

THE RESPONSE OF 'BUTTERCRUNCH' LETTUCE TO
APPLICATIONS OF CYANOBACTERIUM (NOSTOC MUSCORUM)
IN NUTRIENT SOLUTION ,

by

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CHAPTER I

INTRODUCTION

The urgency of providing an adequate supply of renewable-source nitrogen for crops has become increasingly evident in view of population growth projections and limitations of non-renewable resources. On a worldwide basis, increases in N fertilizer applications over the past three decades have been closely associated with increased average crop yields in many agricultural regions. However, the reliance on natural gas, a non-renewable resource, in the commercial manufacture of nitrogen fertilizers will result in future shortages and continually increasing production costs.

Development of renewable-source N for crops has received widespread attention in recent years (5, 6, 9, 10, 11, 12, 13, 16, 20). The efficiency of biological inputs in improving soil fertility and crop productivity has been well recognized. A classic example is the nitrogen input of rhizobia in symbiotic association with the root nodules of legumes. Certain free-living, autotrophic cyanobacteria (blue-green algae) are also of ecological and agricultural importance.

Free-living cyanobacteria occur in soil, freshwater, and marine habitats from the tropics to the polar regions (8). Annual fixation of nitrogen by these microorganisms at the rate of 15-50 kg/ha, sometimes as much as 80 kg/ha, has been observed in tropical rice fields (1, 11). Evidence indicating the effectiveness of algal applications in increasing rice crop yields is extensively documented (11, 16, 17, 19). Increases

in grain yields of 10-15% have been noted even in combination with inorganic nitrogen fertilization regimes.

Evidence of growth response to algal applications in vegetable crops is limited. To date, use of commercial preparations of 'blue-green algae soil inoculants' have provided no significant benefits to plants or soil (3). There are indications from India that massive doses of algal applications may positively affect yields and percentage nitrogen in tissues of green peppers and lettuce (7, 18) and increase vitamin C content in tomatoes (2). Large algal inoculations on cabbage and tomato crops in field conditions in Russia have also shown increased yield response (4).

Preliminary studies (Adler and Morse, unpublished data) with cyanobacterium applications on selected greenhouse vegetable crops indicated the necessity of providing sufficiently high rates of inoculum to affect consistent growth responses in a variety of soil and artificial-mix media. Growth response by crops from residual nitrogen in these media was corrected by utilization of nutrient solution culture with an inert root-support medium. 'Buttercrunch' lettuce was selected due to the utilization of butterhead type lettuce in greenhouse production and to its apparent greater sensitivity to N deficiency than several other varieties of lettuce based on preliminary studies. Nostoc muscorum is known for its widespread distribution in various soil and aquatic habitats, and was selected as inoculum material due to its capacity for rapid growth and nitrogen-fixation under a wide range of nutrient, aeration, and temperature parameters.

Two separate experiments were initiated to determine the growth response of lettuce to nutrient solution cultures containing different rates of nitrogen fertilizer with and without N. muscorum.

CHAPTER II

MATERIALS AND METHODS

Cultures of N. muscorum were kindly provided by Dr. G.A. Peters of Kettering Research Laboratory. The cyanobacterium was subsequently cultured in a semi-continuous 12-ℓ glass culture bottle in a nitrogen-free modified BG-11 medium of Stanier et al. (15). The culture was continuously illuminated by a four-foot-long, double-tubed lamp fixture with two Sylvania Gro-Lux F40-Gro tubes suspended 8 cm from the upper vessel surface. Agitation was provided by bubbling glass wool filtered air through an airstone at the bottom of the culture vessel (Figure 1). Removal of inoculum culture during preliminary studies was accomplished through a pressure-siphon tube and occurred at the rate of approximately 2-ℓ every 2-3 weeks over the course of 18 months. Autoclaved nitrogen-free BG-11 medium was added to the culture to maintain a constant solution level and stimulate semi-continuous culture growth.

Two separate greenhouse experiments were conducted during early spring and summer of 1979 at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. Lettuce seeds were germinated in autoclaved vermiculite media in 9-cell count trays under a greenhouse propagation misting system. Seedlings were fertilized at the two true leaf stage with 25 ml per plant of soluble Peters 20-20-20 dissolved at the rate of 2.5 g/ℓ of distilled water. Transplanting into GRO-JARS was done at the three leaf stage for the spring crop and at the four to five leaf stage for the summer crop.

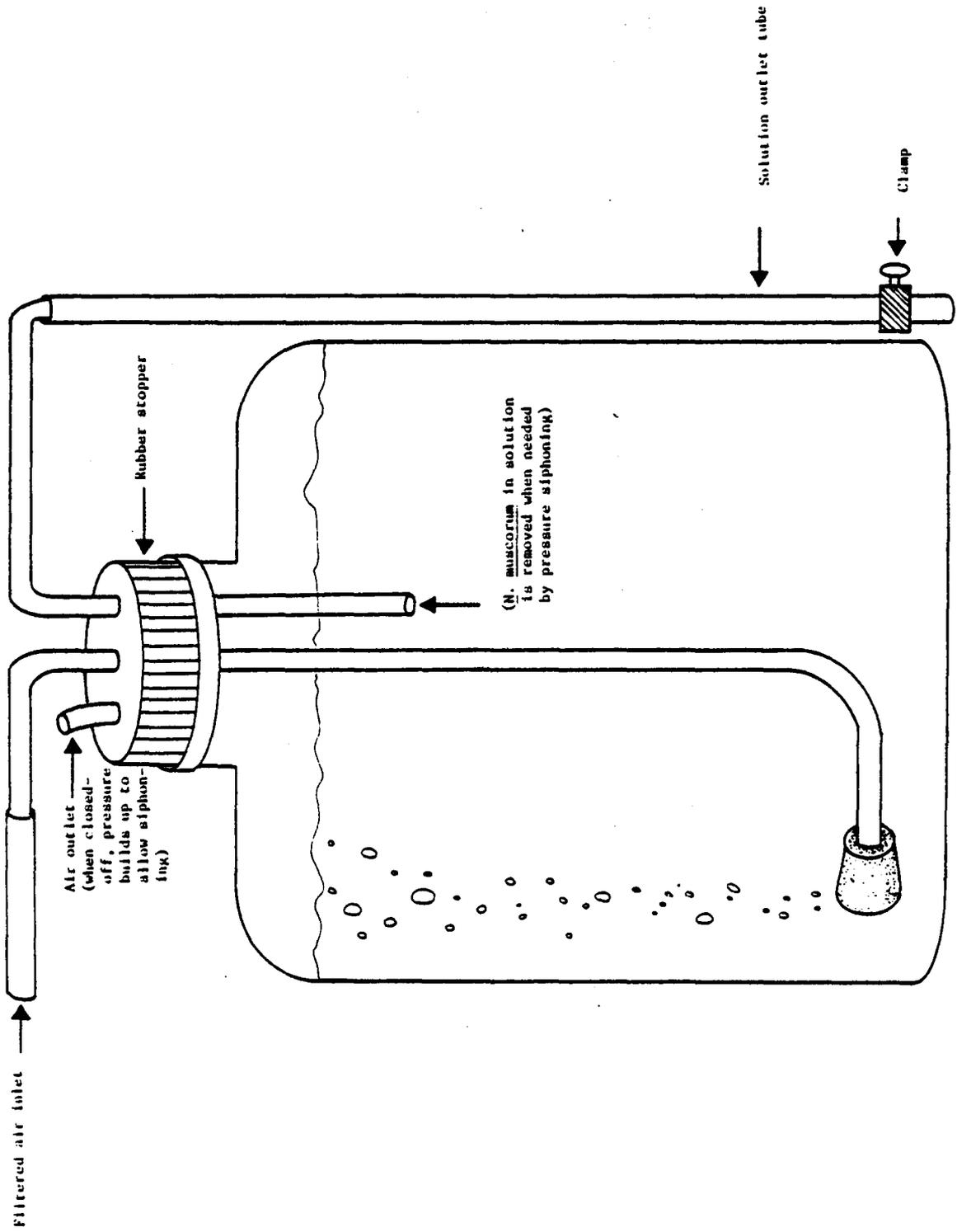


Figure 1. N. muscorum culture vessel.

A system was designed to separate nutrient solution treatments as well as to provide optimum cultural conditions for the growth of both lettuce and N. muscorum. Use of an inert media eliminated background nitrogen interference. Pea-sized acid and distilled water rinsed gravel was used for plant root support. Individual GRO-JARS were designed and constructed from 1-gal glass jars, opaque 1-liter nalgene polyethylene cylindrical bottles, rubber latex tubing, glass tubing, sand airstones, and non-toxic silicon aquarium sealant (Figure 2).

Both spring and summer crops were arranged in a complete randomized design with 5 single plant replicates. Sodium nitrate served as the inorganic N source. Nostoc muscorum inoculum concentration was approximately 2.07 g/l on a dry weight basis as determined from culture samples. Tissue analysis indicated Total Kjehldahl Nitrogen (TKN) at 4.89%.

The spring crop treatments included two rates of N (0 PPM and 165 PPM) with and without N. muscorum at 33.3% by volume (1/3 BG-11 medium and 2/3 stock nutrient solution) (Table 1).

The summer crop treatments included three rates of N (0 PPM, 83 PPM, and 165 PPM) with and without N. muscorum at 16.7% by volume (1/6 BG-11 medium and 5/6 stock nutrient solution) (Table 1).

All treatments were brought up to 3 liters final volume with the stock nutrient solution containing:

| | | | |
|------------|---|-------------------------------|---|
| 263 PPM K | (as KH_2PO_4) | 840 PPM SO_4 | (as $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) |
| 91 PPM P | (as KH_2PO_4) | 10 PPM Fritted Trace Elements | (Peters FTE) |
| 194 PPM Ca | (as $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) | 5 PPM Fe | (as Chelated Fe) |
| 49 PPM Mg | (as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) | | |

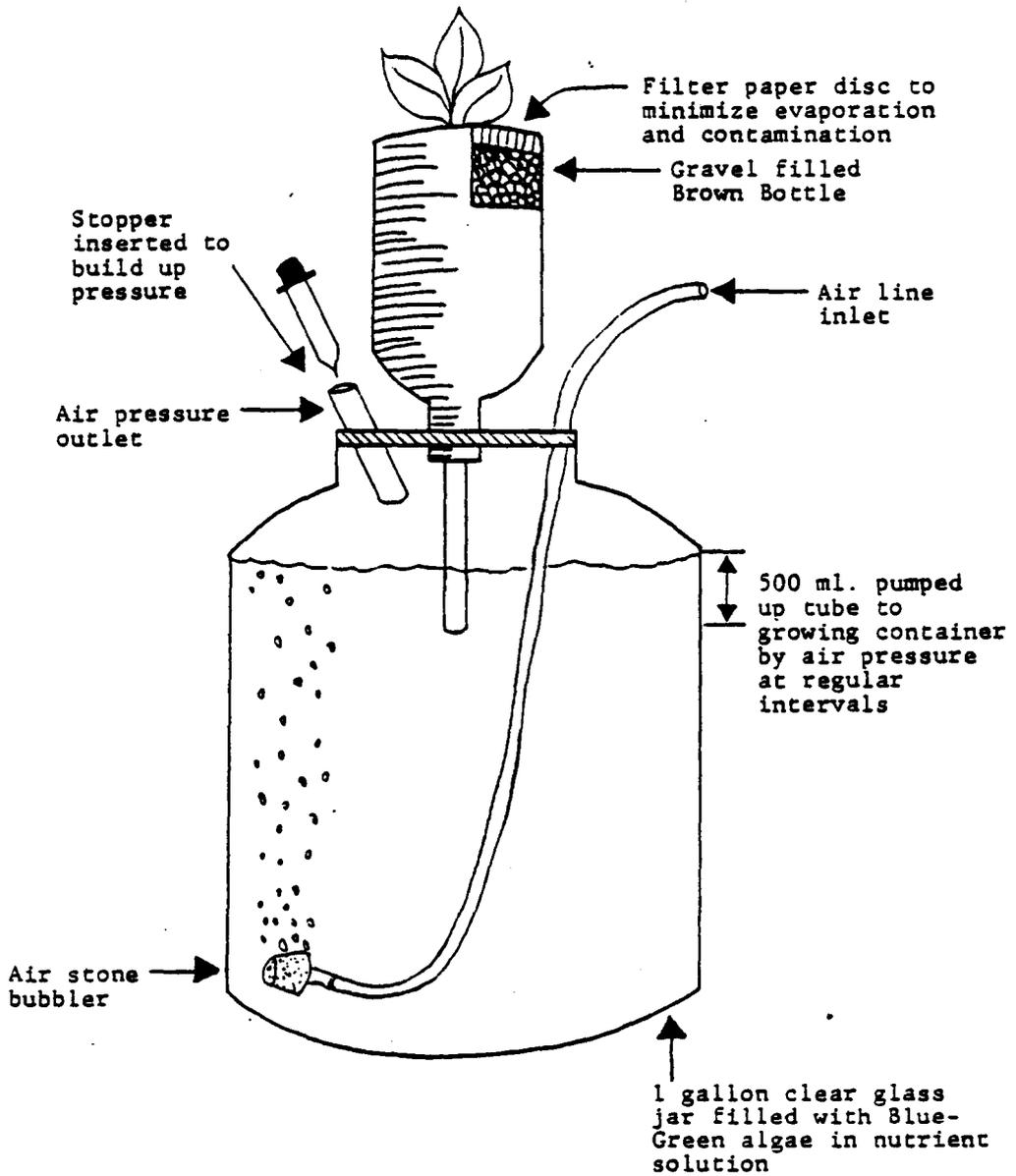


Figure 2. GRO-JAR apparatus.

Table 1. Nutrient solution nitrogen regimes.

| Crop | Treatment ^z | ppm N from NaNO ₃ |
|--------|--|---------------------------------|
| Spring | 1. 1 liter BG-11 medium | 0 |
| | 2. 1 liter <u>N. muscorum</u> in BG-11 | 0 |
| | 3. 1 liter BG-11 plus NaNO ₃ | 165 |
| | 4. 1 liter <u>N. muscorum</u> in BG-11 plus NaNO ₃ | 165 |
| Summer | 1. .5 liter BG-11 | 0 |
| | 2. .5 liter <u>N. muscorum</u> in BG-11 | 0 |
| | 3. .5 liter BG-11 plus NaNO ₃ | 83 |
| | 4. .5 liter <u>N. muscorum</u> in BG-11 plus NaNO ₃ | 83 |
| | 5. .5 liter BG-11 plus NaNO ₃ | 165 |
| | 6. .5 liter <u>N. muscorum</u> in BG-11 plus NaNO ₃ | 165 |

^zTreatments were added to stock nutrient solutions containing 263 ppm K, 91 ppm P, 194 ppm Ca, 49 ppm Mg, 840 ppm SO₄, 10 ppm FTE, and 5 ppm Fe.

GRO-JARS were filled with nutrient treatments and bubblers were adjusted to obtain an even flow by manually adjusting clamps on the rubber tubing for each GRO-JAR. One 3390 rpm pressure electric air pump was utilized for the spring crop, while an additional 3000 rpm pump was needed to supply adequate air pressure to additional summer crop GRO-JARS.

Bottom irrigation with nutrient solution was provided to each lettuce plant by stoppering off the air outlet tube; the buildup of air pressure forcing the solution up into the gravel media (Figure 2). The spring crop was irrigated in this manner and immediately drained 2-3 times daily depending on ambient temperatures. To minimize water stress in the summer crop, plants were irrigated in the mornings and drained in the evenings on a daily basis. The constant aeration of the nutrient solutions helped to prevent root oxygen depletion during the day. Nutrient solution levels were maintained constant for both crops by daily topping-off with distilled water. No additional nutrients were added during the growth of the crops.

Nutrient solutions were monitored at ten day intervals to determine pH and conductivity readings. Samples of 10 ml each were analyzed for each replication with a Barnstead Conductivity Bridge model pm-70 cb, set at millimhos x 10, and with an Orion Model 601A/digital ionalyzer pH specific ion electrode.

Nutrient solution samples were also taken at the beginning and the end of each experiment. After centrifugation at 2,500 g for 10 min at 5° C, the separated liquid solutions were analyzed for Total Kjeldahl Nitrogen (TKN), ammonium nitrogen ($\text{NH}_4\text{-N}$), nitrates ($\text{NO}_3\text{-N}$), and total

digested phosphorus (TP) in cooperation with Dr. M.D. Smolen at the Southern Piedmont Center in Blackstone, Virginia. Ammonium-N and $\text{NO}_3\text{-N}$, analyses were performed on 10 ml samples that were frozen and subsequently thawed for analysis on a Technicon II Analyzer using procedures modified by Smolen (14). TKN and TP analyses utilized 20-ml samples with 50 microliters of concentrated sulfuric acid added to fix $\text{NH}_4\text{-N}$. TKN samples were stored at 5°C in the dark. Due to problems with analysis arrangements, solution samples from the spring crop were stored for as long as six months.

Lettuce plants were harvested by careful cutting of the stem directly below the basal point of the lowest leaf scar, approximately at surface level. Each plant was immediately weighed to determine fresh weight. Entire plants were dried at 75°C in a forced air oven for 72 hours and dry weights were determined. The plants were ground in a Cyclone grinder to pass a 40 mesh screen. Replicates of dried tissue materials were pooled by treatment and subsamples were analyzed for N, P, K, Ca, Mg, Mn, Fe, Al, Cu, Zn, Na by the University of Georgia Plant Analysis Laboratory. N levels were determined by the Kjeldahl method. Other elemental determinations were by spectrographic analysis. The number of replicates subjected to analysis varied for each treatment due to limitations in dried material available.

CHAPTER III

RESULTS

Dry weight growth response of 'Buttercrunch' lettuce was evaluated with regard to nutrient solution variables, tissue mineral content, and treatment interaction in both spring and summer greenhouse crops. Differences in rates of N. muscorum inoculation, watering procedures and environmental conditions suggest different complex biological systems in both spring and summer crops.

Spring Crop

Treatments receiving N. muscorum, in addition to basic stock nutrients, showed more growth, as measured by dry weight, than treatments with similar basic stock nutrients not receiving N. muscorum at the 165 ppm N rate of fertilization. A similar trend was observed at the 0 ppm N rate of fertilization, although not statistically significant (Table 2). Overall, the effect at both rates combined showed a doubling of dry weight yields in treatments with N. muscorum.

No growth response at 0 ppm N and succulent growth at the higher rate of 165 ppm N indicated that N was a factor limiting to lettuce growth. All other nutrients appeared to be available in sufficient supply for crop growth.

Evaluation of final nutrient solution analyses reveals several strong relationships (Table 5). The positive correlation between final $\text{NO}_3\text{-N}$ and yield appears in all treatments. Those treatments receiving

Table 2. Interaction and main effects of *N. muscorum* and two rates of N fertilization on dry weights and percentage N of leaf tissue in spring greenhouse lettuce, and on NH₄-N, NO₃-N, Total Kjeldahl Nitrogen (TKN), Total Digested Phosphorus (TP), pH and conductivity (SS) in final nutrient solutions.

| Treatments | Dry weight lettuce (g) | N in leaf (%) | NH ₄ -N (ppm) | NO ₃ -N (ppm) | TKN (ppm) | TP (ppm) | pH | SS (10 ⁵ x mhos) |
|--|------------------------|---------------------|--------------------------|--------------------------|------------------|-----------------|------------------|-----------------------------|
| Interaction Effects | | | | | | | | |
| 0 ppm N, BG-11 | 0.1c ^z | -- | 0.0b | 0b | 19 ^y | 37 ^y | 7.1 ^y | 16 ^y |
| 0 ppm N, BG-11 plus <i>N. muscorum</i> | 0.6c | 1.44 | 0.1b | 0b | 19 | 22 | 7.6 | 15 |
| 165 ppm N, BG-11 | 3.9b | 3.45 | 0.1b | 51a | 18 | 10 | 7.5 | 18 |
| 165 ppm N, BG-11 plus <i>N. muscorum</i> | 7.4a | 2.15 | 1.6a | 11b | 25 | 3 | 7.9 | 15 |
| Main Effects | | | | | | | | |
| Combined treatments: | | | | | | | | |
| BG-11 without <i>N. muscorum</i> | 2.0 ^w | (3.45) ^x | --- | --- | 18a ^v | 24a | 7.3b | 17a |
| BG-11 with <i>N. muscorum</i> | 4.0 | 1.80 | --- | --- | 22a | 12a | 7.8a | 15b |
| Combined treatments: | | | | | | | | |
| 0 ppm N | 0.4 ^w | (1.44) ^x | --- | --- | 19a ^v | 30a | 7.3b | 15a |
| 165 ppm N | 5.6 | 2.80 | --- | --- | 21a | 7b | 7.7a | 16a |

^zMean separation within columns by Duncan's multiple range test, .05 level.

^yInteraction effects within column were not significant at the .05 level. See main effects for comparative analysis.

^xOnly one sample was available for this determination.

^wInteraction within column effects were significant at the .05 level. See interaction effects for appropriate analysis.

^vMean values within columns with different letters differed at the .05 level.

N. muscorum, however, appear to be related in a cubic rather than a linear manner. This cubic response may be a result of interaction of the N. muscorum with nutrient uptake or a fruititious result of limited data.

Final amounts of $\text{NH}_4\text{-N}$ in solution, although statistically greater in the N. muscorum treatments at 165 ppm N than in the without N. muscorum treatments, did not appear to add substantial amounts of N to the total N content of the nutrient solution, but the possibility also exists that $\text{NH}_4\text{-N}$ was rapidly utilized by the lettuce. The $\text{NH}_4\text{-N}$ may have acted in a stimulatory manner on lettuce growth. A strong cubic relationship between final $\text{NH}_4\text{-N}$ and dry weight yields was indicated, but may be in part a result of limited sampling data.

Differences were noted between combined treatments of 0 ppm N and combined treatments of 165 ppm N for total digested phosphorus (TP), and pH (Table 2). Plants showing greater growth predictably utilized greater quantities of phosphorus from the nutrient solution. The use of sodium nitrate ($\text{NO}_3\text{-N}$) as a N source resulted in increased alkalinity at the higher rate of 165 ppm N fertilization.

Treatments with N. muscorum, when compared to without N. muscorum treatments, exhibited a greater ability for raising the pH and removing soluble salts, as measured by conductivity. This suggests a utilization of nutrients from the solution by the N. muscorum.

Summer Crops

Summer crop treatments, which received N. muscorum inoculum at one-half the rate of similar spring crop treatments, exhibited some similar

Table 3. Interaction effects of *N. muscorum* and three rates of N fertilization on dry weights and percentage N of leaf tissue in summer greenhouse lettuce, and on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, Total Kjeldahl Nitrogen (TKN), Total Digested Phosphorus (TP), pH and conductivity (SS) in nutrient solutions.

| Treatment | Dry Weight in Lettuce Leaf (g) | N (%) | $\text{NH}_4\text{-N}$ (ppm) | | $\text{NO}_3\text{-N}$ (ppm) | | TKN (ppm) | | TP (ppm) | | pH | | SS ($10^5 \times \text{mhos}$) | | | | | | |
|--|--------------------------------|-------------------|------------------------------|------------------|------------------------------|----------------|----------------|------------------|----------|------------------|-------|-----------------|----------------------------------|------------------|------------------|------------------|-----------------|------|------|
| | | | begin | end | begin | end | begin | end | begin | end | begin | end | begin | end | begin | end | | | |
| 0 ppm N, BG-11 | 0.2 ^y | 1.06 ^x | 0.1 ^y | 0.1 ^y | 0.0 ^y | 0 ^y | 0 ^y | 16c ^z | 5c | -11 ^y | 136ab | 35 ^y | -107a | 6.5 ^y | 7.2 ^y | 0.8 ^y | 16 ^y | 12c | -4b |
| 0 ppm N, BG-11 plus <i>N. muscorum</i> | 0.6 | 1.60 | 0.1 | 0.1 | 0.0 | 1 | 0 | 15c | 7bc | -8 | 155ab | 38 | -121ab | 6.7 | 7.0 | 0.3 | 14 | 15b | 1a |
| 83 ppm N, BG-11 | 5.5 | 2.66 | 0.1 | 0.1 | 0.0 | 106 | 3 | 20bc | 6bc | -15 | 165a | 5 | -162b | 6.6 | 7.5 | 0.9 | 21 | 14b | -7c |
| 83 ppm N, BG-11 plus <i>N. muscorum</i> | 6.3 | 2.14 | 0.6 | 0.3 | -0.4 | 82 | 0 | 22ab | 18a | -4 | 121ab | 2 | -118ab | 6.9 | 7.5 | 0.6 | 21 | 14b | -6c |
| 165 ppm N, BG-11 | 7.0 | 3.13 | 0.2 | 0.2 | 0.1 | 147 | 36 | 16c | 12ab | -6 | 112b | 4 | -108a | 6.9 | 7.8 | 0.9 | 27 | 16a | -11d |
| 165 ppm N, BG-11 plus <i>N. muscorum</i> | 5.9 | 3.23 | 0.3 | 0.8 | 0.6 | 184 | 33 | 25a | 15a | -9 | 164a | 0 | -164b | 6.9 | 8.3 | 1.4 | 26 | 16ab | -10d |

^zMean separation within columns by Duncan's multiple range test, .05 level.

^yAnalysis of variance indicated interaction effects within column were not significant at the .05 level. See Table 4 analysis of main effects.

^xInsufficient number of samples for statistical analysis.

Table 4. Main effects of N. muscorum and three rates of N fertilization on dry weights and percentage N of leaf tissue in summer greenhouse lettuce and on NH₄-N, NO₃-N, Total Kjeldahl Nitrogen (TKN), Total Digested Phosphorus (TP), pH and conductivity (SS) in nutrient solutions.

| Treatment | Dry Weight in Lettuce leaf (g) | N (%) | NH ₄ -N (ppm) | | NO ₃ -N (ppm) | | TKN (ppm) | | TP (ppm) | | pH | | SS (10 ⁵ x mbos) | | |
|----------------------------|--------------------------------|-------|--------------------------|------|--------------------------|-------|-----------|--------|----------|------|--------|-------|-----------------------------|--------|-------|
| | | | begin | end | change | begin | end | change | begin | end | change | begin | end | change | begin |
| Combined treatments | | | | | | | | | | | | | | | |
| BG-11 without N. muscorum | 4.2a ² | 2.28 | 0.1b | 0.1b | .04a | 84a | 13a | -69a | y | -11a | 14a | 6.7a | 7.5a | 0.9a | 21a |
| BG-11 with N. muscorum | 4.3a | 2.32 | 0.4a | 0.4a | .08a | 100a | 11a | -86a | | -6a | 14a | 6.8a | 7.6a | 0.8a | 20b |
| Combined treatments | | | | | | | | | | | | | | | |
| 0 ppm N | 0.4b ² | 1.33 | 0.1b | 0.1b | .02ab | 1c | 0b | 0a | y | -10a | 145a | 6.6b | 7.1c | 0.6b | 15c |
| 83 ppm N | 5.9a | 2.40 | 0.3a | 0.2b | -.21b | 94b | 2b | -91b | | -8a | 143a | 6.8ab | 7.5b | 0.8ab | 21b |
| 165 ppm N | 6.5a | 3.18 | 0.2ab | 0.5a | .36a | 166a | 34a | -127c | | -10a | 138a | 6.9a | 8.1a | 1.1a | 26a |

²Mean separation within column by Duncan's multiple range test, .05 level.

^ySignificant interactions within columns were observed, .05 level. See Table 3 for factor interaction effects.

trends to the spring crop but failed to be significant in several respects. Treatments receiving N. muscorum, in addition to the basic stock nutrient solution, showed growth similar to treatments not receiving N. muscorum (Tables 3 and 4).

An evaluation of beginning, end, and changes of nutrient solution contents revealed some strong relationships which were consistent with data from the spring crop (Table 5). Nitrate-N was strongly correlated both linearly in the without N. muscorum treatments and in a cubic manner in the with N. muscorum treatments. At higher NO_3 -N rates more N was removed from the nutrient solution by actively growing lettuce than the slower growing plants at the lower NO_3 -N rates.

Beginning and end amounts of NH_4 -N in the summer nutrient solutions were greater in the treatments with N. muscorum than in the treatments without N. muscorum. However, the changes in this variable were not significant and it appeared that the NH_4 -N was not being liberated into the solution in any substantial quantity or else was taken up by the plant (Table 4).

The final measurements and changes in pH and conductivity were generally consistent with the spring crop results, showing increased alkalinity and a greater depletion of soluble salts at higher N rates. The effect of N. muscorum, however, did not exhibit similar responses to the spring crop. Those treatments with N. muscorum showed differing responses for soluble salts at the beginning levels, but these slight differences were not reflected in corresponding changes in final amounts. It is interesting to note that a strong linear correlation between the change in soluble salts and dry weight appeared in the without N. muscorum

treatments while a reasonably strong cubic relationship between soluble salts and dry weight yields was indicated in the with N. muscorum treatments. This was consistent with the data for $\text{NO}_3\text{-N}$ relationships with dry weight yields.

The expected relationship between growth and phosphorus utilization in the summer crop was not evident from the data. Similar inconsistent data for TKN indicates possible difficulties in the digestion processes and analyses for these variables.

Table 5. The relationship of nutrient solution $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, Total Kjehldahl Nitrogen (TKN), Total Digested Phosphorus (TP), pH and conductivity (SS) with dry weight yields of spring and summer greenhouse lettuce.

| Nutrient solution content | | Treatment | | | |
|-----------------------------------|--------|--|--|--|--|
| | | BG-11 without <i>N. muscorum</i> | | BG-11 with <i>N. muscorum</i> | |
| | | Spring combined treatments R-Square | Summer combined treatments R-Square | Spring combined treatments R-Square | Summer combined treatments R-Square |
| $\text{NH}_4\text{-N}$ (ppm) | begin | | .20 (L) | | .52 (Q) |
| | end | .18 (L) ^z | .10 (L) | 1.00 (C) ^y | .02 (L) |
| | change | | .01 (L) | | .05 (L) |
| $\text{NO}_3\text{-N}$ (ppm) | begin | | .86 (L) | | .90 (C) |
| | end | .98 (L) | .08 (L) | 1.00 (C) | .01 (L) |
| | change | | .90 (L) | | .89 (C) |
| TKN (ppm) | begin | | .17 (L) | | .87 (C) |
| | end | .05 (L) | .08 (L) | .36 (L) | .54 (Q) |
| | change | | .32 (Q) | | .12 (L) |
| TP (ppm) | begin | | .002 (L) | | .44 (C) |
| | end | .69 (L) | .45 (Q) | .82 (Q) | .77 (L) |
| | change | | .11 (L) | | .002 (L) |
| pH | begin | | .28 (L) | | .002 (L) |
| | end | .53 (L) | .67 (L) | .56 (L) | .65 (Q) |
| | change | | .04 (L) | | .03 (L) |
| SS (10^5 μmhos) | begin | | .85 (L) | | .58 (L) |
| | end | .44 (L) | .71 (L) | .003 (L) | .00 (L) |
| | change | | .81 (L) | | .67 (C) |

^zR-Square values were reported for the most significant relationship as linear (L), quadratic (Q), or cubic (C).

^yHigh cubic relationship values may be due to limited sample size.

CHAPTER IV

DISCUSSION

The positive growth response to increasing rates of N is well-known. However, with the introduction of N. muscorum in the same nutrient media in a closed system and hence into the rhizosphere of the lettuce, this relationship becomes more complex. N. muscorum has the potential for uptake and/or fixation of N from solution and therefore may have been interacting in a separate, competitive and/or symbiotic manner at the various stages of lettuce growth.

N. muscorum increased growth in lettuce in the initial experiment. In the subsequent experiment, this response was not duplicated. Since the first experiment received twice the initial inoculation rate, it is believed that the rate of inoculation may have been of critical importance. Cyanobacteria supplements by Indian and Russian researchers on greenhouse and field vegetable crops showed positive growth responses at inoculation rates from 25 mg dry wt/pot up to 7 double centner fresh wt/ha (approximately 1400 kg/ha). Significant increase in the percentage N in the shoots was proportional to the amount of inoculum (4, 7, 18). These inoculation rates were considerably higher than the N. muscorum added to the lettuce crops grown during the course of the two experiments reported herein, thus serving to reinforce the importance of large application rates. Other factors such as time of growth, temperature, and variations in watering procedures for the lettuce may have contributed to a lesser degree to this response.

The interaction of N. muscorum and available N may have been a function of several mechanisms of action. Nostoc muscorum can utilize $\text{NO}_3\text{-N}$ from the nutrient solution in preference to fixing N from the air (8, 11). Initial inoculum placed in solutions with higher rates of N fertilization appeared to visually grow more rapidly than in solutions with lower rates of N--apparently using $\text{NO}_3\text{-N}$ in the nutrient solution for growth, fixing little if any N from the atmosphere. As this $\text{NO}_3\text{-N}$ in the nutrient solution became depleted and was a limiting factor for further growth, the N. muscorum either: (1) began fixing nitrogen (a portion of which was released into solution in forms readily available for lettuce growth), (2) began producing relatively small quantities of growth promoting substances and/or $\text{NH}_4\text{-N}$ which may have acted to stimulate lettuce growth, or (3) terminated growth with subsequent release of nitrogenous substances upon decay and mineralization, or (4) as some combination of these mechanisms.

In a similarly speculative manner, it appears that previous to reaching this "critical point of N depletion", the N. muscorum may have been competing with the lettuce for the same N in solution. This may explain the observed trend in the summer crop for a decrease in lettuce growth at the highest rate of N fertilization with N. muscorum as compared to the without N. muscorum treatments at the same level. The lower N. muscorum rate of the summer crop may have taken longer to reach this "critical point" and may have been competing with the lettuce for N up to this "point"; whereas in the initial spring crop, with the higher inoculation rate, N. muscorum may have begun nitrogen fixation or release of substances that promoted growth into solution at an earlier stage in

regards to the lettuce growth. The N use index reinforces this speculation, by indicating that in all treatments with N. muscorum that show any trends for increased dry weight yields over treatments without N. muscorum there also appeared to be a greater dry weight increase per mg of N removed from the solution (Table 6). In other words, the N. muscorum treatments, except for the summer crop at the highest rate of N, showed an increased efficiency per unit of N removed from the solution and/or were providing additional N to the lettuce.

The failure to measure the dry weight of the N. muscorum per liter during the various stages of lettuce growth or to measure acetylene-reduction of the system has limited the capacity to determine contributions of fixed nitrogen to the lettuce-N. muscorum-nutrient solution complex. If any N was fixed, which was likely, then it appeared to be readily available for lettuce growth or N. muscorum growth, as accumulation of N in the nutrient solution had not been noted. Similarly, if lettuce growth increases resulted from mineralization of N. muscorum, then this process occurred at a relatively rapid rate.

These preliminary data are consistent with the original hypothesis that additions of the free-living, cyanobacterium N. muscorum may increase yields of lettuce grown under specific conditions in nutrient solution culture. However, the indications are not strictly of a linear fertilizer response, but rather a complex interaction within a biological equilibrium. Attention to N. muscorum concentration in solution at various stages in the growth of the lettuce and analysis for possible growth promoting substances is indicated for future research efforts and will assist in developing a clearer picture of the mechanisms of this

Table 6. Evaluation of dry weight growth response and nitrogen removal from nutrient solutions in spring and summer greenhouse lettuce crops.

| Treatments | Dry weight lettuce (mg) | Estimated total N in leafz (mg) | Total N removed from nutrient solution ^y (mg) | Nitrogen use index ^x |
|--|-------------------------|---------------------------------|--|---------------------------------|
| <u>Spring crop</u> | | | | |
| 0 ppm N, BG-11 | 118 | --- | --- | --- |
| 0 ppm N, BG-11 plus <u>N. muscorum</u> | 638 | 9 | --- | --- |
| 165 ppm N, BG-11 | 3887 | 134 | 290 ^u | 13 ^u |
| 165 ppm N, BG-11 plus <u>N. muscorum</u> | 7390 | 159 | 388 ^u | 19 ^u |
| <u>Summer crop</u> | | | | |
| 0 ppm, N, BG-11 | 207 | 2 | 35 | 6 |
| 0 ppm N, BG-11 plus <u>N. muscorum</u> | 566 | 9 | 27 | 21 |
| 83 ppm N, BG-11 | 5483 | 125 | 350 | 16 |
| 83 ppm N, BG-11 plus <u>N. muscorum</u> | 6339 | 135 | 255 | 25 |
| 165 ppm N, BG-11 | 7016 | 220 | 336 | 21 |
| 165 ppm N, BG-11 plus <u>N. muscorum</u> | 5897 | 190 | 470 | 13 |

^zMg dry weight X %N in lettuce leaf.

^yChange in NO₃-N + change in TKN of nutrient solution.

^xNitrogen use index = mg dry weight lettuce/mg N removed from nutrient solution.

^wSamples unavailable.

^uValues are estimates based on final samples only.

interaction. A greater understanding of inoculation rate, timing of inoculation, and plant-microorganism-nutrient responses is also necessary before specific application recommendations may be suggested. Sufficiently high N. muscorum inoculation rates or inoculation far enough in advance of transplanting of the lettuce may result in more consistent positive growth response.

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APPENDIX I

PRELIMINARY REVIEW OF CURRENT
RESEARCH AND LITERATURE

PRELIMINARY REVIEW OF CURRENT RESEARCH
AND LITERATURE (September, 1977)

Due to a variety of political, economic, social, and environmental factors, the process of converting atmospheric nitrogen into ammonia or nitrates by biological micro-organisms has been the focus of considerable research in recent years. Rather than attempt to develop these factors which indicate the importance of nitrogen input for crop production, this review is concerned with a brief overview of current emphasis in research with particular attention to potentials for utilization of free-living blue-green algae as nitrogen biofertilizers.

Significant research advances in nitrogen fixation since 1960 have been listed by Hardy (1976) under the headings of engineering and chemical, biochemical, genetic, physiological, and agronomic in an approximate chronological sequence. The most intense recent advances occur in the areas of genetic, physiological, and agronomic research. In another article, Hardy and Havelka (1975) summarize present emphasis:

The breadth of the objectives of current research includes the enhancement of symbiotic nitrogen fixation by grain legumes, the domestication of associative symbioses for cereals; the development of abiological nitrogen-fixing systems with high rates of nitrogen fixation under mild conditions; the extension of rhizobial infection or the development of man-made associative symbioses for cereals; and the transfer of the genetic information for nitrogen fixation to cereals. However, no advance has produced a new technology that is suitable for direct application to high-yield crop production. Until alternative technologies are in hand, it is critical that the production of fertilizer nitrogen continue to be increased throughout the world.

There exists a diversity of biological nitrogen-fixing organisms and relationships. These include some bacteria and blue-green algae in both free-living and symbiotic relationships. A synthetic diazotroph (nitrogen-fixing organism) has been produced in the form of a strain of nitrogen-fixing Escherichia coli. In addition to the more common associative symbioses between Rhizobium and legumes, similar associative symbiotic associations have been observed in tropical grasses including maize, sugarcane, and rice and free-living nitrogen-fixing bacterium (Dobereiner and Day, 1974). Current research concerning the Rhizobial potentials and nitrogen fixation is more thoroughly surveyed by Child (1976).

Particular emphasis has been placed on development of symbiotic associations for increasing nitrogen input to crops. According to Hardy and Havelka (1975):

In general, those relationships in which the site of nitrogen fixation is located on or in the plant will permit direct and thereby efficient coupling of the fixed nitrogen to the plant in amounts that parallel the changing needs of the plant throughout its complete growth cycle.

Hardy (1976) further elucidates on the limitations of free-living nitrogen-fixing bacteria, while indicating that free-living blue-green algae might offer potentially significant agronomic contributions:

The contribution of fixed N by free-living bacteria to crop production is small, and I do not foresee a major change. Limitations include poor coupling to crop plant and low N₂ fixed:CH₂O consumed ratio. Soil addition of Azotobacter or Clostridium that are constitutive in nitrogenase probably will not be of agronomic significance. Similarly, use of such organisms as a biological N-fertilizer factory does not appear attractive without improvements of more than an

order of magnitude in $N_2:CH_2O$ ratio, and the maximum ratio based on calculations is 1:4. The potential role for free-living algae is more difficult to assess. They are not coupled to crop plants, but they produce their own carbohydrate and make significant contributions of N in irrigated rice culture.

Nitrogen Fixation by Blue-Green Algae (Cyanobacteria)

Proof of the capability for assimilation of molecular nitrogen (N_2) by blue-green algae was not published until 1928 (Drewes, 1928), although the suspicion that certain members of this group had been current for forty years before this (Fogg, 1974). A considerable amount of research has occurred since this time in regards to identification of blue-green algae with nitrogen-fixing (nif) properties. According to Mishustin and Shil'nikova (1971):

Nitrogen assimilation is quite widespread among blue-green algae (Watanabe, 1951-1963; Allen and Arnon, 1955; Singh, 1961; Fogg, 1962; Land, 1942; Takha, 1963; Laloraya and Mitra, 1964; Cox et al., 1964; Fogg and Stewart, 1965). The ability to utilize molecular nitrogen has now been recorded in almost eighty species. With the exception of Chloroglea, these species all belong to the order Harmogonales. They are different species of Amorphonostoc, Anabaena, Anabaenopsis, Aulosira, Calothrix, Cylindrospermum, Fischerella, Gleotrichia, Hapalosiphon, Mastigocladus, Nostoc, Scytonema, Scytonematopsis, Stigonema and Tolypothrix. Chloroglea fritschii (synonym Nostoc sp. according to data of Stewart et al.) (Mitra, 1961) belongs to the order Chroococcales. The list of nitrogen-fixing algae is growing very rapidly.

Since 1967, particularly rapid advances have been made in the understanding of nitrogen-fixing algae. This has been due not only to increased research by physiologists, biochemists, ecologists, and electron microscopists, but also to improved techniques for growing the algae in bacteria-free culture in the laboratory and to the advent of

new techniques for measuring nitrogen fixation--particularly the acetylene-reduction assay (Stewart, 1974). Rather than examine the volumes of research data, emphasis will be focused upon potentials for increasing crop productivity by application of biologically produced nitrogen.

Potential Agronomic Contributions

The efficacy of biological inputs in improving soil fertility and crop productivity is well recognized. The prime example is nitrogen input from the legume-Rhizobium association, which has now assumed industrial proportions in many countries. Certain non-symbiotic, autotrophic, blue-green algae, because of their ability to carry out both photosynthesis and nitrogen fixation, are ecologically and agriculturally important, particularly in tropical rice fields (De, 1939; Singh, 1961). This ability to carry out both photosynthesis and nitrogen fixation within the same organism is an advantage over symbiotic micro-organisms which fix nitrogen. The significance of this increased efficiency may be potentially great enough to offset the advantages of symbiotic systems which fix nitrogen in the root zones of plants at rates that correspond to crop needs. Shields and Durrell (1964) review some comparative rates of nitrogen assimilation in micro-organisms:

From culture solution, Anabaena cylindrica is estimated to fix gaseous nitrogen in an organic form equivalent to 480 pounds per acre per month (5), Azobacter is reported to fix up to 40 pounds of nitrogen per acre per year, in fertile soil. The contribution of the legume-Rhizobium combination to total nitrogen is approximated at 200 pounds per acre per crop (169) ...Relative rates of nitrogen fixation are low in the photosynthetic bacteria, ...amounting to a doubling of cell nitrogen in three to eight days.

Mishustin and Shil'nikova (1971) offer a most comprehensive review of literature regarding the amounts of nitrogen fixed by blue-green algae:

There are very conflicting reports about the actual level of nitrogen accumulated by blue-green algae. This is easy to explain; experiments have not always been carried out in the same conditions and have not all lasted the same length of time. For example, in calculations of accumulation per unit area, different principles of conversion have been applied. Nevertheless, we feel that some generalizations are possible. We shall use the material obtained in laboratory conditions by several investigators (including Allison et al., 1937; Stokes, 1940; Muzafarov, 1953; Allen, 1958; Watanabe, 1959-1963; Venkataraman, 1961), who used different cultures of blue-green algae (Amorphonostoc paludusum, Nostoc muscorum, Anabaena cylindrica, Anabaenopsis circularis, Cylindrospermum sphaerica and others). Disregarding findings that differ sharply from the mean or are not readily explicable, we can say that in 1.5-2 months algae in laboratory conditions, in the light, usually fix 2-5 mg of nitrogen per 100 ml of medium. In rare cases this value is more than 10 mg.

Individual investigators have not agreed as to which are the most vigorous nitrogen fixers. Singh (1961) considers that in India the most active form is Aulorisa fertilissima; Watanabe (1950) said Tolypothrix tenuis for Japan and Ley Shang hao (1959) said Anabaena azotica for China.

There have been many investigations of the amount of nitrogen accumulated by blue-green algae in the soil especially in rice fields (for example De, 1939; Singh, 1942; Prasad, 1949; Willis and Green, 1948; Watanabe, 1951b; Watanabe and Kiyohara, 1963; Tamiya, 1957; Venkataraman, 1961a; Iha et al., 1965). In rice fields, conditions are optimum for blue-green algae during a considerable part of the growing season. Most investigators found that in these conditions the annual nitrogen increment due to the activity of blue-green algae varies between 15 and 50 kg/hectare, although sometimes it is 80 kg/ha. Watanabe (1962), who summarized the findings of many Japanese investigators, put the mean quantity of nitrogen accumulated by blue-green algae at approximately 20-25 kg/ha. Watanabe considered that in countries where nitrogenous mineral fertilizers are used in small quantities blue-green algae are very important in the maintenance of soil fertility.

There are also indications that blue-green algae can accumulate very large quantities of nitrogen in the soil. Thus, Allen (1956) believes, on the basis of laboratory experiments, that after conversion nitrogen accumulation by blue-green algae may reach in one month 53 kg per hectare. Keller (1948) calculating the nitrogen in the excrescences of algae, obtained a maximum figure of 205 kg of nitrogen per hectare per season. There have been references to even more nitrogen accumulation by algae, but we consider them to be overestimates.

Evidence indicating the effectiveness of algal applications in increasing crop yields in rice fields is extensively documented by Venkataraman (1975). In summarizing:

Recent investigations show that irrespective of the varietal differences among the high-yielding, fertilizer responsive dwarf rice varieties, all respond to algal inoculation (10-15% increase in grain yield) even in the presence of high levels of nitrogen fertilization (100-150 kg N/ha). This results in an increased output per unit of added fertilizer (3-5 kg grain/kg N) with a consequently enhanced economic return.

Stewart (1974) further documents the potential increases of crop yields by utilization of algal fertilizers and also suggests that blue-green algae may produce growth promoting substances.

Evidence that nitrogen fixed by the algae becomes available to associated plants comes from studies using pot and field experiments. Early studies by Allen (1956) showed that rice plants grew better and increased in nitrogen content when blue-green algae were added to plants in sand-culture and the beneficial effect of blue-green algae has been confirmed on many occasions both in the greenhouse and under field conditions (De and Sulaiman, 1950; Watanabe et al., 1951; Singh, 1961; Subrahmanyam et al., 1964a,b, 1965 a,b; Subrahmanyam and Manna, 1966; Sang et al., 1972; Subrahmanyam, 1972). Usually increase in total crop yield has been taken as evidence of a transfer of fixed nitrogen but this is not necessarily the case because there is evidence that blue-green algae may produce growth promoting substances, in addition to combined nitrogen (Gupta et al., 1967; Venkataraman and Neelakantan, 1967; Kushwaha and Gupta, 1970; Gupta and Kushwaha, 1972).

Gupta and Kushwaha (1972) reported, for example, that when extracts of the aerobically non-nitrogen fixing Phormidium faveolarum were sprayed on to wheat seeds there was a stimulation of growth, which did not appear to be attributable to an increased supply of combined nitrogen. The review by Subrahmanyam (1972) on the beneficial effects of inoculating crop soils with algae is particularly exhaustive and should be read. In it he also makes the interesting observation that weed growth may be reduced in paddy fields treated with blue-green algae.

Potentials of Large-Scale Production of Cyanobacteria

A variety of environmental factors influence nitrogen fixation in blue-green algae, and the degree to which these factors influence assimilation varies amongst the various species. Distinctive responses have been recorded to temperature, light, pH, oxygen levels, nutrient levels of growth media, and trace elements. Manipulation of environmental factors can be important in obtaining maximum nitrogen fixation.

An example from the experiments of Allen and Arnon (1955b) illustrates the effect of trace elements on the growth of algae. Anabaena cylindrica in a medium without vanadium and molybdenum yielded a biomass of 0.36 g per litre; within the same period in a medium containing these trace elements, the weight of the biomass became 5.8 g per litre. This of course in turn influences nitrogen fixation, for it usually correlates with algal growth. According to some findings, algae accumulate several trace elements in their cells (Sr, Ba, Ti, Mn, Cu, and Co) (Mishustin and Shil'nikova, 1971).

Although blue-green algae have been found to be able to survive in a variety of adverse conditions and habitats, it seems that optimum growth levels can be best maintained in controlled environmental growth facilities where ideal conditions are assured.

Several methods have been devised for continuous culturing of algal fertilizers. For the large-scale application of algae in rice cultivation,

the "open-air soil culture" method developed by Venkataraman (1975) appears most useful for tropical farmers allowing for continuous culture in algal nursery beds in their own fields. Venkataraman also suggests:

The other possibility of growing these algae for agricultural purposes is to grow them in sewage. This will be economically attractive, because of the involved reclamation of the sewage water that can be subsequently used for irrigation purposes. However, the presence of cyanophages in sewage requires the use of phage-resistant strains.

Open-air systems, although more practical and economically competitive at this point, are subject to failure of algal establishment in the field due to contact with acid fertilizers, lack of competitive ability, restricted tolerance to pesticides, presence of predators and/or phages, or nutrient deficiencies. Temperature restrictions limits open-air methods to tropical zones for continuous culture.

Several more sophisticated devices have been developed. Mishustin and Shil'nikova (1971) describe several of these:

Watanabe (1959c and d) devised a very refined apparatus. In this, the algae multiply in a tank of mineral medium containing nitrates and lit by electricity (Fig. 34), and air enriched with carbon dioxide (up to 2-3 percent) is blown through the tank. It is economically advantageous to heat the apparatus by making use of hot springs and to use natural gases as a source of carbon dioxide. One litre of culture medium gives up to 2 g of algal mass each day... Vessels of capacity 250 l are used, and for each square metre of apparatus, 7.9 g of algae is obtained daily. From each apparatus it is possible to obtain as much as 7 tons of algae.

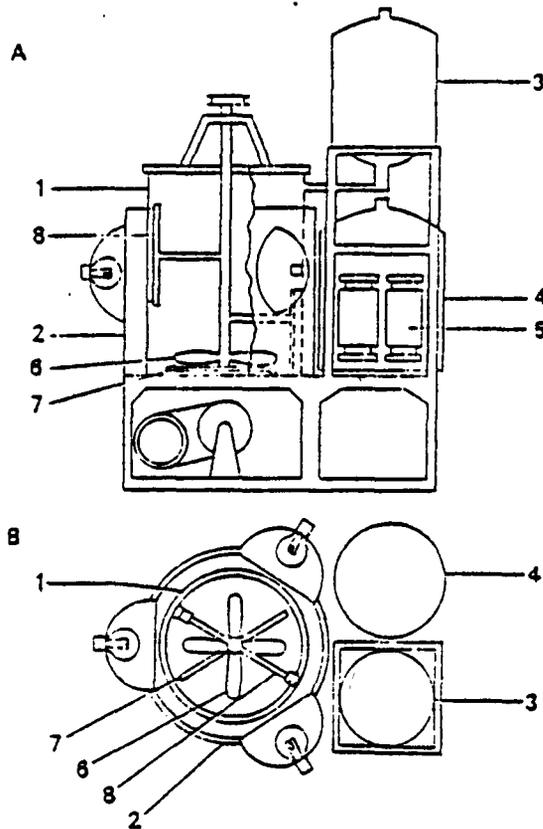


Fig. 34. Diagram of tank for culturing algae by Watanabe's method
 A. Longitudinal section; B. transverse section. 1. Tank; 2. thermostat;
 3, reservoir for medium; 4, heater; 5, cotton wool filter; 6, ventilator;
 7, aeration pipe; 8, stirrer.

Pinevich and Verzilin (1963) designed a column type apparatus with natural lighting. The upper part of the column is made of transparent plastic and the lower part of stainless steel placed in a thermostat. The culture is aerated. Various other devices for culturing algae have been described (Florenzano, 1958).

For agricultural purposes most of the culture obtained can be multiplied further in open systems, and then spun down and dried. Blue-green algae rapidly settle and are easily centrifuged at 2,500-3,000 r.p.m.

Many ways of drying algae have been proposed. Lyophilization is expensive, and so Venkataraman (1961b) recommended sunlight and Tseng Chi-Mien (1959) a current of hot air. Watanabe et al. lyophilized algae and then dried them adding serum albumin. In dried preparations of algae 50 percent of the cells are lost in

two years. Watanabe et al. also cultivated algae on the surface of volcanic gravel prepared as a fine porous mass (Fig. 35). The algae that developed were kept in chlorovinyl bags or dry glass bottles, retaining their activity for 2-5 years, although some of them died.

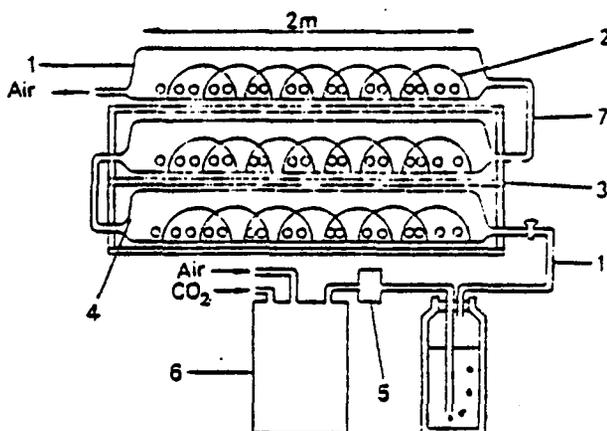


Fig. 35. Diagram of apparatus for cultivating blue-green algae on gravel (after Watanabe). 1, Polyvinyl reservoir for cultivating the algae; 2, wire frame; 3, glass plate; 4, gravel inoculated with algae; 5, cotton wool filter; 6, reservoir for gas; 7, pipe for aeration.

No well formulated techniques for large-scale production are indicated at this point. Setlick, Sust, and Malek (1968) deal with basic design considerations and scheme of a pilot plant. Recent indications of economic significance in rice cultivation (in part due to the increasing cost of industrial manufacture of nitrogen fertilizers) have encouraged further research on agronomic and economic merits of algal fertilizers. Only limited references have been made with regard to potential utilization of algal fertilizers on vegetable crops. Most of the research to date has been involved with more agronomically significant crops such as rice.

Utilization of algal fertilizers on vegetable crops, particularly in controlled environmental growth systems using hydroponic nutrient solution methods might offer potential economic advantages. By incorporating algal production into a nutrient solution system, several problems associated with drying, packaging, storage, and transportation of algal fertilizers are eliminated.

What is required now are more detailed studies evaluating the potential role and contribution of blue-green algal fertilizers for the production of vegetable crops. Such research could lead to development of controlled environmental growth production-units capable of being utilized in rural areas and in developing countries where fertilizer availability, water, and land are limiting resources. Emergence of appropriate solar energy technologies even suggest the possibilities for designing a prototype unit capable of functioning relatively independent of industrialized support networks (electricity, fertilizer plants, transportation, etc.) which involve prohibitively high initial capital investments for rural areas in less developed nations.

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APPENDIX II

PRELIMINARY STUDIES--SUMMARY OF
OBJECTIVES, DESIGN, AND DATA

LIST OF APPENDIX CONTENTS

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PRELIMINARY STUDY #1

BLUE-GREEN ALGAE AND
NUTRIENT SOLUTION SCREENING--FALL, 1977OBJECTIVES:

- Selection of a species of blue-green algae that will grow at a maximum rate under a variety of nutrient solutions
- Selection of a nutrient solution that will encourage maximum growth of blue-green algae species

DATA: Relative growth of blue-green algae species and Azolla caroliniana in various nutrient solutions.

| Relative Rank | Species of Organisms | Relative Rank | Nutrient Solutions ^y |
|-----------------|----------------------------------|---------------|---------------------------------|
| 1. ^z | <u>Anabaena cylindrica</u> (636) | 1. | St., ES, WA, Alf. (597) |
| 2. | <u>Anabaena flos-aquae</u> (582) | 2. | ML, B, WA, Alf. (569) |
| 3. | <u>Nostoc muscorum</u> (569) | 3. | St., ES, WA, Dol. (559) |
| 4. | <u>Tolypothrix tenuis</u> (450) | 4. | Hoagland's (493) |
| 5. | <u>Oscillatoria sp.</u> (423) | 5. | BGA (429) |
| 6. | <u>Nostoc sp.</u> (391) | 6. | OL, WA, B, Dol. (411) |
| 7. | <u>Calothrix sp.</u> (391) | 7. | ES, WA, Alf. (386) |
| 8. | <u>Aulosira terrestre</u> (376) | 8. | ML, WA, B, Dol. (338) |
| 9. | <u>Anabaena 7120</u> (333) | 9. | St., OL, FA, B, CC (332) |
| 10. | <u>Azolla caroliniana</u> (253) | 10. | B, CC, Dol. (296) |

FORMAT: 10 x 10 two-way factorial with 3 replications.

^zBased on weekly visual relative growth ratings on a 1-10 scale. Numbers in parentheses indicate total rating.

^yAbbreviations for nutrient sources:

St. = Straw ash

ES = Egg shells ash

WA = Pine wood ash

Alf. = Alfalfa sprout rinse water

ML = Maple leaf ash

B = Bonemeal

Dol. = Dolomitic limestone

BGA = Allen and Arnon Blue-Green Algae nutrient solution

OL = Oak leaves

FA = Coal fly-ash

CC = Corn cob ash

PRELIMINARY STUDY #2

LETTUCE AND TOMATOES IN SOIL MIX
AND SAND CULTURE--SPRING, 1978OBJECTIVES:

- Examine growth response in lettuce and tomatoes to applications of Nostoc muscorum as compared with ammonium sulfate and control treatments
- Observe effects on growth response in two different media--soil mix vs. sand

DESIGN:

TREATMENTS: 10 ml every 2 weeks.

1. Ammonium Sulfate (110 ppm N)
2. Blue-Green Algae Mix (Anabaena flos-aquae, A. cylindrica, Nostoc sp., N. muscorum, Tolypothrix tenuis, and Calothrix sp.)
3. Nitrogen-free BG-11 nutrient solution

All plots received initial Dolomitic limestone and FTE with application of mono-potassium phosphate at weekly intervals.

FORMAT: 3 treatments (6 plants/treatment) x 2 types of growing media. Randomized complete block with 3 replications of above. (Six benches were used. Three with soil mix, three with sand. Each bench was partitioned for three different treatments. Each treatment was applied on both lettuce and tomatoes.)

DATA: Growth response of greenhouse lettuce and tomatoes grown in soil mix to N regimes.

LETTUCE--SPRING, 1978

| Treatment | Fresh wt means of blocks |
|---------------------------------|--------------------------|
| 1. $(\text{NH}_4)_2\text{SO}_4$ | 561.4a ^z |
| 2. BGA mix | 735.1a |
| 3. N-free BG-11 | 452.6a |

^zMean separation within columns by Duncan's multiple range test, .05 level.

TOMATOES--SPRING, 1978

| Treatment | Fresh wt means of blocks | Avg. wt/tomato |
|---------------------------------|--------------------------|----------------|
| 1. $(\text{NH}_4)_2\text{SO}_4$ | 1020.7a ^z | 32.2a |
| 2. BGA mix | 1209.9a | 42.3a |
| 3. N-free BG-11 | 1111.8a | 39.0a |

^zMean separation with columns by Duncan's multiple range test, .05 level.

PRELIMINARY STUDY #3

SWEET CORN IN GREENHOUSE SOIL POT
CULTURE SUMMER, 1978OBJECTIVES:

- Examine biological nitrogen sources in several forms as compared to standard chemical treatments for growth response on sweet corn grown in the greenhouse in soil pot culture

DESIGN:

| Treatments | Nitrogen Source ¹ | PKCaMgFTE |
|------------|--|-----------|
| 01 | NONE | - |
| 02 | NONE* | + |
| 03 | Dried <u>Nostoc</u> (8.6 lb N/A) ¹ | - |
| 04 | Dried <u>Nostoc</u> (8.6 lb N/A) ¹ | + |
| 05 | NONE (N-free BG-11) | - |
| 06 | <u>Nostoc</u> in BG-11 (1 lb N/A) ¹ | - |
| 07 | <u>Nostoc</u> in BG-11 (1 lb N/A) ¹ | + |
| 08 | Ammonium Nitrate (60 lb N/A) ¹ | + |
| 09 | Ammonium Sulfate (60 lb N/A) ¹ | + |
| 10 | Sodium Nitrate (60 lb N/A) ¹ | + |
| 11 | Agrilife 10X (10 ml/plant) ¹ | - |
| 12 | Agrilife 10X (10 ml/plant) ¹ | + |

* Received PKCaMgFTE at planting only.

¹ Received treatments at time of planting and two weeks following germination.

FORMAT: 12 treatments with 4 replications; complete randomized.

DATA: Growth response of sweet corn in greenhouse pot culture to various N regimes.

| Treatment | | Dry wt gms | Relative rank |
|-----------|-------------------------------------|-------------------|---------------|
| 01 | Control ⁻ | 4.1b ^z | 9 |
| 02 | Control ⁺ | 6.3b | 4 |
| 03 | Dried <u>Nostoc</u> ⁻ | 3.3b | 12 |
| 04 | Dried <u>Nostoc</u> ⁺ | 4.6b | 7 |
| 05 | Control BG-11 | 3.6b | 11 |
| 06 | <u>Nostoc</u> in BG-11 ⁻ | 4.7b | 6 |
| 07 | <u>Nostoc</u> in BG-11 ⁺ | 4.3b | 8 |
| 08 | Ammonium Nitrate ⁺ | 18.6a | 2 |
| 09 | Ammonium Sulfate ⁺ | 20.8a | 1 |
| 10 | Sodium Nitrate ⁺ | 17.9a | 3 |
| 11 | Agrilife 10x ⁻ | 3.8b | 10 |
| 12 | Agrilife 10x ⁺ | 5.8b | 5 |

^zMean separation within column by Duncan's multiple range test, .05 level.

PRELIMINARY STUDY #4

LETTUCE AND TOMATOES IN
SOILLESS MEDIA--FALL, 1978OBJECTIVES:

- Examine growth response in lettuce and tomatoes to applications of Nostoc muscorum as compared with ammonium nitrate and control treatments.
- Observe effects on growth response in a nonsoil media mix of peat, vermiculite, and perlite.

DESIGN:

TREATMENTS:

1. Ammonium Nitrate (300 ppm N) 50 ml/week
2. Nostoc muscorum in BG-11 nutrient solution (0 ppm N) 50 ml/week
3. BG-11 nutrient solution (0 ppm N) 50 ml/week

All treatments received 50 ml mono-potassium phosphate in water @ 2T/gal also at weekly intervals.

FORMAT: 3 treatments per bench, 4 replications. Randomized complete block.

DATA: Growth response of greenhouse lettuce and tomatoes in soilless media to N regimes.

| Treatments | <u>Lettuce</u> | <u>Tomatoes</u> | |
|---------------------------|--------------------|-------------------|---------------------|
| | Means fresh wt | Means fresh wt | Means No. fruits |
| 1. Ammonium Nitrate | 10.3a ^z | 1962.2a | 69.3a |
| 2. <u>Nostoc muscorum</u> | 4.2b | 1447.7b | 55.5a |
| 3. Control | 2.3c | 1354.5b | 53.5a |

^zMean separation within columns by Duncan's multiple range test, .05 level.

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THE RESPONSE OF 'BUTTERCRUNCH' LETTUCE TO
APPLICATIONS OF CYANOBACTERIUM (NOSTOC MUSCORUM)
IN NUTRIENT SOLUTION

by

Barry Adler

(ABSTRACT)

Two separate greenhouse experiments were conducted to determine the effects of additions of a blue-green algae (Nostoc muscorum) to nutrient solution cultures with different rates of nitrogen fertilizer on the growth of lettuce (Lactuca sativa L. cv. Buttercrunch). Lettuce yields increased with increased rates of N. Yields were significantly greater in treatments containing 165 ppm N with N. muscorum culture added (at the rate of 33.3% by volume) than in treatments containing the same rate of N without N. muscorum. A similar growth increase was not noted at lower N. muscorum inoculation rates (16.7% by volume). These preliminary data indicate the potential for increased yields of lettuce grown under specific conditions in nutrient culture. Further research of the complex interactions within this biological equilibrium are required before specific application recommendations may be suggested.