount of chlorophyll could not be due to chance (0.036 mg produced in N-free media), nor to nitrogenous impurities (0.107 mg produced in NO_3, at 1 mg

N/l; 0.112 mg produced in NH_4, at 1 mg N/l). A low MPR is another useful measure of yellowing since a low value indicates more chlorophyll pigment compared to carotenoids. Here the minimum would be any ratio lower than 2.50, since again this would not be due to chance (2.99 in N-free media), nor to nitrogenous impurities (2.55 in NO_3, at 1 mg N/l; 2.52 in NH_4, at 1 mg N/l). The lowest concentration of urea allowing chlorophyll production higher than 0.150 mg, and an MPR lower than 2.50, was 7.5 mg N/liter (Table X). It was felt that perhaps other sources of N such

TABLE X

Effect of low concentrations of urea on total yield after 28 days.

UREA (Mg N/L)	Dry Weight (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR	Diff. in Final pH from Orig. (6.5)
0.1	15.1	28	0.064	0.052	2.63	-0.42
0.5	17.0	30	0.075	0.049	2.71	-0.52
2.5	19.0	43	0.172	0.170	2.50	-0.44
5.0	19.3	52	0.208	0.249	2.51	-0.46
7.5	20.1	52	0.213	0.241	2.44	-0.51
10.0	20.4	57	0.216	0.274	2.43	-0.52

as amino acids might affect growth, especially if supplied along with this minimum N level in SMI. Of the many choices available those three amino acids were chosen which are normally found in abundance among the free amino acids in *Salvinia* (LÄHDESMÄKI, 1968): alanine, glutamic acid and glutamine. They comprise 7.1, 12.7, and 64.7% of the total free amino acid content of *S. natans* respectively. Table XI summarizes the results. High concentrations of the three amino acids definitely inhibited growth. The lowest concentrations, which were below physiological, had no significant effect.

Nitrate is usually present in the plant's environment (Table II). When used with urea, nitrate produced a significant increase in leaf number (Table XII). Because of this increase in leaf number, 1 mg N/liter as nitrate was added to SMI. This modification changed the level of potassium and consequently the new medium is referred to as "SMII" (Table II). In this medium after 21 days, *Salvinia minima* contained 3.5—4.8% total nitrogen on a dry weight basis. LÄHDESMÄKI (1968) found lower levels in *S. natans*, since under continuous light his cultured plants contained 2.9% total nitrogen on a dry weight basis (versus 3.7% when the plants were shifted to continuous darkness.)

TABLE XI

Effect of 3 amino acids on total yield after 28 days.

Amino Acid (Mg/L)	Dry Weight (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR
Alanine					
0.05	22.6	71	0.227	0.217	2.45
0.25	19.9	61	0.180	0.187	2.45
0.50	19.2	59	0.185	0.195	2.34
1.00	19.1	50	0.107	0.204	2.34
Glutamine					
0.75	22.2	63	0.223	0.178	2.38
1.50	21.6	62	0.239	0.237	2.34
3.00	21.5	60	0.228	0.213	2.43
4.50	19.3	57	0.175	0.182	2.41
Glutamic Acid					
0.15	18.4	52	0.163	0.129	2.45
0.30	17.9	53	0.191	0.209	2.41
1.50	17.3	49	0.167	0.207	2.35
3.00	7.5	46	0.163	0.194	2.38
95% Conf. Levels for SMI (7.5 Mg N/L)	23.0 19.2	65 53	0.252 0.204	0.271 0.223	2.35 2.43

TABLE XII

Effect of nitrate on growth of Salvinia in SMI.

Treatment (Mg N/L)	Dry Weight (Mg/Flask)	Leaf Number (#/Flask)	Leaf Area (Dm^2/Flask)	Chloro-phyll (Mg/Flask)	MPR
UREA (56)	25.0	67	0.298	0.386	2.24
NO_3 (1)	19.1	42	0.160	0.107	2.55
UREA (56) + NO_3 (1)	22.6	72	0.295	0.394	2.35

Growth under Standard Conditions

Under standard conditions, because nutrient are available, and high temperatures are used, the growth of *S. minima* should be limited mostly by light intensity. An increase in light intensity should result in an extension of the exponential phase of growth. But, over long periods of time, such growth results in a great number of leaves per flask with consequent crowding and self-shading. During the first two weeks in culture in White's nutrient or under greenhouse conditions (Fig. 3A) exponential growth was achieved and self-shading had not yet occurred. After this time, in White's medium, the plants have covered the surface of the medium and subsequent growth is less than exponential. In order to avoid such crowding effects, growth in SMII over 2 weeks was selected as the standard growth period. In fact, during the first two weeks at three different light intensities, growth was exponential (Fig. 7 and Table

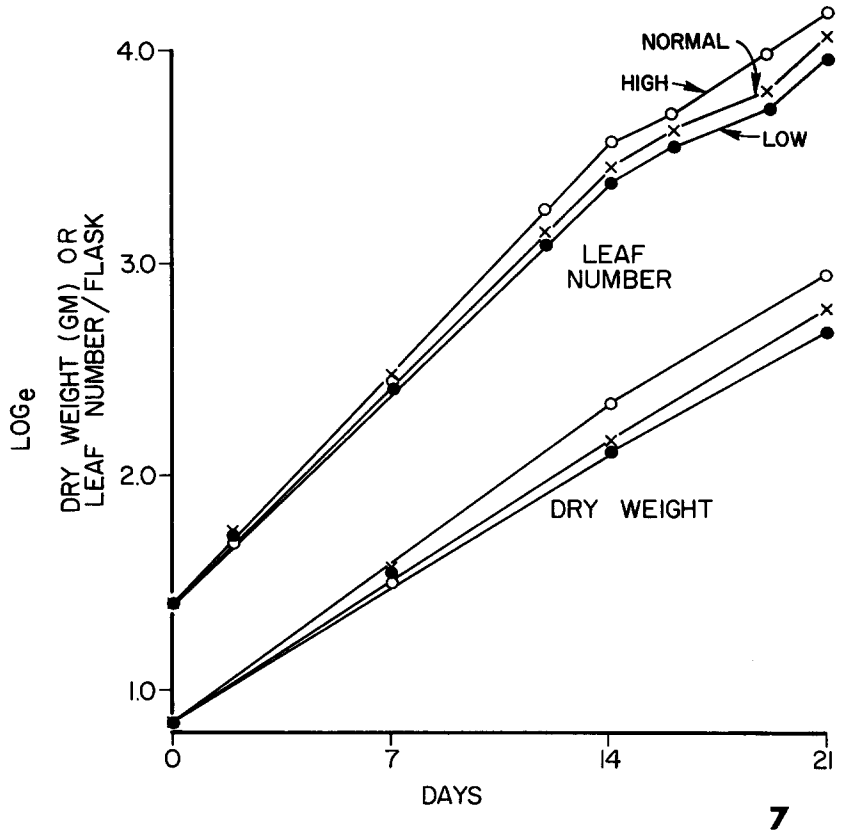

Fig. 7. Effect of light intensity on growth of *Salvinia minima* in SMII.

XIII). As could be expected, the growth rate in SMII was dependent on light intensity with the higher rate achieved under higher light intensity. ZUTSHI & VASS (1971) using cultures of *S. natans* in lake water under laboratory conditions found that low light intensity (partial shading) caused a large increase in fresh weight over the high light intensity (no shading). But in this case nutrients may have been limiting under the high light intensity conditions. Thus SMII was a distinct improvement over the earlier media tested, since it prolonged the exponential phase of growth to slightly beyond two weeks.

MAC DOWALL (1969) suggested that the simplest kinetically valid measure of growth for higher plants could be obtained as follows. Under non-limiting circumstances, the logarithm of some measure of growth (e.g., dry weight, leaf number, leaf area, etc.) taken during the exponential phase is plotted against light intensity. The slope

TABLE XIII

Effect of light intensity on growth of Salvinia in SMII.

Treatment	Day							
	0	2	7	12	14	16	19	21
Low								
Leaf #	3.8	5.7	11.2	22.6	29.3	35.7	42.0	53.7
(± S. E.)	(0.1)	(0.3)	(0.7)	(1.1)	(1.5)	(1.6)	(1.5)	(1.9)
Dry Wt. (Mg)	2.23		4.66		8.33			14.73
(± S. E.)	(0.11)		(0.42)		(0.40)			(0.72)
Normal								
Leaf #	3.8	5.6	12.1	23.4	31.7	38.1	45.7	59.0
(± S. E.)	(0.1)	(0.3)	(0.6)	(1.5)	(1.7)	(1.8)	(2.7)	(3.3)
Dry Wt. (Mg)	2.23		4.59		8.71			16.11
(± S. E.)	(0.11)		(0.33)		(0.49)			(0.84)
High								
Leaf #	3.8	5.3	11.4	26.4	36.4	40.7	54.8	65.7
(± S. E.)	(0.1)	(0.2)	(0.8)	(1.4)	(1.6)	(1.8)	(2.6)	(2.4)
Dry Wt. (Mg)	2.23		4.49		10.50			19.21
(± S. E.)	(0.11)		(0.19		(0.81)			(1.20)

of the resulting straight line would be proportional to the growth rate constant. Figure 8 shows such a plot for *Salvinia minima* with doubling times for each point. The line was fitted by regression ($Y = 3.1628 + 0.3583\ X$, and $r = 0.98$). A prediction based on the regression formula indicated that at the greenhouse light intensity, *Salvinia minima* after 2 weeks should produce approximately 69.4 $\pm$ 3.5 (S.E.) leaves with a doubling time of 3.5 days. This would be so, since tank growth was comparable to flask growth in so far as the inoculum consisted of a 4-leaved plant, the tank medium resembled SMII, and exponential growth was still occurring. The actual number of leaves produced in the tanks after 2 weeks was 68.0 $\pm$ 10.0 (S.E.) with a doubling time of 3.4 days. Thus, knowing the growth rate constant in culture and the average light intensity in nature, it should be possible to predict the growth rate for different species of *Salvinia*. This experiment was repeated several times, subsequently, and the slope of the line remained constant. However, the means for each light intensity fall within broad confidence limits. Thus, in Fig. 11 the slope of the line for leaf number of *S. minima* would be the same as that in Fig. 8 if plotted using similar axes, but the means would still be higher. This indicates that any prediction formula should be based on several separate experiments. Such a prediction, if based on growth data obtained during 2 weeks under conditions similar to those described above would enable one to identify those species which could be potential tropical weeds.

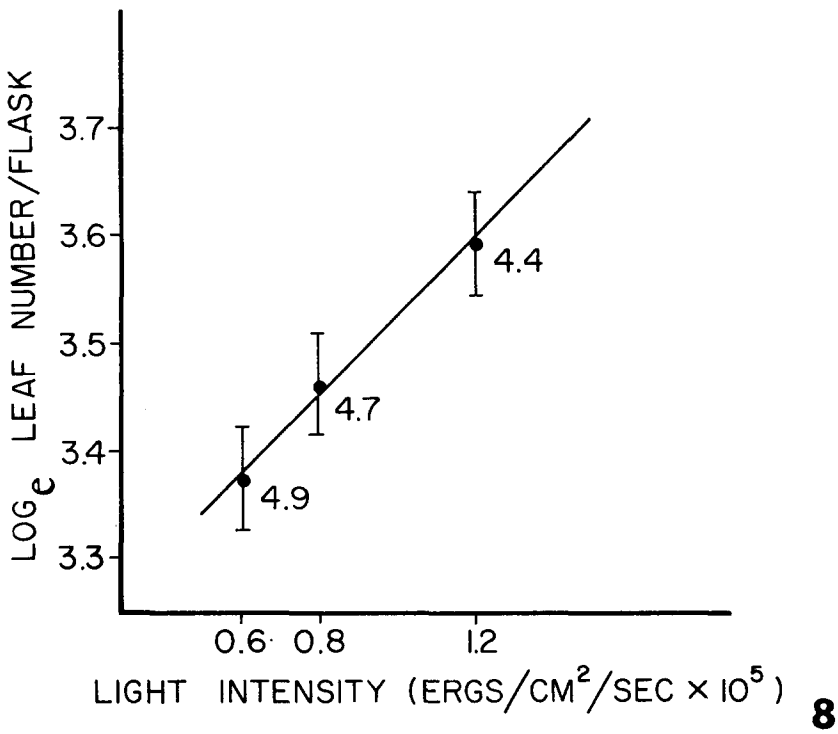

Fig. 8. Relationship between growth and light intensity. Each vertical line represents two standard errors. Line fitted by regression analysis, see text for equation and correlation coefficient.

The decrease in growth rate after 2 weeks may be due to several limiting factors, such as the rate of diffusion of CO_2. But increasing the amount or source of CO_2 did not extend the period of exponential growth, since all of the graphs in Fig. 9 are less than exponential. Both air and 5% CO_2 (in air) had an inhibitory effect on stem length and leaf expansion but allowed increases in leaf number over the control. The inhibitory effect on growth in length might have been due to the lower pH values in the medium caused by these two treatments (Fig. 9) since bicarbonate caused an increase in pH as well as leaf number, but had no inhibitory effects. That the plant can readily take up Bicarbonate-CO_2 is shown in Table XIV.

The chemical composition of an aquatic plant will vary with the season, (BOYD & BLACKBURN, 1970) but, in general, certain elements such as K seem to be accumulated by many aquatic plants (BOYD, 1970, and DENTON, 1965). This is also true of *Salvinia* (Table XV) since it contains levels of K which are generally higher than the mean for aquatic vegetation. Other elements commonly assayed

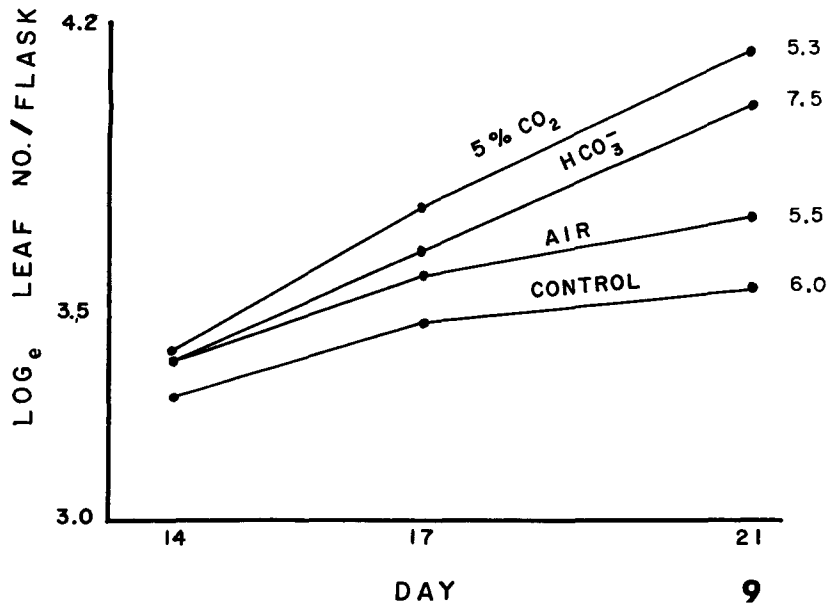

Fig. 9. Effect of different sources and levels of carbon dioxide on growth at high light intensity. Final pH of medium indicated at the right of each graph. (Original pH = 6.5).

TABLE XIV

C^{14} uptake in Salvinia after one hour in light in SMII.

	Age of Plants in Culture (Weeks)	^{14}C Uptake (cpm)
Control (no ^{14}C added)	2	3
	5	7
$Na_2{}^{14}CO_3$ (5^2 µc/200 ml SMII)	2	140,000
	5	20,000

vary considerably depending on the species and growth conditions. In general, some elements are obviously lower than the mean for vegetation (Ca) and some are not much different (Fe). P is much higher in plants from sterile culture or greenhouse tanks. Mg is higher in cultured plants of *S. minima* than in nature. Na is lower in plants from sterile culture than in either the greenhouse tanks or in nature.

According to OPHEL & FRASER (1970) most aquatic plants discriminate strongly against calcium, that is, they can utilize calcium less effectively than strontium. Consequently, aquatic plants may serve as an enrichment step for strontium in aquatic food

TABLE XV

Mineral composition of Salvinia. Plants from sterile culture after 21 days growth in SMII; plants from greenhouse tanks several months old from compact mats; data on plants from small pond from Denton (1965).

	S. minima	S.molesta				
	Sterile Culture	Greenhouse Tanks	in Small Pond[1]	Greenhouse Tanks	Lake Kariba[2]	Aquatic Plant Mean[3]
Ash	19.95	22.55	12.80	20.20	17.2	16.70
Potassium	6.20	4.75	5.20	6.30	2.98	2.48
Sodium	0.22	0.37	2.00	0.87	1.29	0.56
Magnesium	1.10	1.50	0.36	0.41	0.44	0.48
Calcium	0.14	0.39	0.90	0.28	1.11	2.11
Phosphorous	0.64	0.95	0.22	0.92	0.17	0.18
Nitrogen	4.10	3.70	4.05	3.60	1.35	1.97
Iron	0.026	0.021	0.023	0.029	0.079	0.19
Silicon	0.720	0.185	-	0.300	-	-
Boron	0.001	0.002	-	0.002	0.001	-
Manganese	0.0021	0.0460	0.0150	0.0340	0.0380	0.18
Molybdenum	0.0019	0.0005	-	0.0000	-	-
Aluminum	0.0014	0.0008	-	0.1700	0.0680	-
Tin	0.0007	0.0000	-	0.0000	-	-
Copper	0.00026	0.00092	0.0090	0.0034	0.0017	0.0018
Titanium	0.00024	0.00090	-	0.0030	-	-
Nickel	0.00100	0.00140	-	0.00069	-	-
Strontium	0.00180	0.00190	-	0.01300	-	-
Chromium	0.00028	0.00050	-	0.00025	-	-

1. Denton, 1965, % composition of dry plant.
2. Mitchell, 1970, means.
3. A mean based on 12 sets of data, in turn based on various aquatic weeds, organs, sites and authors, from Denton, 1965.

chains (relative to calcium). This is true only if the observed ratio (OR) is greater than one $OR = \left(\frac{(Sr/Ca)\ plant}{(Sr/Ca)\ medium}\right)$ In the *Salvinia* cultures the Sr/Ca (atoms/1000 atoms) for dry plant material was 5.85 and for the medium it was 2.60. The OR was 2.25, thus *Salvinia* also discrimates against calcium relative to strontium.

The level of N (ca. 4%) in *Salvinia minima* is high when compared to the mean for aquatic vegetation, but it is not unusual, since several submerged or floating aquatic weeds often contain 4% N (STEWARD, 1970). This high N content is not due solely to environmental differences since similar levels of N have been detected in *S. minima* in nature (DENTON, 1966; see also Table XV). Thus *S. minima* exhibited luxury consumption of N in culture and in nature but only the plants growing in culture contained the high levels of P indicative of luxury consumption of that element. Since the plants from nature were taken from old mats, it would seem that P, in some cases, may be limiting growth of *S. minima* under natural conditions. The growth of several aquatics *(Elodea spp., Erocaulon septangulare, Lobelia dortmanna,* and *Potamogeton epihydrus)* growing in Lake Nebish in Wisconsin were shown to be limited by the same factor, i.e., P was the limiting element (GERLOFF, 1969). This is also

true of *Eichornia crassipes* on the Guadalupe River in Texas (GOSSETT & NORRIS, 1971). In water polluted with sewage, or in the greenhouse and sterile cultures above, P would occur in excess and the opposite case most likely would prevail, i.e., N would then become a limiting factor. *S. molesta* in the greenhouse tanks exhibited high levels of N and P similar to *S. minima*. But on Lake Kariba, MITCHELL (1970) has shown that the N and P content of this species may vary considerably (0.83—2.65% N, and 0.03—1.13% P). Thus, whether or not any one element is limiting at any one time for *Salvinia* is still an open question, which hopefully will be resolved in MITCHELL's forthcoming work.

The amount of ash present in *Salvinia* varied somewhat, being high in culture but low in nature. From the data of ZUTSHI & VASS (1971) the C : N ratio for *S. natans* would be 9.0—18.0. DENTON reported a C : N for *S. minima* of 11.1 and MITCHELL's data (1970) indicate a ratio of 30.8 for *S. molesta*. In sterile culture an estimated C : N for *S. minima* would be ca. 17. Such high C : N ratios indicate that *Salvinia* would not be a favorable source of animal food as is the case with most other aquatic weeds (BOYD, 1970). It seems possible that *Salvinia* could be used in nutrient removal schemes such as proposed by BOYD (1970). In fact, MITCHELL (1969) has shown that *S. molesta* is capable of depleting appreciable amounts of nutrients from the water in Lake Kariba in Africa. In fertilized greenhouse tank cultures of *S. minima*, the annual production amounts to 1270 Kg dry matter per acre which could remove 53 Kg N and 14 Kg P per acre. ZUTSHI & VASS (1971) in natural populations of a temperate species, *S. natans*, noted an average annual increment of 1133 Kg/acre, which could remove 41 Kg N per acre annually from eutrophic lakes in Kashmir. Based on annual per capita contributions of 4.1 Kg N and 1.4 Kg P (STEWARD, 1970) greenhouse cultures of *Salvinia* would remove the annual contributions of N from 13 people and the P from 10 people. Annual production under natural sunlight in the tropics should be higher by at least one third.

The floating *Salvinia* mats thus may act as a sump for large quantities of nutrients in tropical aquatic ecosystems. The total amount of nutrients depleted and stored by these mats increases with the age of the mat (MITCHELL, 1969). Also, the rate of nutrient uptake may differ with age of the plant, thus, *Salvinia* still in the exponential phase of growth takes up bicarbonate much faster than plants growing at less than exponential rates (Table XIV). The success of a *Salvinia* species might be related to its ability to increase absorptive surfaces. Thus, of two species growing at exponential rates the more successful species may be the one with a larger leaf

area. A comparison of the growth of two species growing at exponential rates, *S. minima* and *S. molesta*, shows this to be true. For example *S. minima* produced leaves at a higher rate (Fig. 10). On

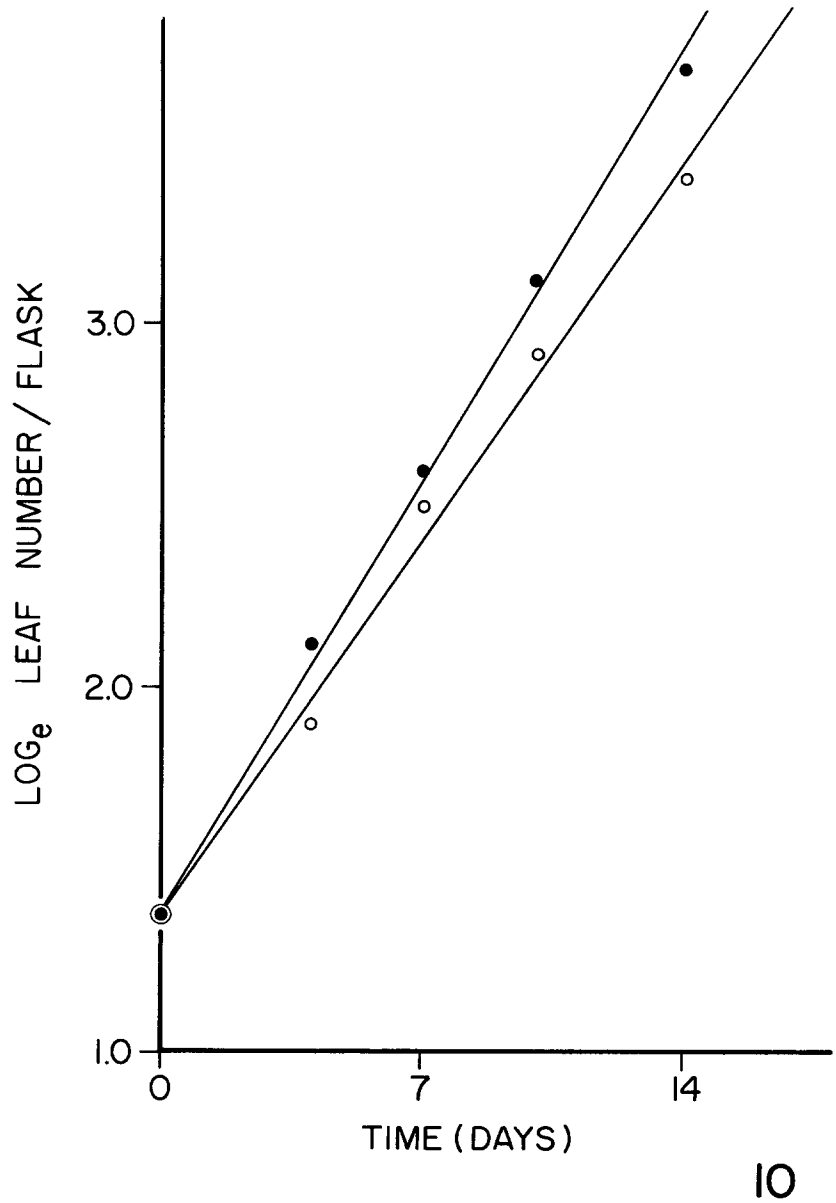

Fig. 10. Growth of *S. minima* (○) and *S. molesta* (●) under standard conditions.

the other hand, *S. molesta* maintained more dry weight and a larger leaf area depending on the light intensity (Fig. 11). Thus *S. molesta*

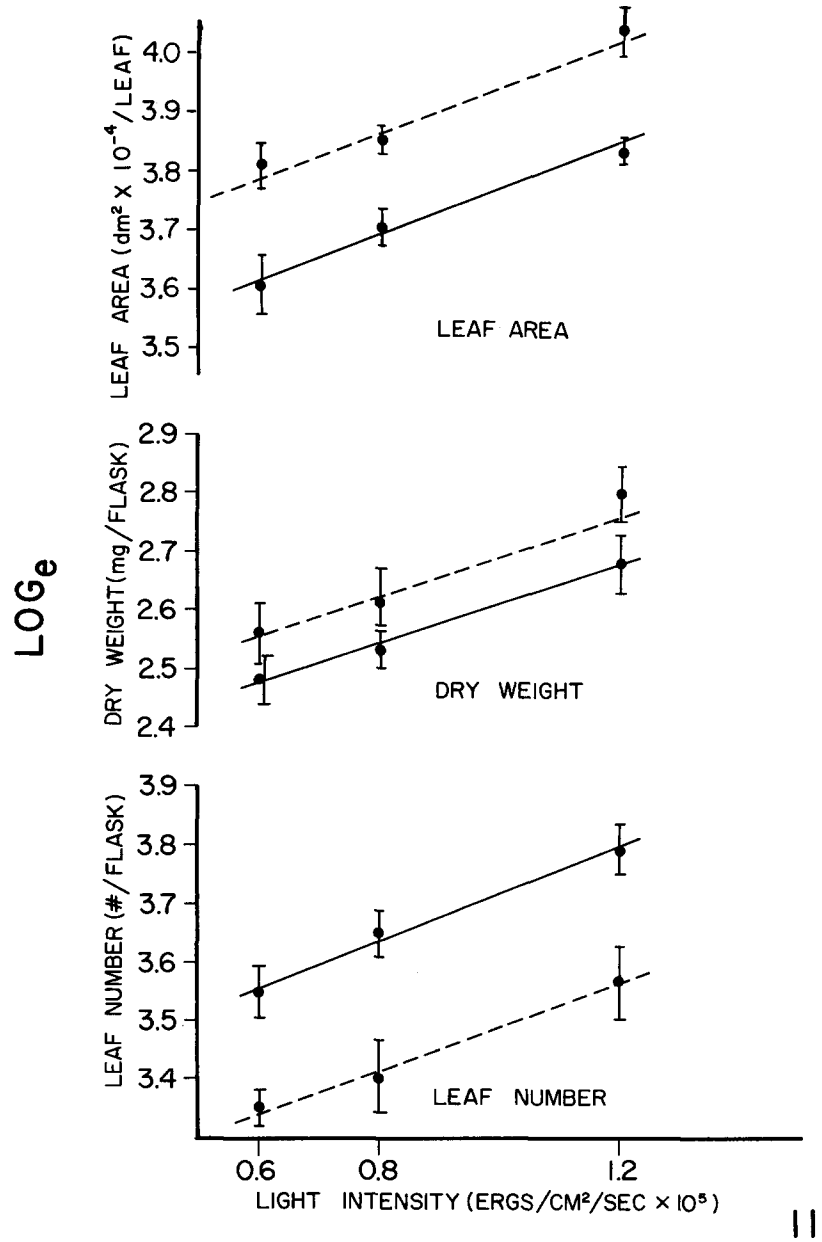

Fig. 11. Relationship between growth and light intensity for *S. minima* (———) and *S. molesta* (— — —).

rapidly covers tropical waterways to the exclusion of many other aquatic weeds (MITCHELL, 1969) whereas *S. minima* is seldom as successful. Such conclusions have yet to be tested under field conditions, but it is hoped that the standardized growth conditions described herein will be of some help as a tool to supplement field work.

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