Blue-Green Algae and Rice

P.A. ROGER and S.A. KULASOORIYA
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The responsibility for this publication rests with the International Rice Research Institute.

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Rising costs of energy and, in turn, chemical fertilizers have focused attention on biological sources of nitrogen, the element commonly most limiting for crop production. For paddy rice, one of the important biological sources of combined nitrogen is blue-green algae, the subject of this publication.

The importance of blue-green algae was emphasized in 1973 by G. E. Fogg and Associates in their book *The Blue-Green Algae.*

"Since in many eastern countries peasant farmers do not fertilize their fields in any way, it appears that blue-green algae may often permit a moderate rice harvest to be gathered when in their absence there would be only a poor one. Indeed, it does not seem unreasonable to suppose that many millions of people survive largely because of nitrogen fixation by blue-green algae."

More recently, the successful use of algal inoculation in India demonstrates that blue-green algae can serve as an alternative or supplemental source of nitrogen for rice cultivation. Blue-green algae may give some advantages not necessarily associated with nitrogen fixation, such as through the production of growth-promoting substances.

Unfortunately, uncertainties about the ecology of blue-green algae and their mode of action on the rice plant limit their practical use. A compilation of all relevant information on blue-green algae that is related to rice is needed to orient the agronomic research on the subject. This publication provides such information. It is a survey of the ecological, physiological, and agronomic aspects of blue-green algae in rice fields and is the result of the compilation and analysis of 369 references.

Dr. P. A. Roger, as senior author, drew on more than 10 years of research and field experience with blue-green algae in West Africa. He prepared this book at the International Rice Research Institute (IRRI) while on a leave from the Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), France. Dr. S. A. Kulasooriya co-authored the book while on sabbatical leave from the University of Peradeniya, Sri Lanka. Dr. Kulasooriya conducted laboratory research on blue-green algae at Westfield College, University of London, UK, and field experiments in Sri Lanka.
Hence, while the authors refer to the work of others, they have also used their direct experience in preparing a very useful compilation of information on blue-green algae. We are indebted to them for their efforts.

This source book of information on the role and potentialities of blue-green algae in wetland rice culture will be helpful not only to microbiologists concerned with algae from a scientific viewpoint but to agronomists and practical field workers who want to use blue-green algae to supply nitrogen to the rice plant.

N. C. Brady
Director General
International Rice Research Institute
Most field experiments conducted in rice-growing countries indicate that the application of organic or chemical nitrogen fertilizers plays a dominant role in increasing rice yield and the efficiency of agronomic practices. As pointed out by Patnaik and Rao (105), the overhead labor cost of tillage, irrigation, water control, and other operations will not vary much whether 1 t·ha$^{-1}$ is produced without fertilizers or 5 t·ha$^{-1}$ is produced with fertilizer, but the productivity of man-hours utilized will increase. For these reasons, fertilizer N may be considered the kingpin in rice farming mainly since the introduction of improved, high N-responsive rice varieties.

Unfortunately the increasing cost of N fertilizer and the widening gap between supply and demand of N in the developing countries have placed heavy constraints on the farmers.

Realizing the influence of energy cost on current and probable future prices of fertilizer N and the need to stimulate research on alternative sources of nitrogen for rice cultivation, the International Rice Research Institute (IRRI) organized the symposium on Nitrogen and Rice in September 1978 (105). Arising from the final discussions, research priority for biological nitrogen fixation by azolla, blue-green algae (BGA), and heterotrophic microorganisms in the root zone was recommended. In particular, IRRI was asked to compile all relevant information on the BGA in relation to rice cultivation. This review on BGA and rice is primarily in response to that request.
Table 2. Analysis of the literature on blue-green algae and rice. (Data are the number of papers dealing with the indicated topics.)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Sub-Topics</th>
<th>Number of References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECOLOGY OF BGA IN PADDY FIELDS</strong></td>
<td>Reviews</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Descriptive ecology</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Environmental factors</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Agronomic practices</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>124</td>
</tr>
<tr>
<td><strong>PHYSIOLOGY OF BGA IN PADDY FIELDS</strong></td>
<td>Books and reviews</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Photosynthesis</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Nitrogen fixation</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63</td>
</tr>
<tr>
<td><strong>BGA AND THE RICE PLANT</strong></td>
<td>66</td>
<td></td>
</tr>
<tr>
<td><strong>ALGALIZATION</strong></td>
<td>Books and reviews (≥ 5 pages)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Short reviews and popularization</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Effects on rice</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Effects on soil</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Strain selection</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Limiting factors</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Technology</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>369</td>
</tr>
</tbody>
</table>

A qualitative analysis of the literature (Table 2) indicates the topics on which the research has been focused and the gaps. Out of 369 references, 255 deal with the ecology and physiology of BGA in the rice field ecosystem; 142 concern algalization. However, the relatively high number of papers on algalization has to be corrected taking into account two characteristics of the literature on this subject. First, an analysis of the size of the papers (Fig. 2) indicates an abundance of short notes of one or two pages. Second, among the published papers about one-third are classified as “book, reviews, and popularization papers.” This may indicate a fragmentary and, to a slight extent, verbal aspect of this literature.

The analysis of the literature on the ecology of BGA in paddy fields (Table 3) indicates that about half of the work has been done on a descriptive basis with a large dominance of taxonomic studies. Quantitative estimations and quantitative studies of the algal successions are few. Among the environmental factors, biotic factors have been less studied. The highest number of references dealing with soil properties is mainly related to pH, and relatively little attention has been paid to the other soil properties. Among agronomic practices, the effect of pesticides has drawn about half of the papers, but little attention has been paid to land preparation and management.
Concerning the physiology of BGA in paddy fields (Table 4) photosynthesis appears to be a deserted topic. On the contrary, nitrogen fixation has been largely documented. However, knowing that algal nitrogen-fixing activity varies throughout the day and the growth cycle of rice, it is surprising that more papers deal with total estimation of nitrogen fixation than with diurnal variations and variations along the cycle.

Of the relationships between BGA and the rice plant (Table 4), most attention has been on production of growth-promoting substances by the algae and their effect on the rice plant. In comparison, the important topic of the availability of
Table 3. Analysis of the literature on the ecology of blue-green algae in paddy fields. (Data are the number of papers dealing with the indicated topics.)

<table>
<thead>
<tr>
<th>DESCRIPTIVE ECOLOGY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence of BGA in paddy fields</td>
<td>14</td>
</tr>
<tr>
<td>Records of species and taxonomy</td>
<td>73</td>
</tr>
<tr>
<td>Quantitative estimations</td>
<td></td>
</tr>
<tr>
<td>Methodology</td>
<td>4</td>
</tr>
<tr>
<td>Enumerations</td>
<td>14</td>
</tr>
<tr>
<td>Biomass measurements</td>
<td>10</td>
</tr>
<tr>
<td>Variations of the algal flora along the growth cycle</td>
<td>8</td>
</tr>
<tr>
<td>Total flora</td>
<td>17</td>
</tr>
<tr>
<td>Qualitative studies</td>
<td></td>
</tr>
<tr>
<td>Quantitative variations</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ENVIRONMENTAL FACTORS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Climatic factors</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>10</td>
</tr>
<tr>
<td>Temperature</td>
<td>8</td>
</tr>
<tr>
<td>Desiccation and rewetting</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
</tr>
<tr>
<td>Biotic factors</td>
<td>2</td>
</tr>
<tr>
<td>Pathogens</td>
<td>6</td>
</tr>
<tr>
<td>Antagonisms</td>
<td></td>
</tr>
<tr>
<td>Grazers</td>
<td>7</td>
</tr>
<tr>
<td>Soil properties</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>21</td>
</tr>
<tr>
<td>Other properties</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGRONOMIC PRACTICES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Land preparation and management</td>
<td>8</td>
</tr>
<tr>
<td>Inorganic fertilizers</td>
<td>39</td>
</tr>
<tr>
<td>Organic manure</td>
<td>15</td>
</tr>
<tr>
<td>Pesticides</td>
<td>45</td>
</tr>
</tbody>
</table>

fixed nitrogen for the plant has been poorly documented.

In the studies concerning algalization (Table 5), the two major topics are the effects on grain yield and the technology of algalization. The effects of algalization on soil properties and the effect on the plant other than grain yield are poorly documented. Compared with the large number of references on grain yield and technology, the limiting factors for algalization also appear to be neglected.
Table 4. Analysis of the literature on the physiology of blue-green algae in paddy fields and their relation with the rice plant. (Data are the number of papers dealing with the indicated topics.)

<table>
<thead>
<tr>
<th>PHYSIOLOGY OF BGA IN PADDY FIELDS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis</td>
<td>9</td>
</tr>
<tr>
<td>Nitrogen fixation</td>
<td></td>
</tr>
<tr>
<td>Methodology</td>
<td>29</td>
</tr>
<tr>
<td>Daily variations</td>
<td>7</td>
</tr>
<tr>
<td>Variations along the cycle</td>
<td>10</td>
</tr>
<tr>
<td>Global estimations</td>
<td>22</td>
</tr>
<tr>
<td>Relative contribution</td>
<td>19</td>
</tr>
</tbody>
</table>

|  |
|-----------------------------------|---|
| 63 |

<table>
<thead>
<tr>
<th>BGA AND THE RICE PLANT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Availability of fixed N for rice</td>
<td>13</td>
</tr>
<tr>
<td>Growth promoting substances</td>
<td>30</td>
</tr>
<tr>
<td>Detrimental effects</td>
<td>16</td>
</tr>
<tr>
<td>Epiphytism</td>
<td>5</td>
</tr>
<tr>
<td>Other effects</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 5. Analysis of the literature on algalization. (Data are the number of papers dealing with the indicated topics.)

<table>
<thead>
<tr>
<th>EFFECTS OF ALGALIZATION ON RICE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect on grain yield</td>
<td></td>
</tr>
<tr>
<td>Pot experiments</td>
<td>25</td>
</tr>
<tr>
<td>Field experiments</td>
<td>47</td>
</tr>
<tr>
<td>With non-N fertilizers</td>
<td>7</td>
</tr>
<tr>
<td>With fertilizer N</td>
<td>26</td>
</tr>
<tr>
<td>With soil sterilization</td>
<td>10</td>
</tr>
<tr>
<td>Cumulative and residual effect</td>
<td>16</td>
</tr>
<tr>
<td>Effect on other than grain yield</td>
<td></td>
</tr>
<tr>
<td>Nitrogen content</td>
<td>5</td>
</tr>
<tr>
<td>Morphology of the plant</td>
<td>11</td>
</tr>
<tr>
<td>Growth cycle</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EFFECTS ON SOIL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical properties</td>
<td>1</td>
</tr>
<tr>
<td>Soil nitrogen</td>
<td>8</td>
</tr>
<tr>
<td>Soil organic matter</td>
<td>4</td>
</tr>
<tr>
<td>Other chemical properties</td>
<td>5</td>
</tr>
<tr>
<td>Soil microflora</td>
<td>2</td>
</tr>
</tbody>
</table>

Continued on opposite page
It can be concluded that among the topics recorded in 369 papers on BGA and rice, the less documented ones are:

- quantitative studies of the algal successions in paddy fields and role of biotic factors;
- influence of soil properties other than pH on BGA;
- effects of land preparation and management on BGA;
- photosynthetic activity of BGA in paddy fields;
- availability of fixed nitrogen for rice;
- effects of algalization on soil properties and soil microflora; and
- limiting factors for algalization.
Blue-green algae (BGA) are photosynthetic prokaryotic microorganisms, some of which are capable of nitrogen fixation. Their main photosynthetic pigments are chlorophyll a, carotenes and xanthophylls together with phycobiliproteins, c-phyccyanin (blue) and c-phycoerythrin (red). Due to the presence of these latter pigments and mucilage, the color of BGA in nature may range from dirty yellow, through various shades of blue-green, to brown or black. Their range in vegetative form extends from simple unicells to multiseriate, true branching thalli (Table 6).

Some BGA can fix nitrogen because they contain nitrogenase, an O2-sensitive enzyme. This ability was first related to the presence of specialized non O2-evolving cells called heterocysts in which the enzyme is protected from O2. It is now clearly demonstrated that N2-fixing ability in air is not confined to heterocystous BGA and also that a large variety of BGA, not known to fix N2 a few years ago, have nitrogenase and fix N2 under microaerophilic or anaerobic conditions (281). Now, over 125 strains are known to fix N2 (Table 7).

Such trophic independence from carbon and nitrogen, together with a great adaptability to variations of environmental factors, enables BGA to be ubiquitous. This was demonstrated by Watanabe in his study of soil samples collected from different countries in the South East Asian region (339), India, and Africa (349). However, his results indicated that N2-fixing BGA are not present in every environment: of 911 samples only 46 (5%) harbored N2-fixing species. His results also suggested that N2-fixing BGA grow more abundantly in tropical and subtropical regions and that they are less common in temperate and subtemperate regions.
Table 6. Schematic representation of the morphological diversity among blue-green algae.

<table>
<thead>
<tr>
<th>BGA</th>
<th>Unicellular</th>
<th>Filamentous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple unicells: <em>Anacystis</em></td>
<td>Without true branching</td>
</tr>
<tr>
<td></td>
<td>Cell aggregates of irregular shape</td>
<td>With false branching</td>
</tr>
<tr>
<td></td>
<td>Compact colonies: <em>Gloeocapsa</em></td>
<td>Nonheterotrichous thalli</td>
</tr>
<tr>
<td></td>
<td>Flat plates: <em>Merismopedia</em></td>
<td>Heterotrichous thalli (primary branches prostrate,</td>
</tr>
<tr>
<td></td>
<td>Cuboidal colonies: <em>Eucapsis</em></td>
<td>secondary branches erect)</td>
</tr>
<tr>
<td></td>
<td>Loose colonies: <em>Microcystis, Aphanothece</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without false branching</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With false branching</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without true branching</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Filament with polarity (tapering)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With false branching</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No polarity (non-tapering)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without cell differentiation:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oscillatoria</em> (thin sheath)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lyngbya</em> (thick sheath)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Spirulina</em> (spiral filament)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Microcoleus</em> (several trichomes within one sheath)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With cell differentiation:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Nostoc</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Anabaena</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cylindrospermum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Noncolonial: <em>Calothrix</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colonial:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rivularia</em> (non-sporing)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Gloeotrichia</em> (sporing)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Without cell differentiation:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Plectonema</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>With cell differentiation:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Scytonema</em> (geminate branches)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(single branches)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both primary and secondary branches uniseriate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mastigocladus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hapalosiphon</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primary branches uniseriate secondary branches multiseriate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Fischerella</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Both primary and secondary branches multiseriate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Stigonema</em></td>
<td></td>
</tr>
</tbody>
</table>

*a*Fix nitrogen in air.
Table 7. Nitrogen-fixing cyanobacteria (reproduced from Stewart et al., 281).

<table>
<thead>
<tr>
<th>Group</th>
<th>Genus</th>
<th>Total</th>
<th>Aerobic</th>
<th>Anaerobic/micro-aerobic</th>
<th>Assay conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcacean</td>
<td>Aphanothece</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Gloeocapsa</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Synechococcus</td>
<td>27</td>
<td>0</td>
<td>3</td>
<td>C₂H₂</td>
</tr>
<tr>
<td>Pleurocapsalean</td>
<td>Dermocarpa</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Xenococcus</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Myxosarcina</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Chroococcidiopsis</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Pleurocapsa</td>
<td>12</td>
<td>0</td>
<td>7</td>
<td>C₂H₂</td>
</tr>
<tr>
<td>Nonheterocystous filamentous forms</td>
<td>Oscillatoria</td>
<td>9</td>
<td>0</td>
<td>5</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Pseudanabaena</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>C₂H₂</td>
</tr>
<tr>
<td></td>
<td>Lyngbya-Plectonema</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Phormidium</td>
<td>25</td>
<td>0</td>
<td>16</td>
<td>C₂H₂, ¹⁵N₂, T.N.</td>
</tr>
<tr>
<td>Heterocystous filamentous forms</td>
<td>Anabaena</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>C₂H₂, ¹⁵N₂, T.N.</td>
</tr>
<tr>
<td></td>
<td>Anabaenopsis</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>C₂H₂, ¹⁵N₂, T.N.</td>
</tr>
<tr>
<td></td>
<td>Aulosira</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Calothrix</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>C₂H₂, ¹⁵N₂, T.N.</td>
</tr>
<tr>
<td></td>
<td>Cylindrospermum</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>C₂H₂, T.N.</td>
</tr>
<tr>
<td></td>
<td>Fischerella</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Hapalosiphon</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Mastigocladus</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Nostoc</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>C₂H₂, ¹⁵N₂, T.N.</td>
</tr>
<tr>
<td></td>
<td>Scytonema</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Stigonema</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Tolypothrix</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Westiella</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>T.N.</td>
</tr>
<tr>
<td></td>
<td>Westiellopsis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>¹⁵N₂, T.N.</td>
</tr>
</tbody>
</table>

*Certain, or all of the cyanobacteria have been tested by these methods; T.N. = total nitrogen.
*bIncludes strains previously designated as N₂-fixing Gloeocapsa strains. cThe data given here are those of Rippka and Waterbury (1977) but the exact numbers of strains tested and shown to have N₂ase may be larger since various earlier workers (Stewart and Lex 1970, Stewart 1971, Stewart et al. 1978a) had examined and obtained positive results with strains which may or may not correspond to those tested by Rippka and Waterbury (1977).

2.1. DESCRIPTIVE ECOLOGY

2.1.1. Occurrence of blue-green algae in paddy fields

The paddy field ecosystem provides an environment favorable for the growth of BGA with respect to their requirements for light, water, high temperature, and nutrient availability. This may account for the higher abundance of BGA in paddy soils than in other cultivated soils (349) as reported under widely different climatic conditions of India (167), Japan (185, 188, 189), and the Ukraine (206).
In the paddy fields, the relative occurrence of BGA varies within large limits. In southeast Iraq, BGA constituted up to 86% of the total algal flora (11). In north and south India they comprised more than half the total number of species recorded (167). In acidic soils of Kerala state (India), their abundance varied between 0 to 76% of the total algae (5). In countries where high levels of N fertilizers are commonly used, green algae were most frequently the naturally dominant species (33, 38, 39, 208), but BGA have been isolated from the soil (33, 155). Venkataraman (13, 328), however, has pointed out that “Contrary to the general belief, N2-fixing BGA are not invariably present in tropical rice soils and that an all India survey showed that out of 2,213 soil samples from rice fields, only about 33% were found to harbour nitrogen-fixing forms.” Reasons for the heterogeneous and sometimes limited distribution of N2-fixing BGA are still not well known, as no systematic analysis has correlated their presence or absence with environmental factors (144).

2.1.2. Record of species and taxonomic studies
Most of the preliminary studies on the ecology of BGA in rice fields have been identifications and records of species. These studies are compiled in Table 8, by geographical region of the world. Four report the observation of new species in paddy fields (42, 140, 202, 236).

It has to be kept in mind that the species recorded depends upon the methodology used: either direct observation or soil culture. As pointed out by Gupta (82) who compared both methods, while many species can be observed both in situ and in soil cultures, certain BGA (Gloeotrichia and Aphanothece) were observed only in situ and others (like Fischerella) grew only in soil cultures.

One other aspect of these preliminary studies has been the demonstration of the ability of the isolated BGA to fix nitrogen (65, 66, 129, 130, 133, 180, 188, 189, 205, 263, 264, 284, 294, 332, 339, 343, 349, 366). Most of these demonstrations were based upon the ability of the strains to grow in nitrogen-free culture media.

2.1.3. Quantitative estimations
2.1.3.1. Methodology
Ecological studies on BGA in submerged soils are limited by problems in methodology, primarily in estimating algal biomasses qualitatively and quantitatively. In addition, problems in sampling techniques in relation to spatial distribution of BGA and their nitrogen-fixing activity (NFA) increase the inaccuracy of quantitative measurements.

Algal abundance is usually determined by three principal methods: plating techniques, measurement of pigments, and direct observation. However, not one is completely satisfactory.

Plating techniques are advantageous in providing qualitative and quantitative results simultaneously; however, the accuracy of the counts depends on the reliability of the particular dilution method. Filamentous forms like Oscillatoria
Table 8. References reporting records of blue-green algae in rice fields.

<table>
<thead>
<tr>
<th>Region</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa:</td>
<td>66 (Egypt); 217 (Morocco); 302 (Mali).</td>
</tr>
<tr>
<td>Europe:</td>
<td>27 (Spain); 39 (Italy); 208 (Italy, Pavia area).</td>
</tr>
<tr>
<td>Central Europe:</td>
<td>101 (Ilfov); 123; 124 (Hungary); 181 (Kazakhstan); 183 (Kazakhstan); 200 (Kocani); 201 (Kochane); 205 (Ukraine); 206 (South of Ukraine); 231 (Lenkoran).</td>
</tr>
<tr>
<td>Middle East:</td>
<td>11 (Iraq).</td>
</tr>
<tr>
<td>Indian Region:</td>
<td>15 (Kerala); 26 (Bengal); 72 (Bombay); 79 (Allahabad); 92 (Sri Lanka); 115 (Nagpur); 119; 129; 139; 167; 180 (Lyallpur); 191 (Uttar Pradesh); 254 (Panki); 269 (Chota Nagpur); 270 (Chota Nagpur); 297 (Sri Lanka); 299; 300; 301; 309 (Allahabad).</td>
</tr>
<tr>
<td>Central Asia:</td>
<td>177</td>
</tr>
<tr>
<td>South East Asia:</td>
<td>113 (Malaysia); 114 (Java); 154 (Philippines); 185 (Japan); 188 (Japan); 189 (Japan); 194 (Philippines); 196 (Philippines).</td>
</tr>
<tr>
<td>Australia:</td>
<td>33</td>
</tr>
<tr>
<td>America:</td>
<td>34 (California).</td>
</tr>
<tr>
<td>Cuba:</td>
<td>76</td>
</tr>
</tbody>
</table>

and Lyngbya are difficult to separate into individual cells, whereas moniliform filaments such as Anabaena and Nostoc, which are easily separated, may give inflated figures of abundance. Plating techniques can be improved by determining the mean volume of each “count unit” (cell, filament, or colony, according to species) by directly examining the first dilution and multiplying the results by the corresponding “volume unit.” This permits the expression of the results of enumeration in terms of biomasses (228). Use of selective media enables the enumeration of algae separately as N₂-fixing, prokaryotic, and eukaryotic (228). Although plating methods do not distinguish between active and inactive forms, they can provide an index of soil potentiality and an evaluation of the evolution of algal populations when compared with a control.

Pigment analysis, although frequently used in limnological studies, does not indicate the composition of the algal flora and, in most instances, is not suitable for field material because colored organic substances such as humic acids may also be extracted in acetone and affect the results (232).

Direct observation under either ordinary or fluorescence microscopy, generally used for qualitative measurements, has been adopted for quantitative evalua-
tion of algal biomasses of floodwater or surface soil. Floodwater was filtered on membrane filters, and gelatinized soil suspension was smeared on glass slides (239).

The validity and accuracy of algal enumerations depend principally on the density of sampling that, for a given degree of accuracy, varies with the distribution law of the variable. A study of the correlation between means and variances of groups of duplicate measurements indicated that algae in a paddy field had an approximately log-normal distribution (logarithms of numbers were normally distributed) (231).

The first implication of this result was that the confidence interval and parametric statistical variables (i.e. $t$-variable of Student-Fisher) must be calculated using only the logarithms of algal enumerations. The confidence interval so calculated was dissymmetrical. Its lower limit was generally a little less than those incorrectly calculated using the $t$-variable of Student-Fisher; the upper limit was frequently much higher. The dissymmetry increased as the number of duplicates was reduced and was markedly strong with less than five replicates (231).

The second implication of the distribution of algae concerned the density of sampling. For a given accuracy, more replicates are needed when the distribution is log-normal than when it is normal. For example, the mean value of *Anabaena* biomasses based upon 40 samples of 10 cores each, taken in a 2,500-m$^2$ paddy

<table>
<thead>
<tr>
<th>References</th>
<th>Location</th>
<th>Values (no.·g$^{-1}$ dry soil)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Thailand</td>
<td>$10^3$ to $10^4a$</td>
<td>9 soil types studied</td>
</tr>
<tr>
<td>22</td>
<td>Thailand</td>
<td>$10^3a$</td>
<td>103 sites studied</td>
</tr>
<tr>
<td>71</td>
<td>Senegal</td>
<td>$0$ to $10^4a$</td>
<td>40 soils studied during the dry season</td>
</tr>
<tr>
<td>107</td>
<td>Japan</td>
<td>$10^6a$</td>
<td>Fertilized plots</td>
</tr>
<tr>
<td>122</td>
<td>Thailand</td>
<td>$10^3a$ to $10^5a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>$10^4$ to $10^7a$</td>
<td></td>
</tr>
<tr>
<td>156 to 160</td>
<td>Thailand</td>
<td>$10^3a$</td>
<td>Brackish water, alluvial soil and Regosol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^5a$</td>
<td>Noncalcic brown soil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^6a$</td>
<td>9 other soil types</td>
</tr>
<tr>
<td>304</td>
<td>Mali</td>
<td>$10^3$ to $10^6b$</td>
<td>12 measurements in the same field along a 2-year period.</td>
</tr>
<tr>
<td>262</td>
<td>India</td>
<td>$2 \times 10^7 \cdot \text{cm}^{-2}c$</td>
<td><em>Aphanothece pallida</em> from the water surface</td>
</tr>
</tbody>
</table>

$^a$Most-probable-number method. $^b$Plating method. $^c$Method not indicated.
field, had a confidence interval of +32% and −27% of the mean. Such an evaluation made on three selective media required 1,800 petri dishes (231).

These difficulties may explain the scarcity of quantitative ecological studies of the algal flora in paddy fields.

2.1.3.2. Results

Results of BGA enumerations (Table 9) are too fragmentary to allow general comments. They indicate that BGA populations in paddy soils vary within large limits, from a few to \(10^7\) g\(^{-1}\) dry soil. Results of algal biomass measurements (Table 10) indicate that BGA can develop large biomasses of several tons per hectare. The available literature suggests that BGA potentiality is higher in paddy fields than in other cultivated soils (232).

Table 11 indicates the mean composition of different \(\text{N}_2\)-fixing genera and the respective biomasses corresponding to 10 kg N ha\(^{-1}\). The results show that \(\text{N}_2\)-fixing algal biomasses of agronomic significance have generally a value suffi-

Table 10. References reporting algal biomass measurements in rice fields.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Dry wt (kg·ha(^{-1}))</th>
<th>Fresh wt (kg·ha(^{-1}))</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>China</td>
<td>3 to 300</td>
<td>7,500</td>
<td>After inoculation</td>
</tr>
<tr>
<td>147</td>
<td>India</td>
<td>32</td>
<td>60 to 6,000(^a)</td>
<td>Green algae dominant</td>
</tr>
<tr>
<td>176</td>
<td>UzbSSR</td>
<td>16,000</td>
<td>Total algal biomass</td>
<td></td>
</tr>
<tr>
<td>219</td>
<td>Senegal</td>
<td>2 to 6,000</td>
<td>Total algal biomass</td>
<td></td>
</tr>
<tr>
<td>239</td>
<td>Philippines</td>
<td>2 to 114</td>
<td>N(_2)-fixing algal biomass</td>
<td></td>
</tr>
<tr>
<td>261</td>
<td>India</td>
<td>480</td>
<td>9,000(^a)</td>
<td>\textit{Aulosira} bloom</td>
</tr>
<tr>
<td>280</td>
<td>India</td>
<td>100 to 2,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>353</td>
<td>Philippines</td>
<td>177</td>
<td>24,000</td>
<td>\textit{Gloeotrichia} bloom</td>
</tr>
</tbody>
</table>

\(^a\) Data extrapolated on the basis of 95% water content.

Table 11. Mean composition of different BGA genera and biomasses corresponding to 10 kg N ha\(^{-1}\) (unpubl.).

<table>
<thead>
<tr>
<th>Genera</th>
<th>Strains tested (no.)</th>
<th>Dry matter (% of fresh wt)</th>
<th>Protein (% of dry wt)</th>
<th>Fresh wt (t·ha(^{-1})) corresponding to 10 kg N·ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Calothrix}</td>
<td>6</td>
<td>5</td>
<td>32</td>
<td>3.9</td>
</tr>
<tr>
<td>\textit{Nostoc}</td>
<td>4</td>
<td>2.2</td>
<td>30</td>
<td>9.4</td>
</tr>
<tr>
<td>\textit{Gloeotrichia}</td>
<td>8</td>
<td>0.74</td>
<td>29</td>
<td>29.0</td>
</tr>
</tbody>
</table>
ciently high to permit direct measurements (weight, chlorophyll or other pigments, proteins, etc.) and to avoid enumerations that are tedious and imprecise and cannot distinguish between active and inactive forms. These results also indicate that, due to the very variable water content of the strains, fresh weight measurements are not reliable in evaluating the potential contribution of N by BGA in the field.

From the highest dry weight recorded (480 kg·ha\(^{-1}\)) (261) and assuming a protein content of 30-50%, it appears that under favorable conditions, the N\(_2\)-fixing algal bloom may contribute 30-40 kg N·ha\(^{-1}\) in the ecosystem.

2.1.4. Evolution of the algal flora along the cultivation cycle

2.1.4.1. Quantitative variations of the total flora
From reports concerning the variations of the algal flora along the cultivation cycle, it appears that maximal biomass could develop any time and is mainly related to climatic conditions. Development of dense algal blooms just after transplanting, due to fertilizers or plowing, or both, and a high light availability have been reported (239) (see also section 43). In paddy fields in Japan, the maximal algal biomass was observed about 2 weeks (137) or 1 month (98) after transplanting; the subsequent decrease of the biomass was related to the consumption by grazers (137) and to a deficient light under the rice canopy (98, 137). In the Ukraine, maximal algal growth was observed just before tillering (205). In paddy fields in Senegal, maximal biomass developed between tillering and panicle initiation (228). In dryland rice fields in India, a similar evolution was observed; in wetland fields, however, the density of the biomass was maximal a little later than in Senegal (82). In the Philippines, during the dry season, algal density was highest just after heading of the rice crop. During the wet season, development was maximum after harvesting (353, 355), probably because of an increase in light availability.

2.1.4.2. Qualitative and quantitative variations of the components of the algal flora
Algal populations appear to be highly susceptible to environmental changes and exhibit rapid qualitative and quantitative variations along the cultivation cycle.

A qualitative study of algae in paddy fields of Allahabad district (India) by Gupta (82) describes the evolution of the algal flora in dryland rice fields planted to an early rice variety, and in wetland paddies planted to a late variety:

- In the dryland fields, the algae began to grow 1 month after the first monsoon rain; the algal community was poor in both quality and quantity and was dominated by *Spirogyra* sp. associated with *Anabaena* sp. During the next third of the cultivation cycle, the algal biomass became abundant with a dominant growth of *Anabaena* sp. During the last third of the cycle, more species were observed and the N\(_2\)-fixing forms were well represented by *Anabaena* sp., *Scytonema* sp., and *Nostoc* sp.
In wetland rice fields, fresh water remained muddy 2 months after the beginning of the monsoon and no algae grew. *Anabaena* was the first species to develop, 2 weeks after transplanting. During the next 2 months, an abundant mixed algal flora was observed. N₂-fixing forms (*Scytonema* sp., *Aulosira* sp., *Nostoc* sp.) were associated with *Chara* sp. and filamentous green algae. At the end of the cycle, non-N₂-fixing forms were disintegrating and *Scytonema* sp. was dominant.

In both soils, N₂-fixing forms were present from the beginning of the algal community and became abundant in the second half of the cycle.

The main differences between these two types of rice paddies were:
- a later growth of algae in wetland soil,
- a well-marked dominance of BGA in dryland soils, and,
- a mixed community of eukaryotic and prokaryotic algae in wetland soils.

Gupta concluded that the difference in growth between wetland and dryland fields was associated with ecological conditions, and the overall succession was controlled chiefly by seasonal variations (82).

Studies on the qualitative evolution of the algal flora have been conducted in paddy fields in Senegal by Roger and Reynaud (219, 228, 229). In this area the soils are acidic, with an average pH value of 5.0 at the beginning of rice cultivation and 6.2 after 2 months of submersion. The rainy season is short (15 July-15 November) and rice fields are dry the rest of the year. High light intensities reaching 70-80 klux occur throughout the year. The qualitative composition of total and N₂-fixing algal flora was studied first during the cultivation cycle in a paddy field located north of Senegal and then in 40 paddy fields differing in geographic location, stage of rice growth, and fertilizer treatment (Table 12). Similar results from the two studies can be the basis of a scheme for algal successions in the studied area.

During the early part of the cultivation cycle (planting and tillering), the algal biomass increased and consisted mainly of diatoms and unicellular green algae. From tillering to panicle initiation, the algal biomass reached its highest values, and filamentous green algae and non-N₂-fixing BGA were dominant. After panicle initiation the total biomass decreased. If the plant cover was sufficiently dense, heterocystous BGA developed; if it was thin, filamentous green algae and homocystous BGA remained dominant.

The following interpretation of algal flora variations was proposed:

At the beginning of the cultivation cycle, paddy soils were characterized by:
- a low pH, which favored the development of Chlorophyceae but not of BGA;
- an absence of plant cover and a corresponding high light intensity at the air-water interface that was also favorable for the development of Chlorophyceae and diatoms but unfavorable to BGA;
- a high level of CO₂, caused by soil remoistening, which was favorable to green algae.
Table 12. Algal biomass composition in relation to rice development in 40 paddy fields in Senegal (Reynaud and Roger, 219).

<table>
<thead>
<tr>
<th>Stages of rice development</th>
<th>Dominant flora</th>
<th>% of total biomass</th>
<th>N₂-fixing algae (%) of total biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nature</td>
<td>Mean value</td>
<td>Max value</td>
</tr>
<tr>
<td>Tillering</td>
<td>Diatoms, unicellular green algae</td>
<td>73</td>
<td>99</td>
</tr>
<tr>
<td>Panicle initiation</td>
<td>Filamentous green algae, non-heterocystous blue-green algae</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>Heading to maturity</td>
<td>Filamentous green algae, non-heterocystous blue-green algae</td>
<td>70</td>
<td>91</td>
</tr>
<tr>
<td>Weak plant cover</td>
<td>Blue-green algae</td>
<td>71</td>
<td>99</td>
</tr>
</tbody>
</table>
During the cultivation cycle, a decrease in light intensity and N level related to rice growth and an increase in pH value favored BGA growth. The non-evolution of algal flora composition under a weak plant cover indicated the important role of light in regulating the algal composition.

It is clear that the proposed interpretation of algal successions was incomplete (i.e. other factors of nutrition and competition may affect the sequence of the algae groups) and should be considered characteristic of a definite geographic zone having acidic soils, high light intensities, and a semiarid climate (219, 228, 229). However, the same kind of algal successions has been described in the Kuban area (169) and the Philippines (194) where diatoms predominated during land preparation for transplanting, followed by green algae as the plant grew and BGA just before and during the harvest. Also in Japan, diatoms and unicellular green algae developed at the beginning of the cultivation cycle, followed by filamentous green algae, but the dominant alga at the end of the cycle was *Trachelomonas* and BGA were never dominant (120).

A very frequent observation is that N₂-fixing BGA rarely become dominant at the beginning of the cultivation cycle (82, 120, 169, 181, 194, 206, 219, 228, 229). In a lysimeter experiment in Japan, green algae developed their maximal biomass at the beginning of the cultivation cycle, and then decreased; BGA reached the maximal abundance in the middle of the cycle and became dominant at the end (293). A similar observation was done in paddy fields in India (Varanasi) where nonfixing forms developed first. The appearance of fixing forms was earlier in the unfertilized plots than in the fertilized ones (267).

However, the growth of a dense N₂-fixing algal bloom has been reported in a paddy field in Mali at the earliest stage of growth of rice (304) and an observation done in Sri Lanka indicated that N₂-fixing forms were present throughout the cultivation cycle; *Nostoc* and *Anabaena* appeared during the early stages and continued throughout the cycle, but *Gloeotrichia* and *Rivularia* appeared during the latter part (297).

2.2. PHYSICAL FACTORS

2.2.1. Light

Of the habitat factors, which affect the seasonal fluctuations of the phytoplankton in paddy fields, the most important is light (98, 232). Algae, as phototrophic microorganisms, are restricted to the photic zone and are usually located in the upper 0.5 cm of the soil. However, viable propagules have been found up to a depth of 1 m (115). In the paddies BGA occur especially as surface scum, as a bloom, or as crust-forming aggregates at the soil water interface. During daytime, vertical migration of algae occurs in the water in relation to O₂ production by photosynthesis.

In submerged soils, light availability depends upon the season and latitude, the cloud cover, the plant cover, and the turbidity of the water. In rice fields, the light-screening effect of the crop canopy appears to cause a rapid decrease of light
reaching the floodwater. With transplanted rice, the canopy produced a 50% decrease after 15 days, 85% after 1 month, and 95% after 2 months (137). This was also related to the height of the rice plants: 30 cm plants suppressed 50% of the light and 60 cm plants cut off 90% (137). Depending upon the region, the season, and the plant canopy, light intensity reaching the floodwater could vary from deficiency to inhibitory levels.

Light tolerance differs between algal species and may be roughly correlated with taxonomic groups. Many green algae are adapted to high light conditions, red algae are low-light species, diatoms and chrysophyceae seem more indifferent to light. BGA are generally sensitive to high light intensities and may be regarded as low light species (232). However, certain BGA appear to be more resistant to high intensities. In a paddy field in Mali, a dense bloom of *Cylindrospermum* was observed after harvesting, growing under full sunlight (>100 klux at 13.00) (304). Field growth of inoculated *Anulosira fertilissima* was reported to be better under full sunlight (261). Because algae have different light-adaptation abilities, light may have a selective effect on the composition of the flora.

The effect of high light intensities was observed in Senegal where diatoms and unicellular green algae developed at the beginning of the cultivation cycle and BGA developed later when the plant cover was sufficiently dense to protect them from high light intensities (higher than 80,000 lux at 1300 h). The N2-fixing algal biomass and the density of the plant cover were positively correlated (229). The influence of high light intensities on algal successions was confirmed by incubating submerged unplanted soil under screens to permit the transmission of 100, 60, 22, and 7% of incident sunlight (higher than 80,000 lux at 1300 h). After 1 month of incubation, the acetylene reducing activity (ARA) was highest in the most heavily shaded soil, and green algae and diatoms were dominant in the soil placed in full sunlight (219). On the other hand in a monsoon zone where light intensities were not as high, no such succession was observed and BGA developed from the beginning of the cultivation cycle (82).

Deficiency of light may also act as a limiting factor. In Japan the productivity of plankton increased in early summer but decreased in late July when the rice canopy decreased the light intensity (1% of incident light) below the compensation point of the phytoplankton (98). In the Philippines, during the wet season when light was moderate, ARA was higher in bare soil than in planted soil (98, 343).

### 2.2.2. Temperature

The optimal temperature for the growth of BGA is about 30-35°C, and is higher than that of eukaryotic algae. Temperature extremes inhibiting their growth are beyond the range within which rice grows; thus, rarely is temperature a limiting factor for BGA in paddy fields (232). Temperature influences both algal biomass composition and productivity.

In a paddy field in Japan, *Hydrodictyon reticulatum*, *Spirogyra setiformis*, and *Anabaena oscillarioides* were proliferous in summer but were replaced by
Tetraspora gelatinosa, Draparnaldia sp., and Sphaeroplea annulina in winter. But water blooms of Euglena sp. with different associated species were prolific over the year round without being influenced by water temperature (172). In temperate or sahelian zones during the dry season, a lower temperature at the beginning of the cultivation cycle may favor eukaryotic algae and inhibit BGA growth (229). Low temperatures decrease the phytoplankton productivity. In India both field and pot experiments indicated a setback to the growth of algae during the cold season (287). In Japan the phytoplankton yield was usually higher on the plain or in warm-temperature districts than in the mountain or cold-temperature districts (98).

Very high temperatures have a deleterious effect on BGA and algal NFA (232). Exposure to a temperature of 42°C for 50 minutes reduced the photosynthetic activity of Nostoc sp. and Calothrix sp. to about 20% of the control (317). In Japan, violent daily fluctuations of water temperature were observed in the afternoon from June to mid-July. At temperatures higher than 35°C, the daytime CO₂ assimilation abruptly declined (137). On the other hand, the high temperature in Indian paddy water (34-39°C) was favorable to Aulosira fertilissima growth (261).

As high temperatures are frequently associated with high light intensities, it is important to interpret separately the effect of these two factors in ecological studies, specially in ARA measurements where "a greenhouse" effect may occur (see section 321).

2.2.3. Desiccation and rewetting
Soil algae, especially BGA, have a high capacity to withstand desiccation. Resistance to desiccation increases with the dryness of the biotope and can be related to the floristic composition of desiccated soils (232). In a paddy field in Italy, where the dry period was relatively short, N₂-fixing BGA comprised only about 30% of the algal flora (155); but in Senegal, where the dry season lasts about 8 months, spores of heterocystous BGA constituted more than 95% of potential flora at the end of the dry period and homocystous BGA forms were present primarily because of their introduction by irrigation water (228). In Uttar Pradesh (India), a large number of Chlorophyceae occurred in low-lying fields where the moist habitat apparently was suitable for their growth. Being more resistant to drier conditions, BGA occurred in larger numbers in paddy fields at higher elevations (191, 192).

Alternate periods of desiccation and submersion may also influence the quality and quantity of the algal biomass. In paddy soils in Japan, algae were more abundant when the soil was waterlogged throughout the year than when it was waterlogged during rice growth only (186). A peak of carbon and nitrogen mineralization occurs during rewetting of desiccated soils; the high concentration of mineral nitrogen may decrease the relative competitiveness of N₂-fixing forms, and the high concentration of CO₂ may favor the growth of green algae at the beginning of the cultivation cycle (232). At the end of the cultivation cycle and as
the soil dries, species that form mucilaginous colonies, such as *Nostoc* and *Cylindrospermum* sp., lose water slowly and can develop profusely (191, 219, 304).

### 2.2.4. Other factors

Rain may increase water turbidity, limiting available light to such an extent that phototrophic NFA is significantly decreased. Heavy rains have been shown to suppress the development of *Aulosira fertilissima* inoculated in the field (261). In the Philippines, we measured in situ ARA before and after a typhoon and observed a suppression of any photosynthetic ARA, algae being washed out of the field (unpublished data). In nonsubmerged soils, buffeting rains may mix the algae with the top layer of the soil inhibiting the algal NFA almost completely (304).

Wind may either cause algae to accumulate on one side of the field, decreasing light availability, or cause dense algal masses to disperse and form separate colonies (232).

#### 2.3. BIOTIC FACTORS

Biotic factors capable of limiting BGA growth in rice fields are pathogens, antagonisms, and grazers.

##### 2.3.1. Pathogens

Certain bacteria, fungi, and viruses have been shown to be pathogenic to BGA. Lytic bacteria may cause BGA lysis within 2-10 hours; they frequently caused the decomposition of vegetative cells, but did not affect the heterocysts and spores. Parasitic fungi have a host range limited within a single species and some may even be confined to a specific structure as a heterocyst or a spore. Their seasonal variations are directly correlated with the abundance of the algae on which they occur. However, the presence and the role of parasitic bacteria and fungi in rice fields have not yet been reported (232).

Considering the widespread occurrence of algal viruses and the specific host ranges of the individual strains, it is possible that in natural situations cyanophages may be important in determining algal successions and disappearance. Cyanophages have been isolated from rice fields in India. Two types that are lysogenic for *Plectonema boryanum* were recorded with a maximal density of 1,000 plaque-forming units ml⁻¹. Seasonal variations in their abundance have been demonstrated (259).

##### 2.3.2. Antagonisms

Many algae release substances that inhibit either their own growth or the growth of other species, or both. Such substances may play important roles in the succession of species in aquatic ecosystems, but evidence of antagonism for BGA in natural environment is lacking (232).

Antagonisms between BGA and bacteria are also poorly documented: hydrogen sulfide produced by sulfate reducing bacteria under waterlogged conditions had a toxic effect on algae (59) and evidence was obtained that some
materials toxic to *Azotobacter* are formed in submerged rice soils as a result of BGA growth (293).

In Philippine rice paddies, the colonization of the water by submerged weeds suppressed the algal blooms formed after transplanting (239). At crop maturity, a negative correlation was also observed between the floating algal biomasses and submerged weed biomasses, but whether this was due to antagonisms or to competition, or to both, is still unknown (136).

### 2.3.3. Grazers

Invertebrates like cladocerans, copepods, ostracods, mosquito larvae, snails, etc. are common grazers of algae in rice fields. The development of such populations prevents the establishment of algal inocula (90, 336) and causes the disappearance of algal blooms within one or two weeks (311).

In a study of the succession of phytoplankton and zooplankton in a rice field in Japan, zooplankton was found to appear about 1 week after the growth of phytoplankton and developed its maximal biomass 2 weeks after the maximal abundance of the phytoplankton (137).

Evidence of preferential grazing among algae has been presented by Watanabe et al (336) who showed that unicellular green algae are excellent feed for daphnids while filamentous N$_2$-fixing BGA also served as a nutrient source, although less effectively.

Insecticides have been shown to enhance algal growth and sometimes to favor BGA over green algae and diatoms (see section 2.5.4) and the establishment of an algal inoculum in the field frequently needs a simultaneous application of insecticides (see section 2.5.4, 5.4.2). Development of grazer populations can also be controlled by other methods such as seasonal drying or measures to prevent the growth of unicellular green algae that favor the increase of daphnid populations (336). Introduction into the paddy fields of the freshwater fish *Tilapia mozambica*, which feed on algal grazers such as chironomial larvae, had a beneficial effect on the growth of the *Nostoc* commune (151). On the other hand, *Tilapia nilotica* has been reported to ingest large quantities of BGA (232).

Snails form another group of algal grazers in rice fields, but no work has so far reported their effect on algae. In some preliminary experiments, we observed a rapid consumption of laboratory-grown N$_2$-fixing BGA by snails and a heavy biomass of snails (1 to 1.6 t·ha$^{-1}$) in certain rice fields at IRRI where there were very little algae (unpublished data).

Some biotic factors may also have a positive effect on the BGA population and its N$_2$-fixing activity — synergistic effects (35), production of growth-promoting substances by associated microorganisms, etc. — but no evidence has been reported on these aspects.

### 2.4. SOIL PROPERTIES

#### 2.4.1. pH

Among the soil properties, pH is certainly the most important factor determining
the algal flora composition.

In culture media the optimal pH for growth of BGA seems to range from 7.5 to 10.0 and the lower limit is about 6.5 to 7.0 (232).

In soil-culture experiments, it was found that soils having slightly alkaline reaction fixed much more nitrogen than those with lower pH. In the former, a heavy algal growth appeared soon after waterlogging and exposure to light, but in the latter the growth was poor even after inoculation (49).

Under natural conditions, BGA grow preferentially in environments that are neutral to alkaline (232). In central Sri Lanka, where the soils are acidic, N₂-fixing BGA were not predominant in rice fields and inoculation in such soils was unsuccessful. But in the northern part where the soils are alkaline, the predominant algae in the fields were N₂-fixing BGA (133). In Thailand, marine alluvial, freshwater alluvial, humic gley, and Grumosol soils—which were generally high in pH, organic matter, and phosphorus contents—tended to hold higher populations of Azotobacter, Clostridium, and BGA. Considerably low populations of such microorganisms were obtained in acid-sulfate soils of brackish water alluvium (156 to 160).

There are, however, reports on the presence of certain BGA strains in soils with pH values between 5 to 6. From field observations in India, it was inferred that most BGA preferred a neutral or near neutral pH (6.5-7.5), but others were also capable of thriving over a wider range (5.5-8.5) (203). Aulosira fertilissima and Calothrix brevissima have been reported to be ubiquitous in Kerala rice fields where the pH ranged from 3.5 to 6.5 (5). The development of a dense algal bloom on an acidic soil (pH 5.5) was observed in Japan after surface application of straw (162).

Soil pH has a selective effect on both the indigenous algal flora and the changes of the algal population. The dominant algal species in acidic and alkaline soils often differ, i.e. the growth of Chlorophyceae is favored by low pH values and that of BGA by higher values (192). In acidic soils of Kerala (pH 3.6-6.3), application of lime increased available N and promoted the growth of N₂-fixing BGA; in the untreated plots, predominant algae were Chlorophyceae (15). In Senegal, the rewetting of acidic soils was generally followed by an increase of pH (71). The evolution of the algal flora in these soils, where BGA grew preferentially at the end of the cultivation cycle, was related pro parte to this pH increase (228, 229).

A common observation is a positive correlation between pH and occurrence of BGA. This correlation was observed between:

- water pH and the occurrence of BGA (189);
- soil pH and the number of spores of N₂-fixing BGA in the soil during the dry season (71);
- soil pH and the growth of BGA (186, 187, 189);
- soil pH and the N₂-fixing algal biomass, but this relationship was conspicuous only in samples homogenous for stage of rice development, fertilization, and plant cover density (228, 229).
The positive influence of high pH on BGA growth is further demonstrated in soil amendment experiments in which addition of lime increased BGA growth and N\textsubscript{2}-fixation (186, 187, 189, 250, 363).

2.4.2. Other properties

Very little information is available on the effects of other soil properties on BGA. In a study of the relationship between the growth of BGA and some soil properties in Japan it was found that next to pH, the most decisive factor was the available-phosphorus content. No correlation was found between soil organic matter content or soil texture and BGA growth (186, 188, 189). Similar experiments conducted in 40 paddy fields in Thailand indicated significant differences of the nitrogen-fixing populations and ARA among 9 soil types. These differences were related mainly to pH and phosphorus content of the soils (156 to 160).

In a laboratory experiment where 12 paddy soils were compared, Wilson and Alexander (360) observed that nitrogen fixation and development of indigenous BGA were correlated with pH and the levels of extractable K and of Ca and Mg in these soils, but not with extractable phosphate and Fe.

In saline soils in the USSR, N\textsubscript{2}-fixing BGA were reported to be less often observed than nonfixing ones.

Among the physicochemical properties of the soil, the redox potential may be an important factor, especially in view of the occurrence of unicellular and homocystous BGA, which reduces C\textsubscript{2}H\textsubscript{2} under microaerophilic conditions. When nonheterocystous algae are dominant, N\textsubscript{2}-fixation by them may be important (281), but no information is available on this subject.

2.5. AGRONOMIC PRACTICES

2.5.1. Land preparation and management

The various agronomic practices adopted along the cultivation cycle influence growth of BGA.

Tillage has a disturbing effect, mainly because of the incorporation of algae (or spores) in the soil and dispersion of clay particles in the submersion water, which decreases available light. After a superficial incorporation of algae into the soil, motile forms such as Oscillatoria and Pseudanabaena are probably more adapted to the recolonization of the submersion water (232). Because tillage increases ammonification, excessive tillage may favor eukaryotic algae. On the other hand, midseason tillage, which increases P and Fe availability, may favor BGA growth.

Transplanting provides a discontinuous canopy at the beginning of the cultivation cycle and, compared to broadcasting, may favor algal growth where light becomes a limiting factor by deficiency (27).

Weeding disturbs the ecosystem and can be detrimental for algal growth. However, no significant correlation between this practice and the standing crop
of phytoplankton was observed in Japan (137) and a negative correlation between submerged weed biomass and floating N₂-fixing BGA was observed in a Philippine rice field (136), indicating that the removal of weed may permit better BGA growth after a possible initial depressive period.

Another agronomic practice that directly affects BGA growth is water management. In alkaline lands of northern India, water impoundment was sufficient to permit the profuse growth of BGA (265). A similar observation was found in the Philippines (154). The rate of irrigation may affect the algal growth. In Australian rice soils it was observed that algal development was most marked where the movement of the irrigation water was reduced to a minimum and its turbidity was low (33). Alternating the field's drying and rewetting throughout the phase of rice germination suppressed a detrimental growth of green algae and favored the mass propagation of BGA (169). This practice was also recommended to control grazers that feed on the phytoplankton (see section 2.3).

2.5.2. Inorganic fertilizers
The nature and the quantity of fertilizers as well as application techniques have a considerable influence on the subterranean algal flora.

Combined NPK fertilization has given variable results in different locations. In the Philippines, a much higher N₂-fixing algal biomass (Gloeotrichia) was recorded in unfertilized plots (24 t/ha) than in fertilized plots (<3 t/ha); ARA values were in agreement with these biomasses (352, 355). In Senegal, the study of the qualitative and quantitative composition of algal flora in 30 paddies differing in geographic location, stages of rice growth, and fertilizer treatment indicated that NP fertilization had a positive effect on both total algal biomass and N₂-fixing algal biomass. However, it had a negative effect on the relative N₂-fixing algal biomass expressed in terms of percentage of the total biomass (229).

The mode of fertilizer application influences the algal flora both quantitatively and qualitatively. Surface application of NPK generally results in a profuse growth of algae. To prevent such a growth that might cause seedlings to lodge, incorporation of basal dressing of fertilizers is frequently practiced in Senegal (232). It has also been observed that deep placement of urea supergranules not only prevents the dense growth of green algae observed with surface broadcast urea but also does not inhibit BGA growth and N₂-fixation (234).

Considered separately N, P, K, lime, and other elements may have differential qualitative and quantitative effects on BGA growth. After combined N, P, K, lime, and organic manure treatments, changes in the dominant BGA species were observed. In particular, Aphanothece was predominant under lime treatments, Rivularia aquatica under K, and Plectonema boryanum under P (274).

2.5.2.1. Nitrogen
Under N-deficient conditions, N₂-fixing BGA are greatly favored by a lack of competitiveness of the other algae and can develop profusely if the other en-
vimental factors are not limiting. When nitrogenous fertilizers are applied, their NFA is inhibited or at least affected. As diazotrophic organisms, they can use mineral nitrogen for their growth; but under such conditions, they have to compete with non N₂-fixing BGA and eukaryotic algae.

Little is known about the competition between N₂-fixing and non-N₂-fixing forms as affected by the nature and the concentration of inorganic nitrogen (232); however, the selective action and inhibitory effect of nitrogenous fertilizers on N₂-fixing BGA have been demonstrated in many experiments (222, 224).

In pot experiments, Yoshida et al (368) observed that N fertilizer increased algal growth, but that generally there were more BGA in pots without N fertilizer. Than Tun (296) reported that nitrogenous fertilizers increased the total algal biomass and depressed the growth of *Anabaena* and that in soils treated with ammonium sulfate or calcium cyanamide, only green algae appeared dominant (296). Subrahmanyan et al (286) also observed that after ammonium sulfate treatments, *Spirogyra* sp. and *Euglena* sp. were so abundant that rice farmers had to interfill their crop to prevent the algae from smothering the rice plants. Okuda and Yamaguchi (186) observed algal flora monthly (from April 1944 to November 1945) in soils of lysimeters treated with different N-fertilizers; they reported N₂-fixing forms to become abundant only in the unfertilized control. A survey of Australian rice soils showed that although N₂-fixing BGA were isolated from almost all the soil samples, their presence in the fields was not apparent. This has been attributed partially to the application of heavy dressings of (NH₄)₂SO₄ and the addition of CuSO₄ to irrigation water (33).

However, a report indicated that in a soil where diatoms, *Ulothrix*, and *Spirogyra* were dominant in the absence of fertilizers, addition of 160 kg N·ha⁻¹ induced the dominance of *Nostoc muscorum*, *Anabaena cylindrica*, and *Volvox* sp. (147). No explanation was given for this surprising observation.

In situ ARA measurements confirm the inhibitory effect of N fertilizers on N₂-fixing BGA; however, this inhibition is frequently only partial (see section 3.2), and decreases during the growth cycle because of the uptake of N by plants, especially at the later stages of growth.

In stagnant paddy water, within mixed algal masses, combined N may diffuse at a slower rate than the readily available dissolved N₂, and a local depletion in combined N may favor N₂-fixing algae. Thus, in the fields, the lack of competitiveness of N₂-fixing algae in the presence of mineral nitrogen may not be as clear-cut as it was first thought (232).

2.5.2.2. Phosphorus

The phosphorus requirement for optimal algal growth differs considerably among species when no other external factor is limiting. However, no conclusions could be made about the relationship between P-requirements and taxonomic groups. The P levels within algal cells may fluctuate widely depending on whether or not the algae are growing under P-limited conditions. BGA assimilate more P than they require and store the excess as polyphosphate, which can be
used under P-deficient conditions (232).

In laboratory experiments the addition of phosphates, in either soluble (KH2PO4) or insoluble [Ca3 (PO4)2] form stimulates algal N2-fixation. Anabaena and Tolypothrix have been found to fix more nitrogen in phosphated sets containing basic slags than in unphosphated ones (25). The basic slags had a stabilizing effect on N2-fixation products by the formation of phosphoproteins which appear to resist ammonification, nitrification, and loss of nitrogen (25).

In paddy soils, P-supplying manure enhances algal growth and ARA (53, 54, 276, 296). Okuda and Yamaguchi (186) incubated 117 submerged soils in a greenhouse and noted that BGA growth seemed closely related to the available P content of the soil; algal growth in moist soils was poor at 0 to 5 ppm P, but vigorous above 6 ppm. The populations of BGA, Azotobacter, and Clostridium butyricum were also reported to show a tendency to correspond to total and available P content in the plow-sole (21).

The growth of N2-fixing BGA in paddy fields is most commonly limited by low pH and P deficiency. Application of P together with lime has frequently produced positive results (21, 107, 363), particularly in poor soils (250), but was also reported to produce a loss of N in fertile soils which had frequently received organic fertilizers (250).

2.5.2.3. Liming material
The addition of CaCO3 in paddy soils has been shown to generally enhance both the growth of algae and N2-fixation (15, 179, 185, 186, 250, 296, 363); however, a depressive effect of lime on both total algal flora and Anabaena was observed in soil cultures, but no explanation was given (296).

In general, BGA require more Ca for growth on N2 than on combined nitrogen, but the beneficial effect of liming is more closely related to pH increase than to calcium availability (232). Laboratory incubations of acidic soils in light showed that BGA growth and N2-fixation did not occur. Even inoculations failed unless the pH was adjusted by adding liming material (49). But in experimental paddy fields of Japan, application of superphosphate was found to be more efficient than that of calcium: an increase of the limiting pH in the floodwater, obtainable by liming, was also achieved by the growth of algae once the P deficiency was overcome (189).

2.5.2.4. Molybdenum
Because of its function in nitrate reductase and nitrogenase, Mo is required by all algae obtaining N through either process. The minimum level for optimum growth (0.2 ppm) is often available in rice paddy soils, particularly with water-logged conditions when the soil pH increases (281). In paddy fields, however, Mo is sometimes likely to be a limiting factor for NFA particularly during the drying-out period when algal NFA is often most active (281).

Subrahmanyan et al (285) suggested the addition of sodium molybdate (0.25 kg/ha) to soil to improve N2-fixing algal growth. This addition has been beneficial
in several cases (37, 53, 54, 296). Stewart (281) pointed out the need to ensure that adequate Mo is present in the soil. The cost of its addition is low relative to the addition of fertilizer nitrogen, but the benefit to the rice crop may be enormous.

2.5.2.5. Other elements

Potassium applied singly (51, 147, 149) or in combination with nitrogen and phosphorus (147) was reported to have either no effect (149) or a depressive effect (51, 147) on algal growth.

Magnesium is required for both nitrogenase and glutamine synthetase to function. Because increasing the pH of rice paddy soils reduces the level of soluble Mg and increases BGA growth and NFA activity, Mg$^{2+}$, which is required for the enzyme to function, may become limiting (281). While magnesium individually had a stimulating effect on *Aulosira fertilissima* inoculated in soil, a depressive effect was noticeable when it was combined with calcium and potassium (310). Magnesium sulfate also had a depressive effect on algal growth when used in the absence of phosphate (51).

Iron is seldom likely to be generally limiting except in acid soils where BGA grow poorly. However, some cases of iron deficiency in paddy soils have been reported and in the case of BGA this may be due, in part at least, to the chelating properties of algal extracellular products (281). In laboratory experiments, iron addition stimulated the activity of inoculated BGA in a P-amended flooded soil of pH 7.9, but not in a soil with a pH of 5.5 (360).

On the other hand, BGA growth in iron pans may be inhibited because of iron toxicity (281).

Many other elements (Na, S, Cl, Co, Zn, Cu, etc.) are required for optimal growth of algae, but their ecological implications as limiting factors or as factors affecting the composition of the algal community in paddies have not been demonstrated.

2.5.3. Organic manure

It is generally believed that incorporation of organic matter preferentially enhances heterotrophic nitrogen fixation. Effects of organic manure on BGA and phototrophic nitrogen fixation seem to be variable, and both favorable and inhibitory effects have been reported.

In California rice fields, incorporation of crop residues induced an abundant growth of algae during the early stages of rice development (34). In India, addition of green manure stimulated the growth of a large number of BGA; of the two green manures used, “Sunn Hemp” had a better effect on soil algae than Sesbania (151). A dominance of green algae was observed in chemically fertilized plots with and without organic manures; however, N$_2$-fixing BGA were recorded only in the plots with organic manure (120). Surface application of straw has been reported to have a high stimulatory effect on N$_2$-fixing BGA growth and phototrophic ARA (162). Organic matter in the form of compost or straw
stimulated N\textsubscript{2}-fixation by \textit{Tolypothrix tenuis} inoculated in pots of 20 kg silty loam soil (59).

On the other hand, the incorporation of organic matter has been reported to temporarily depress the algal population, especially during the active decomposition of the organic matter (232). A superphosphate and compost application had a depressive effect on N\textsubscript{2}-fixation, which was interpreted as a toxic effect of sulfate reduction by-products on algae (59). Several reports indicate a negative effect of organic manures on inoculated algae (142, 243, 282, 286, 318); however, a positive effect of farmyard manure was reported when applied in combination with ammonium sulfate and phosphorus (318). Effects of organic manure on algal flora and phototrophic N\textsubscript{2}-fixation vary with its nature. A change in the N\textsubscript{2}-fixing dominant species was observed after application of compost and farmyard manure, but not with green leaf manure (274). Sugar factory waste water that pollutes irrigation water was reported to preferentially enhance homocystous BGA growth (\textit{Oscillatoria, Spirulina, Lyngbya}) (114). Comparison of \textsuperscript{15}N\textsubscript{2}-fixation in light in three types of Philippine soils amended with rice straw, rice roots, green manure, and cellulose showed that in the neutral soil all the amendments gave a positive effect; in the acidic soils, only two amendments (straw and roots) out of eight produced an increase of N\textsubscript{2}-fixation (145).

From the preceding results, it appears that organic manure has very variable effects on N\textsubscript{2}-fixing BGA. Incorporation may be less beneficial because of anaerobic decomposition processes that produce by-products toxic to BGA. Surface application may be more favorable, providing CO\textsubscript{2} and an algal inoculum due to the epiphytic propagules (see section 4.4).

### 2.5.4. Pesticides

#### 2.5.4.1. Methodology

Although field use of pesticides has now become a common practice in rice cultivation, most of the information on their effects on algae has come from laboratory experiments conducted with flask cultures. Among the 38 references cited in Table 13, only 7 refer to field observations.

Flask experiments with algal cultures can give an index of the sensitivity of the strains to the pesticides, but such results can hardly be extrapolated to field conditions for the following reasons:

- Toxicity seems to be higher in flask cultures than in the field; for example, 5 ppm propanil prevented the growth of \textit{Anabaena cylindrica}, \textit{Tolypothrix tenuis}, and \textit{Nostoc endophytum} in flask cultures, but the same concentration did not produce any inhibition in the presence of unsterilized or sterilized soil (362).
- The rate of degradation of pesticides in the field is likely to be more rapid than in flask experiments.
- In the field, toxicity also depends on the initial microbial population, the nutrient status (262, 44), and the mode of application of the pesticides: Pentachlorophenol incorporated in soil with lime stimulated N\textsubscript{2}-fixing...
BGA; but if surface-applied, even at low levels, it was depressive with a long residual effect (106).

- For nonpERSISTENT pesticides, the rate of degradation and the toxicity of products are important in considering the possible effects on algae. Laboratory experiments showed that metabolic products of Aldrin, Dieldrin, and Endrin are inhibitory to algal growth (28). 3,4-Dichloroaniline, the primary product of propanil degradation, is far less inhibitory than propanil, but at the concentration of propanil used in the field (12 ppm), the degradation product can still be inhibitory for some BGA (3627).

From the experiments conducted under laboratory or, less frequently, field conditions, it appears that depending upon the nature of the chemical, its concentration, and the algal strain, the pesticide's effects could be inhibitory, selective, or even stimulatory.

2.5.4.2. Inhibitory effect

Resistance to pesticides varies widely with strain. Among 10 Anabaena strains tested for their resistance to Ceresan, 9 could tolerate 100 ppm, but 1 was inhibited by concentrations higher than 1 ppm (327). Cylindrospermum sp. was observed to be less resistant to insecticides than Aulosira fertilissima and Plectonema boryanum (258). BGA can tolerate fungicide concentrations of 100 to 1,000 ppm depending on the nature of both pesticides and strains tested (70). Testing 27 strains of N2-fixing BGA for their in vitro tolerance for 2 fungicides and 6 herbicides, Venkataraman et al (327) concluded that most of the N2-fixing BGA could tolerate high levels of pesticides. The levels were generally higher than the recommended application rate (262, 325, 327). A similar observation was found with Aulosira fertilissima tested with three herbicides and six insecticides commonly used in the paddy field (4). Cylindrospermum tolerated up to a maximum concentration of 800 ppm of 2, 4-D (260).

2.5.4.3. Selective action

The resistance to high levels of pesticides seems to be more characteristic of BGA than of the eukaryotic algae as indicated by a selective action of certain pesticides on mixed algal flora. Pentachlorophenol used at a concentration of 100 ppm suppressed green algae without any deleterious effect on BGA (346). Gamma-BHC at a low level depressed diatoms, but not the N2-fixing BGA. At a rate of 50 kg·ha⁻¹ (10 times the recommended dose) it had no detrimental effect on total algal population. However, BGA were more abundant in treated soils, whereas green algae and diatoms were more abundant in untreated soils (106, 207). It has also been shown that coccoid algae are generally more resistant to pesticides than are the filamentous ones (66, 106). But some pesticides like Chloropicrin (106) may affect all algae without discrimination.

2.5.4.4. Stimulatory effect

The stimulatory effect of pesticides on BGA can be either direct or indirect. At
low concentrations, a direct stimulatory effect of herbicides on growth and N\textsubscript{2}-fixation by *Tolypothrix tenuis* and *Calothrix brevissima* was observed (97). Low doses of insecticides (<10 ppm) stimulated *Aulosira fertilissima* growth (recommended dose, 1-2 ppm) (4); even high concentrations (300 ppm Difolatan) have produced a stimulatory effect on the same alga (70).

Among the different groups of pesticides, insecticides have been shown to have a stimulatory effect on algal growth. This is primarily an indirect effect due to a decreasing population of algal grazers. It has been observed that application of gamma-BHC for control of rice stem borer caused the development of a dense algal bloom at the surface of the floodwater (207) in spite of the fact that BHC was observed to be more toxic to algae than are other insecticides (258). Growth of BGA, either spontaneous or after inoculation, can be suppressed by grazing daphnids and other small animals. Addition of 1 to 5 ppm parathion in the irrigation water was demonstrated to be harmless to *Tolypothrix tenuis* but sufficient to kill the grazers (90). A similar effect was obtained with 25 ppm Folidol (180), 12.5 g.m\textsuperscript{-2} Furadan, 15 g.m\textsuperscript{-2} 10% B.H.C. (272), 10 g.m\textsuperscript{-2} phorate (272), 6 g.m\textsuperscript{-2} carbofuran (277), and 6 g.m\textsuperscript{-2} Ekalux (277).

### 2.5.4.5. Algicides

To control the detrimental effects of algal blooms (section 4.3) especially during the early stages of rice growth, algicides have sometimes been used. As these chemicals have been selected for their effectiveness against the “scum”-forming algae — most frequently, filamentous green algae — very little information is available on the effects of algicides on BGA. 2-3 dichloro 1-4 naphtoquinone (0.1 ppm), KMnO\textsubscript{4} (6 ppm), alkylidimethyl-benzyl ammonium-chloride (5 ppm) (31), and HOE 2997 (29) have been effective against *Anabaena* sp. Some algicides have a selective effect on the algal flora. Unicellular green algae were more resistant than filamentous ones (63). *Anabaena* spp., *Nostoc* spp., and *Oscillatoria* spp. that developed in Italian rice fields have been considered as “substitutes” (successors) for green algae that were controlled by Fentin derivatives and sodium dithiocarbamate (31).

### 2.5.4.6. Effect of pesticides on nitrogen-fixing activity

In general, pesticides appear to have an initial depressive effect on N\textsubscript{2}-fixation by BGA (94), followed by either an increase or decrease in activity (46). However, some pesticidal compounds limited the N\textsubscript{2}-fixing capacities of BGA, thereby affecting the overall nitrogen economy of soils (46, 97, 100). At concentrations recommended for field application 2, 4-D and MCPA inhibited N\textsubscript{2}-fixation by *Nostoc muscorum*, *Nostoc punctiforme*, and *Cylindrospermum* sp. (100). Insecticides generally have little effect; however, an inhibitory effect of malathion was observed (46). It appears that algicides are used mainly in areas where heavy doses of fertilizer N are applied (58) and under such circumstances, no algal N\textsubscript{2}-fixation can be expected.
2.5.4.7. **Mode of action on blue-green algae**

Little is known about the biochemical interaction between pesticides and BGA. The effect of STAM F-34 on *Nostoc muscorum* was similar to that of DCMU, inhibiting both growth and heterocyst differentiation (308). Some pesticides have been demonstrated to have a mutagenic action on BGA (256, 257). By repeatedly growing and removing BGA from a BHC-containing medium, Das and Singh (46) observed a gradual loss in the toxicity of the pesticide and suggested detoxification by BGA.

Experiments done mainly with flask cultures suggest some general trends on the effect of pesticides on BGA:
- BGA seems to be more resistant than other algae to pesticides.
- Most BGA are capable of tolerating pesticide levels recommended for field application.
- Insecticides are generally less toxic to BGA than other pesticides and have the secondary beneficial effect of suppressing the grazer population.

Some exceptions to these general statements have been reported. Field experiments are badly needed to demonstrate more precisely the effects of pesticides and establish the in situ levels of toxicity. A list of pesticides tested for their effects on algae is given in Table 13.

### 2.6. CONCLUSION

Ecological studies indicate that BGA are ubiquitous and more abundant in rice than in other cultivated soils; however, the relative occurrence of N\textsubscript{2}-fixing BGA varies within large limits and they are not invariably present in rice soils.

Studies concerning qualitative and quantitative variations of the algal community in paddy soils are scarce and are limited by methodological problems. However, numerous observations in fields and experimental plots identify major factors affecting N\textsubscript{2}-fixing BGA. High light intensities, low temperatures, acidic pH, and low level of available P have been found to limit BGA growth in paddy soils. The comparison between the low N\textsubscript{2}-fixing algal biomasses observed in acidic P-deficient paddies in Senegal (max value = 2 t·ha\textsuperscript{-1}) and the high value observed in neutral paddies in the Philippines (24 t·ha\textsuperscript{-1}) illustrates the predominant role of pH and P when both factors are favorable. A beneficial effect of P application and liming on N\textsubscript{2}-fixing BGA has frequently been observed. In contrast, an inhibitory effect on nitrogenous fertilizers has been established. Little is known, however, about the competition between N\textsubscript{2}-fixing BGA and other algae as affected by the nature, concentration, and mode of application of nitrogenous fertilizers. The detrimental effect of mineral nitrogen on N\textsubscript{2}-fixation clearly demonstrated in the laboratory may not be so effective and clear-cut for N\textsubscript{2}-fixing BGA growing in the field.

Major gaps in our knowledge of the ecology of N\textsubscript{2}-fixing BGA in the paddy field are on:
- the mechanism of algal succession and the influence of biotic factors,
Table 13. List of the pesticides tested for their effects on BGA.

FUNGICIDES:
- BENLATE: 94; CERESAN: 325, 327; DIFOLATAN: 70; DITHANE: 325, 327; HECAP: 70; MBC: 70.

HERBICIDES: 85, 100, 170, 178.
- ALACHLOR: 257; BUTACHLOR: 256, 257; COTORON: 325, 327; DELAPRON: 325, 327; DIURON: 325, 327; EPTAM 6F: 97; LINURON: 325, 327; MCPA: 4, 100; MCPB: 4; ORDRAM: 87, 97; PENTACHLOROPHENOL: 106; PROPANI: 362; PROPZINE: 325, 327; TRIFLURALIN: 87, 97; 2,4-D: 87, 100, 260, 262, 325, 327; STAM F34: 4, 87, 97, 308.

INSECTICIDES:

ALGICIDES:
- ALGAEDYN: 14; ALKYLDIMETHYL BENZYL AMMONIUM CHLORIDE: 31; BENZURIDE: 32; BRESTAN: 29; CAPTAFOL: 32; CHLORTALONIL: 29; CuSO4: 14, 32, 45, 56, 117, 118; DICHLONE: 45; DICHLOROPHEN: 29; DIQUAT: 45; FENTIN DERIVATIVES: 31, 32, 45; FERBAM: 45; FOLPET: 32; HOE 2997: 29, 89; K2MnO4: 31; ROCCAL: 45; RICETRINE: 56; SIMAZINE: 45; SODIUM DITHIOCARBAMATE: 31; 2,3 DICHLORO 1-4 NAPHTOQUINONE: 31; ZINEB: 45.

- the reasons for a limited distribution of N₂-fixing forms, and
- the influence of cultural practices other than P application and liming. In particular, the search for N fertilizer forms and methods of application compatible with BGA growth and NFA has to be continued.
3. PHYSIOLOGY OF BLUE-GREEN ALGAE IN PADDY FIELDS

3.1. PHOTOSYNTHESIS
BGA are essentially photoautotrophic. They can also exhibit heterotrophic growth, but no evidence of heterotrophy in the rice fields has been reported. Because of their important role in the N fertility of rice soils, much emphasis has been laid on the NFA of BGA, but the study of their in situ photosynthetic activity has been neglected. However, information on ecological implications of algal photosynthesis can be obtained from laboratory and limnological studies.

In general, limnologists have paid little attention to the possibility that C supply may sometimes be the factor controlling algal growth. This controversial hypothesis is supported by the demonstrated stimulatory effect of CO$_2$ on algal growth. In a laboratory experiment using test tube cultures of rice seedlings, the presence of the rice plant enhanced algal growth and N$_2$ fixation, even at acidic pH levels. This stimulation was maintained by replacing the plant with a stream of 2% CO$_2$ in air passing through the floodwater. The stimulatory effect of the "crop" was therefore interpreted as largely due to the increased supply of CO$_2$ resulting from respiration and root decomposition (51). In laboratory experiments, Wilson and Alexander (360) also observed that 2% CO$_2$ in air bubbled through the floodwater of an acidic soil stimulated algal ARA; the effect was most marked if phosphate was supplied.

Another controversial hypothesis is a possible selective effect of the CO$_2$ concentration on the algal flora. Dissolved inorganic C in the floodwater occurs as a CO$_2$ = HCO$_3^-$ = CO$_3^{2-}$ equilibrium system. This system is affected by pH changes resulting from the extraction of aqueous CO$_2$ by the biomass at a rate higher than that at which it can be replaced. This leads to an increased pH level
and a shift of the equilibrium system, so that \( \text{HCO}_3^- \) and even \( \text{CO}_3^{2-} \) predominate. For a number of algae, \( \text{CO}_2 \) is the only C compound that can support growth. Direct utilization of \( \text{HCO}_3^- \) and even \( \text{CO}_3^{2-} \) by the other algae, including BGA, is controversial, but it appears that BGA extract dissolved inorganic C at high pH values more competently than other algae do (232). This was demonstrated by Shapiro (see 232) who manipulated pH, nutrient concentration, and amount of free \( \text{CO}_2 \) in isolated lake zones. The addition of free \( \text{CO}_2 \) resulted in a large dominance of green algae; lowering the pH had a similar effect. At higher pH values when free \( \text{CO}_2 \) had a lower concentration, BGA predominated. On the basis of these results, it was suggested that high \( \text{CO}_2 \) production in the paddy field either after soil rewetting (priming effect) or organic matter incorporation may be more favorable to green algae than to BGA (232). Some reports support the hypothesis that organic matter incorporation enhances green algae growth (34, 120) but a dense BGA growth was also reported 2 weeks after surface application of straw (162).

Photosynthetic activity of the algal biomasses in the paddy field influences the equilibrium of the ecosystem by increasing \( \text{O}_2 \) concentration and pH of the floodwater during daytime. The primary productivity of the floodwater community (including submerged weeds) was reported to be high and equivalent to that of eutrophic lakes, and corresponded to 10 and 15% of that of rice plants in fertilized and unfertilized plots (239). In laboratory soil-incubation experiments, the concentration of dissolved \( \text{O}_2 \) near the soil surface exposed to light was twice that in water equilibrated with air (363). Implications of BGA photosynthetic activity for the topsoil microflora \( \text{(O}_2 \text{ increase)} \) and N losses by volatilization \( \text{(pH increase)} \) are certainly of agronomic significance, but there is little information on this aspect.

3.2. NITROGEN FIXATION

3.2.1. Methodology of measurements
The methods used for the measurement of \( \text{N}_2 \)-fixation by intact organisms are 1) total N analysis, 2) measurement of gas ratios, 3) \( ^{15}\text{N} _2 \) gas incorporation, and 4) acetylene reduction assay. Of these, N-analysis by the Kjeldahl technique is generally used in N-balance studies. The use of this method to distinguish between phototrophic and heterotrophic NFA by parallel light and dark treatments is suitable only for long-term trials for gross measurements. The method has been used with planted (52, 103) and nonplanted soils (49, 145, 179, 363) incubated under laboratory conditions. App et al (104) reported that balance studies with planted pots corresponding to 10 different treatments needed 2,650 Kjeldahl analyses; that indicates how tedious this method becomes for obtaining accurate results. Estimation of algal NFA by analyses of gas changes in an enclosed atmosphere is not a very sensitive method and has rarely been used (53-54). We are not aware of any studies using \( ^{15}\text{N} \) incorporation to assess specifically algal NFA in rice soils.
The acetylene reduction technique is presently the most widely used, because of its simplicity, rapidity, and sensitivity. Advantages and disadvantages of the general use of this method have been widely discussed, but its adaptability in assessing algal NFA in rice fields has encountered specific problems.

3.2.1. Sampling
It has already been pointed out that the spatial distribution of N\textsubscript{2}-fixing BGA follows a log-normal pattern (see section 2.1). ARA associated with BGA also exhibits the same pattern as demonstrated for soil algae (230, 303) and for BGA epiphytic on wetland rice (233). This results in a wide variability of ARA among individual samples (8). Hence, all implications in sampling for a log-normal distribution are applicable to in situ ARA measurement (see section 2.1). The accuracy of these measurements can be improved more by increasing the number of replicates than by increasing the surface area of the incubated samples (305).

3.2.1.2. Devices and greenhouse effect
Any gastight transparent enclosure that provides for the introduction and removal of gas samples during incubation is basically suitable for algal ARA measurements. The devices used range from flasks (222, 223, 367), through syringes, plastic cylinders, and cut bottles (8, 9, 10) to plastic bags attached to metal cylinders or frames (352, 353, 355, 356). An inevitable problem connected with airtight transparent enclosures is the increase in temperature during incubation. This effect is directly related to the light intensity reaching the enclosure as well as its size. In tropical rice fields where light intensities are frequently very high, this could become a serious problem inducing partial (305) or total (317) ARA inhibition.

3.2.1.3. Diffusion and solubility of gases
Another difficulty is the dissolution and the slow diffusion of acetylene and ethylene in the floodwater. Problems concerning aquatic C\textsubscript{2}H\textsubscript{4}-reduction techniques were reviewed by Flett et al (70) and Lee and Watanabe (141). In situ ARA in rice soils showed a 1-hour lag followed by an almost linear reaction. The lag has been attributed to the slow diffusion of C\textsubscript{2}H\textsubscript{4} in standing water. BGA growing on agar slants did not show any lag phase (8). An incubation period of 30 minutes in situ and 15 minutes in culture was reported to be necessary before measurements were taken (303). Reporting on problems of ARA method applied to water-saturated paddy soils, the slow diffusion of C\textsubscript{2}H\textsubscript{4} through water was shown to be a limitation (141).

Methods adopted to overcome this problem were longer periods of incubation to permit acetylene to diffuse in, and agitation at the end of incubation to release the ethylene (141, 353, 355, 356). However, long-term incubations suffer from the inherent error due to enhancement of acetylene reduction during prolonged incubation. In addition, agitation of the system is inadvisable during time-course measurements because it adversely affects the algae, both directly and indirectly,
by increasing the turbidity of the water. A more suitable method consists of introducing in the floodwater a small quantity of water saturated with acetylene and to measure, after incubation, the ethylene content of both the gas phase and the floodwater (141).

3.2.1.4. Duration of incubation
As algal ARA varies during the day, evaluations have to be done over a 24-hour period. It has been shown that long periods of incubation (2-6 hours) under acetylene led to a multifold enhancement of ARA in *Anabaena cylindrica*, *Anabaenopsis circularis*, *Rhodospirillum rubrum*, and *Azotobacter vinelandii*. It was concluded that assessments based on long-term experimental incubation with acetylene may grossly overestimate actual N<sub>2</sub>-fixation (47). For this reason, it is necessary to perform several separate measurements during the day. The reuse of the same samples, which can facilitate measurements, did not significantly affect the results when the samples were exposed to air at 1-hour intervals between measurements (303).

3.2.1.5. Estimation of algal contribution
The major difficulty in making field ARA measurements specifically with BGA is in determining the relative contribution of the other N<sub>2</sub>-fixing organisms. If methods other than ARA are used, other difficulties appear, like estimating N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing algal biomasses separately, determining what fraction of total algal nitrogen corresponds to reabsorption of already fixed nitrogen, choosing time and density of sampling in relation to the heterogenous distribution of algae, and the rapidity of algal biomass evolution (144).

The commonest practice in estimating algal contribution was to measure ARA in the light and in the dark and to attribute the latter activity solely to N<sub>2</sub>-fixing bacteria. This scheme does not take into account the fact that BGA continue to reduce acetylene for long periods even after light is cut off. It is, therefore, advisable to preincubate in the dark samples destined for subsequent ARA measurements in the dark (134, 233). A technique has been reported whereby algal N<sub>2</sub>-fixation in the field was estimated by one ARA measurement followed by another one, after removing the floodwater and the surface soil. The difference between the two measurements was attributed to algal activity (356). The selective herbicide propanil was used by Hapte and Alexander to estimate specifically autotrophic NFA (pages 243-244 in 103).

3.2.1.6. Conversion rate C<sub>2</sub>H<sub>4</sub>:N<sub>2</sub>
Another error involved in ARA measurements is related to the conversion of ARA values by algae to nitrogen fixation. A molar ratio of C<sub>2</sub>H<sub>4</sub> to N<sub>2</sub> equal to the theoretical value of 3 was observed in an experimental model by the comparative study of N<sub>2</sub>-fixation by ARA and Kjeldahl measurements (224); but in 12 experiments with N<sub>2</sub>-fixing BGA, linear and log-transform regression analyses of the results yielded values of 4.8 and 4.2 (199). In an experiment comparing ARA
with $^{15}$N-fixation in waterlogged soil-straw and sand-clay-straw mixtures, it was found that 6-15 moles of $\text{C}_2\text{H}_2$ reduced corresponded to 1 mole of N fixed. This discrepancy was attributed mainly to the higher solubility of $\text{C}_2\text{H}_2$ than $\text{N}_2$ in water. It was concluded that ARA provides a method for measuring potential nitrogenase activity in waterlogged soil, but it should be calibrated for specific conditions (221).

In a review of field studies of N-fixation in paddy soils, lack of suitable methods for assessing NFA was cited as a limitation in research (354). Problems associated with ARA in waterlogged soils have been highlighted, and the method was therefore considered suitable for qualitative but not for quantitative estimations (144, 354, 359).

Measurements of algal ARA are reliable when the incubation is brief, the problems of gas diffusion and greenhouse effect are minimized, and statistically valid sampling methods are adopted. However, quantitative estimates of algal NFA from ARA measurements are hazardous because ARA is certainly the method “most liable to misinterpretations” (144).

### 3.2.2. Daily variations of algal NFA

Four general forms of diurnal variations of ARA have been reported:

1. Asymmetrical curves with a maximum in the morning and a low decreasing activity in the afternoon (8, 10).
2. Curves with two maxima, one in the morning and another in the afternoon (218, 219, 304).
3. Asymmetrical curves with a maximum in the afternoon (8, 10).
4. Symmetrical curves according to variations of incident light with a slight delay (219).

These four types of curves were also observed with samples of an *Anabaena* bloom placed under screens that permitted the passage of 100, 60, 22, and 7% respectively, of incident sunlight where the maximum light intensity during the day was about 90,000 lux (219).

The observed curves may be explained as follows. Curves of the first form seem artificial; they were obtained with cultures or algal masses not adapted to high light intensities, placed under direct sunlight. Under such conditions, the algae are bleached in stirred diluted cultures. Curves of the second form result from a transitory inhibitory effect of high light intensity in the middle of the day; those of the third form are obtained with optimal light intensities. Curves of the fourth form occur with limited intensity because of cloudy weather or a dense plant cover, or both (232).

Factors other than light may induce diurnal variations of ARA. High temperature in the middle of the day may have an inhibitory effect but in submerged paddy fields the floodwater is a relatively good temperature buffer and inhibitory temperatures probably do not occur frequently. When algae develop large biomasses and a high photosynthetic activity, available CO$_2$ may be depleted at noon, when water pH raises values higher than 8.2. Such a depletion
and its influence on ARA have not been demonstrated (232).

These results indicate that light seems to be an important factor, if not the most important one, in regulating diurnal variations of ARA. Under some conditions BGA can fix nitrogen in the dark. In situ measurements confirmed a low but non-negligible activity during the night (10).

3.2.3. Variations along the cultivation cycle

Most of the work concerning NFA and its variations during the cultivation cycle were those done at the International Rice Research Institute (IRRI).

Yoshida and Ancajas (367) compared algal NFA in planted and unplanted flooded soils during the wet and the dry seasons by measuring the ARA of submersion water. No fertilizers were applied. During the wet season, ARA was higher in unplanted fields; its sharp variations correlated with fluctuations in algal growth. In planted paddy fields, ARA reached its maximal value 3 weeks after transplanting, then decreased from the third to the ninth week, and finally remained very low. The kinetics of ARA was related to limiting light intensities, the deficiency increasing with the density of the plant cover. This interpretation was confirmed with measurements done during the dry season. Under a more intense light intensity, there was no difference between planted and unplanted soils and an approximately bell-shaped curve with a peak around the ninth week was observed. The estimated amount of N fixed in the floodwater, using the theoretical conversion factor, was 3 kg N·ha⁻¹ in a planted flooded field and 11 kg N·ha⁻¹ in an unplanted flooded field during wet season, and 15 kg N·ha⁻¹ in either planted or unplanted field during dry season.

Alimagno and Yoshida (10) compared ARA during a cultivation cycle in a NP (42.4 kg·4 kg) fertilized paddy and in an unfertilized paddy in the Philippines. Samples covered with black cloth exhibited no ARA, suggesting that BGA were the principal N₂-fixing agents in these fields. In both fertilized and unfertilized paddies, maximum NFA occurred about 40 days after transplanting. A much higher NFA was estimated in the nonfertilized field (18 to 33 kg N·ha⁻¹ per cropping season) than in the fertilized one (2.3 to 5.7 kg N·ha⁻¹ per cropping season).

Seasonal changes in ARA in IRRI long-term fertility plots have been summarized by Watanabe and Cholitkul (358). During the wet season, the relative contribution of algal N₂-fixation was large just after transplanting, and just before and after the harvest stage (352). In unfertilized plots, the highest ARA appeared late in the growing cycle in both dry and wet seasons, when the activity of the BGA in the floodwater was highest (353).

In Japan, soil samples removed at different times of the cultivation cycle exhibited a very low N₂-fixing activity before flooding, but a rapid increase after flooding. The maximum value was reached at maximum tillering stage, declined thereafter, and reached a very low value after drainage (333).

In a pot experiment in India, more than 80% of the phototrophic NFA took place in the first 4 weeks after transplanting (53, 54); however, it has been pointed out that pot experiments are not suitable models for assessing photosyn-
thetic NFA in paddy soil (193).

A study of algal ARA variation during the 1976 and 1977 rainy seasons in an upland nonfertilized field in Mali (304) indicated that maximal ARA may occur at both the start and end of the cultivation cycle. The estimated large amounts of N$_2$ fixed (50-80 kg N-ha$^{-1}$ per cultivation cycle) agreed with the high density of N$_2$-fixing algae observed ($2.2 \times 10^6$ cells g$^{-1}$ dry soil).

From the foregoing results, it appears that a peak of algal ARA may occur anytime during the cultivation cycle. The results are too few to allow definite conclusions regarding algal NFA variations during the cultivation cycle. However, a predominant effect of light intensity in relation to the season and the plant cover is clear and an inhibitory effect of nitrogen fertilization is uncontestable.

3.2.4. Estimations of algal NFA during the cultivation cycle
Reported data on nitrogen fixation related to BGA activity are presented in Table 14. In most cases, the activity measured in light in the presence of a visible growth of algae has been attributed to BGA. A few measurements have used specific methods that assess algal activity more closely (71, 223, 304, 353, 356). The estimated amounts of N$_2$ fixed varies from a few to 80 kilograms, and the value of 30 kg N-ha$^{-1}$ per crop indicated by Watanabe et al (353) seems to constitute a satisfactory reference value when environmental factors are favorable for BGA growth.

3.2.5. Relative contribution of BGA
Most of the results concerning the relative contribution of photosynthetic nitrogen fixers have been obtained by comparing soil samples incubated in the dark and in light.

In 1936 De was the first to demonstrate that N$_2$-fixation in waterlogged soils occurs in light (49). He claimed that algae were the main agents of nitrogen fixation in the rice fields and that the part played by bacteria was relatively unimportant (50). This was confirmed by the determination of nitrogen at the end of a 5-year pot-culture experiment that showed considerable increase in soil nitrogen where algae have grown abundantly, and a loss of this element in soil where algal growth was absent (52).

There is much evidence that soils incubated under light developed a higher N$_2$-fixing activity than those incubated in the dark (49). In Japan, pot experiments indicated that nitrogen fixation in waterlogged soil occurred vigorously in light in contrast with very little or no fixation in the dark (179, 187, 363). The nitrogen gains were in proportion to the surface area of soil when an equal amount of soil was used (363). Dark-incubated soil samples from the Ivory Coast had a negligible activity compared to the light-incubated ones (222, 224) and a similar result was observed with Philippine soils incubated with N$^{15}$ under different conditions (368, 369). However, among 15 comparisons of nitrogen fixation by dark- versus light-incubated soil samples from the Philippines, 8 showed a
Table 14. References reporting algal NFA in paddy fields.

<table>
<thead>
<tr>
<th>Reference (no.)</th>
<th>Location</th>
<th>Treatmenta</th>
<th>Method</th>
<th>N₂-fixing microorganisms involved</th>
<th>ARA</th>
<th>N fixed (kg·ha⁻¹)b</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Philippines</td>
<td>F</td>
<td>Unfertilized field</td>
<td>ARA</td>
<td>Total activity</td>
<td>46</td>
<td>Crop</td>
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<td></td>
<td></td>
<td></td>
<td>Planted</td>
<td></td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experimental plot</td>
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<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Planted</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Philippines</td>
<td>F</td>
<td>No nitrogen</td>
<td>ARA</td>
<td>Mainly BGA</td>
<td>18–33</td>
<td>Crop</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>56 kg N·ha⁻¹ added</td>
<td></td>
<td>2–6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53–54</td>
<td>India</td>
<td>P</td>
<td>6 soils planted with rice</td>
<td>Disappearance of N₂ gas</td>
<td>ARA</td>
<td>Mainly BGA</td>
<td>15–50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0–8 nMol C₂H₄·(g dry soil)⁻¹·h⁻¹</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>71</td>
<td>Senegal</td>
<td>P</td>
<td>21 different soil samples incubated both in the dark and in light</td>
<td>ARA</td>
<td>Mainly BGA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>161</td>
<td>Japan</td>
<td>F</td>
<td></td>
<td>N₁⁵</td>
<td>Total activity</td>
<td>0.5</td>
<td>Crop</td>
</tr>
<tr>
<td>187</td>
<td>Japan</td>
<td>P</td>
<td>6 soil samples incubated both in the dark and in light</td>
<td>Kjeldahl</td>
<td>Mainly BGA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td>4–20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ lime</td>
<td></td>
<td>9–29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ P and lime</td>
<td></td>
<td>11–23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>204* (in 286)</td>
<td>India</td>
<td>F</td>
<td>Algal &quot;incrustations&quot;</td>
<td>Kjeldahl</td>
<td>BGA</td>
<td>14</td>
<td>Season</td>
</tr>
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Continued on opposite page
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<thead>
<tr>
<th>Reference no.</th>
<th>Location</th>
<th>Treatment</th>
<th>Method</th>
<th>N(_2)-fixing microorganisms involved</th>
<th>ARA</th>
<th>N fixed (kg·ha(^{-1}))</th>
<th>Time</th>
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<tbody>
<tr>
<td>219</td>
<td>Senegal</td>
<td>F</td>
<td>40 fields studied</td>
<td>ARA &amp; N(_2)-fixing BGA biomass evaluation</td>
<td>Mainly BGA</td>
<td>0–60 nMol C(_2)H(_4)·cm(^{-2})·h(^{-1})</td>
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<td>Ivory Coast</td>
<td>P</td>
<td>3 soil samples</td>
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<td>BGA</td>
<td>7 µg N·(g dry soil)(^{-1})·day(^{-1})</td>
<td>Day</td>
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<td>250</td>
<td>Japan</td>
<td>P</td>
<td>Soil samples exposed to light</td>
<td>Kjeldahl</td>
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<td>Mg N·(100 g dry soil)(^{-1})</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>+ Lime</td>
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<td>8.5</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ Organic manure</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ Lime &amp; organic manure</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ Lime &amp; P</td>
<td></td>
<td>3</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ Nitrogen</td>
<td></td>
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<tr>
<td>266</td>
<td>India</td>
<td>F</td>
<td>Aulosira fertilissima</td>
<td>BGA</td>
<td></td>
<td>53</td>
<td>Crop</td>
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<tr>
<td>304</td>
<td>Mali</td>
<td>F</td>
<td></td>
<td>ARA</td>
<td>Mainly BGA</td>
<td>50–80</td>
<td>Crop</td>
</tr>
<tr>
<td>331</td>
<td>India</td>
<td>F</td>
<td>Results of extensive trials</td>
<td>Grain yield</td>
<td>BGA</td>
<td>25–30</td>
<td>Crop</td>
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Continued on next page
Table 14 continued

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Location</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Method</th>
<th>N&lt;sub&gt;2&lt;/sub&gt;-fixing microorganisms involved</th>
<th>ARA</th>
<th>N fixed (kg·ha&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time</th>
</tr>
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<tr>
<td>352</td>
<td>Philippines</td>
<td>F Non-fertilized field</td>
<td>ARA</td>
<td>BGA</td>
<td></td>
<td>30</td>
<td>Crop</td>
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<td>ARA</td>
<td>BGA</td>
<td></td>
<td>0.26</td>
<td>Day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 days after transplanting heading stage</td>
<td></td>
<td></td>
<td></td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>355</td>
<td>Philippines</td>
<td>F Wet season unfertilized</td>
<td>ARA</td>
<td>Total activity</td>
<td></td>
<td>10–14</td>
<td>Crop</td>
</tr>
<tr>
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<td></td>
<td>Wet season fertilized</td>
<td></td>
<td>Total activity</td>
<td></td>
<td>9–11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season unfertilized</td>
<td></td>
<td>Mainly BGA</td>
<td></td>
<td>11</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Dry season fertilized</td>
<td></td>
<td>Total activity</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>356</td>
<td>Philippines</td>
<td>F Wet season</td>
<td>ARA</td>
<td>BGA</td>
<td></td>
<td>mMol C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dry season</td>
<td></td>
<td></td>
<td></td>
<td>307</td>
<td></td>
</tr>
<tr>
<td>367</td>
<td>Philippines</td>
<td>F</td>
<td>ARA &amp; &lt;sup&gt;15&lt;/sup&gt;N</td>
<td>Total activity</td>
<td></td>
<td>3–63</td>
<td>Crop</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Floodwater</td>
<td></td>
<td>3–11</td>
<td></td>
</tr>
<tr>
<td>368–369</td>
<td>Philippines</td>
<td>F Planted</td>
<td>ARA</td>
<td>Floodwater</td>
<td></td>
<td>3</td>
<td>Crop</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unplanted</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

Average = 27 kg N·ha<sup>-1</sup>·crop<sup>-1</sup> (n = 38; S.D. = 26).

<sup>a</sup>F = field experiment, P = pot experiment. <sup>b</sup>If no other unit indicated.
positive effect of light, 5 no effect, and 2 a negative effect (145).

Results of field measurements indicate a large variability of the relative contribution of BGA; both heterotrophic bacteria and phototrophic microorganisms have been reported to be the main N₂-fixing agent in rice soils.

In a Philippine paddy soil the greatest fixation was reported to be in the rhizosphere and was more pronounced in flooded than in upland conditions (367). In plot experiments in Japan, a rough estimate of the N₂-fixing capacity of each part of a paddy field showed that the most important site was the reduced Apg horizon, and that the importance of floodwater and the oxidized layer in N₂-fixation was rather low except in infertile soils (333).

In situ ARA measurements made at the International Rice Research Institute assessed the contribution of BGA and the rice root zone to nitrogen fixation in the submerged rice soils (the method could not detect NFA occurring in the bulk of anaerobic soil). In unfertilized plots ARA fluctuated greatly with the activity of BGA, and peaks of activity were highest when BGA biomass was highest. Nitrogenase activity in the rice root zone was at an almost constant but low rate (353, 355). From these it was concluded that BGA contribute more to ARA than microorganisms near the rice roots (355) and that the contribution of rhizospheric nitrogen fixation was probably not as important as previously estimated (354). In further experiments, algal ARA activity was estimated to be 200 mmol C₂H₄·m⁻² in the wet season (163 days) and 300 mmol C₂H₄·m⁻² in the dry season (168 days). ARA associated with the rice plant (stems and roots) was calculated to be 90 mmol C₂H₄·m⁻² in the wet season and 50 mmol C₂H₄·m⁻² in the dry (356).

Nitrogenase activity in Banaue rice terrace soils in the Philippines was also reported to be mainly due to BGA alone or in association with azolla (352).

To our knowledge there is only one report indicating quantitative estimations of the relative contribution of floodwater, plant, and soil to N₂-fixation (359). The results (Table 15) indicate that the BGA contribution was marginally higher (61%) than that of the soil and the plant in unfertilized plots (–NPK), and largely lower (16%) in fertilized plots (+ NPK).

It appears from the results that the relative contribution of BGA as a percentage of the total N fixed in the paddy field varies widely. The factors affecting the variations are mainly chemical, climatic, and biotic. Their mode of action is at present poorly understood.
It is well documented that the rice field ecosystem is a favorable environment for the growth of BGA, which frequently produce luxuriant growths in rice fields. As certain BGA fix nitrogen they are also believed to support the crop by the production of growth-promoting substances. However, algal growth has also been reported to be detrimental to rice plants. A few reports are also on algal epiphytism in rice fields. As the rice plants and the algae represent two principal components in this ecosystem, a proper understanding of their various interrelationships is important.

4.1. AVAILABILITY OF FIXED NITROGEN FOR RICE

It is now well established that N$_2$-fixation by BGA plays a vital role in the buildup and maintenance of soil fertility, but it is equally important to understand how much, when, and in what ways the fixed N is made available to the rice plants. Evidence on these aspects is still scanty and mostly hypothetical.

Nutrients fixed by the algae are released either through exudation or through microbial decomposition after the cells die. Laboratory experiments have frequently shown that BGA liberate large portions of their assimilated nitrogenous substances (232, 284); however, the large amounts recorded may be a methodological artifact due to osmotic shock in resuspending the cells or a physical damage of the algal material (144). No information is available on the exudation of fixed nitrogen by BGA under field conditions but it is clear that only part of it is available to rice, some being either reincorporated by the microflora or volatilized.
Release of nutrients through microbial decomposition after the death of the algae appears to be the principal means by which N is made available to the crop (232). The susceptibility to decomposition and the amount of nutrients released depend on:

- the physiological stage of the algae,
- the composition of the associated microflora (342),
- the suitability of the cell wall as a substrate for microorganisms, and
- the relative biodegradabilities of specific components of the algal walls.

Some algae are decomposed in 2-3 days, others withstand microbial digestion for more than 4 weeks. Laboratory experiments demonstrated that more than half of the nutrients contained in an algal biomass can be regenerated in less than 1 month with the aid of microbial degradation (see 2.3.2). A strain of *Bacillus subtilis* was found to decompose several N₂-fixing BGA very rapidly, about 40% of algal nitrogen being converted to ammonia within 10 days (342). Grazers, through their digestive tracts, also make algal nitrogen available to rice, but there is no information on this aspect.

In paddy fields the death of the algal biomass is most frequently associated with soil desiccation at the end of the cultivation cycle and algal growth has frequently resulted in a gradual buildup of soil fertility with a residual effect on, rather than an immediate benefit to, the standing rice crop. In the Philippines algal growth did not significantly increase the yield of rice but a buildup of N in the soil was observed (8) (9). In a 5-year pot experiment it was found that during the first, second, or third year, crop yield in the presence and absence of algae did not differ. But thereafter, yield increased progressively in the presence of algae, and fell in their absence. In the fourth and fifth years, the yields in the presence of algae were much higher than yields in their absence, and also those at the start of the experiment. Soils where algae grew abundantly showed a considerable increase in N, while there was a loss in N soils where algal growth was absent (52).

Field experiments conducted with *Tolypothrix tenuis* for 4 consecutive years indicated that only one-third of the field algae were decomposed in the first year; the rest remained as residual soil N. This was cited as the reason for continued yield increases in successive years (337). A study of C-N ratios and mineralization of nitrogen in inoculated and noninoculated pots for a 4-year period showed that more humus was formed under algae and that the humus was more easily decomposable (91).

The pattern of distribution of total organic and mineral nitrogen studied in inoculated and noninoculated plots indicated a higher mineral nitrogen content and a low mineralizable index of N in the inoculated plots, a phenomenon very much desirable for slow release of soil reserve (37).

The evidence cited shows that although BGA increase the available soil N, their influence on the rice plants is a delayed phenomenon. ¹⁵N studies of availability of algal nitrogen to rice are very scarce (218, 361).

Recently, Wilson et al (361) recovered from a rice crop 39% of the nitrogen from ¹⁵N-labeled *Aulosira* spp. spread on the soil and 51% from the algae incor-
porated into the soil. That shows that BGA nitrogen is readily available to rice; however, more direct information on quantification and dynamics of transfer of fixed N from BGA cells to rice plants is needed.

4.2. GROWTH-PROMOTING EFFECTS OF BGA

Besides increasing nitrogen fertility, BGA have been said to benefit rice plants by producing growth-promoting substances. Most of the documentation from field experiments is based on indirect evidence, the additive effects of BGA inoculation in the presence of nitrogenous fertilizers (40-120 kg N·ha⁻¹) being interpreted as an index of a contribution through biologically potent substances produced by the algae (7, 77, 255, 285, 287, 290, 292, 314, 315, 324, 331). Such interpretations have to be treated with caution since there are other possibilities by which a crop would perform better in the presence of algae. For example, an initial algal growth could temporarily immobilize the added fertilizer N and thereby minimize losses. Subsequent algal decomposition during the growth of the crop may result in a slow release of nitrogen and a more efficient utilization by the crop.

More direct evidence for hormonal effects has come primarily from treatments of rice seedlings with algal cultures or their extracts. Presoaking of rice seeds in BGA cultures or extracts has decreased losses from sulfate-reducing processes and this has been attributed to the enhancement of germination and a faster seedling growth due to algal exudates (108). N₂-fixing BGA have supported early recovery of transplanted seedlings and prolonged the period of tillering, which has resulted in increased length and number of ears, and number of grains per ear (95). Water-soluble products from *Calothrix* spp., *Anabaena* sp., and a *Strattonostoc* sp. had a rhizogenous effect and stimulated plants organs (127). Presoaking of rice seedlings in extracts of *Phormidium* (a non-N₂-fixing BGA) has been shown to accelerate germination (80), promote the growth of roots and shoots (81, 83, 84), stimulate vegetative growth of the plants (75, 83, 253), and increase the weight and protein content of the grains (83, 84, 253).

The probable nature of these substances has been likened to that of a gibberellin (83, 84). Also the growth pattern of rice seedlings treated with algal filtrate from *Aulosira fertilissima* resembled seedlings treated with gibberellic acid (268). On the other hand, extracts of *Cylindrospermum muscicola* that have given a positive effect on root growth of rice seedlings had an action similar to that produced by vitamin B₁₂, which was found to be present in the algal cells (1.5 μg ·g⁻¹) (318, 320). Vitamin B₁₂ has also been extracted from *Tolypothrix tenuis* (190). It has also been shown that amino acids (cysteine, tyrosine, phenylalanine) obtained from algal extract had a rhizogenous effect on rice (318, 320).

The production by BGA of substances that have a growth-promoting effect on rice plants is well established, but whether these substances are hormones, vitamins, amino acids or any others, as well as their mode of action, is still unclear. It has also been established that algal-growth-promoting substances are
beneficial to crops other than rice, (323) and that the production of such substances is not confined to BGA. Beneficial effects of algal inoculation in paddy fields may be partially due to growth-promoting substances, but the relative contribution by algae of N or other substances is still not clear.

4.3. DETRIMENTAL EFFECTS OF ALGAE

Blooms caused by filamentous algae can be detrimental to the rice plants, particularly to direct-seeded rice before the tillering stage. If the bloom forms before the rice seedlings have emerged, it may present a physical barrier that prevents the seedlings from penetrating the floodwater (57). The algal bloom is also harmful when the shoots and rice seedlings have not yet emerged from the water and, being in active growth, are passing through a particularly delicate stage (32). The occurrence of a thick mat of algae during planting of seedlings damages the plants by entangling with them (262) and choking the seedlings (225). Wind may also move the algal bloom, pushing the young plants beneath the surface (57). Another harmful action develops when the water dries up and the algae form a layer at the bottom of the field. This layer envelops the seedlings, which are not yet deeply rooted, and drag them to the surface when the water is let in again (32). Heavy dressings of ammonium sulfate have been reported to induce the growth of an algal "scum" that interferes significantly with the early growth of rice seedlings (262). There is one report of a loss of a rice crop mainly due to interference of algae with tiller formation (26).

The most harmful genera for rice are the filamentous or reticulated colonial types. Among these, the most frequently reported belong to the Chlorophyceae (27, 32, 33, 45, 56). Of the different algae identified in Louisiana rice fields, the two worst genera were Spirogyra and Hydrodictyon (57). BGA are occasionally cited but rarely as a dominant species (27, 32, 33, 262). In a review of algal weeds and their chemical control, the only BGA cited was Oscillatoria (45). However, a report cites BGA as detrimental: the Cyanophyceae which succeeded Chlorophyceae that have been controlled by algicides may cause up to 25% losses (31). On the other hand, Nostoc and Gloeotrichia blooms that developed in California rice fields after incorporation of crop residues interfered with seedling development, but variations in yield did not indicate significant differences between plots where residues were incorporated or burnt (34). A better growth and yield of rice were also observed in the presence of algae (Cyanophyceae and Chlorophyceae) than in plots treated with CuSO4 or Algaedyn (14).

Among the algae that are detrimental to rice, BGA can be considered incidental, and even where they had produced a bloom at the beginning of the cultivation cycle, their effect on yield was very rarely negative.

4.4. EPIPHYTISM

Epiphytic BGA have been observed on wetland rice (233), deepwater rice (102, 135, 153), and on weeds growing in rice fields (134). In the wetland rice field
ecosystem, BGA epiphytic on rice and weeds make a limited contribution to the nitrogen input but play an important role, providing inoculum for the regeneration of algal blooms that are periodically affected by adverse conditions (134, 233). In deepwater rice, which offers a much greater biomass for colonization, the nitrogen contributed by the epiphytic BGA is agronomically significant (135). BGA were found to grow preferentially on submerged decaying tissues (134, 233). An endophytic growth inside the leaf sheath was also observed in deepwater rice (135). All these cases support the observation that algal epiphytism and endophytism are probably related to abiotic effects rather than biotic relationships.

4.5. OTHER EFFECTS

The presence of BGA in the rice field has other effects on the crop. Excretion of organic acids by Anabaena sp. and Tolypothrix tenuis has been shown to increase the availability of phosphorus to the rice plant, but this action, also observed in Chlorella, was not specific to BGA (24).

Inoculation of the field (6) and presoaking of the rice seeds (108) decreased sulfide injury to rice crop. This was related to an oxygenation of the medium unfavorable for sulfate-reducing bacteria (6) and to a growth-promoting effect that enhances seedling development which increases resistance to sulfide (108).

A successful colonization of rice fields by BGA has been reported to prevent the growth of weeds (287); a negative correlation was observed between submerged weeds and floating BGA biomasses (136), but these interactions have not been fully explained.
Blue-green algae were one of the first $N_2$-fixing agents recognized to be active in flooded rice soils. Since De in 1939 (50) attributed the natural fertility of the tropical paddy fields to these organisms, many trials have been conducted to increase rice yield by algal inoculation of the soil. This practice, also called algalization, a terminology introduced by Venkataraman (311), has been reported to have a beneficial effect on grain yield in China, Egypt, India, Japan, Philippines, and the USSR (see Table 17). However, there are also reports indicating failure of algalization (see section 5.5).

5.1. METHODOLOGY

Most of the work on algalization has compared the grain yield in treatments receiving and not receiving an algal inoculum. Additional treatments like fertilizer application have also been simultaneously tested. Both pot and field experiments have been conducted, most frequently on a single crop; only a few long-term experiments have been reported.

It is necessary to critically examine the significance of the results obtained using a method where in fact only the last indirect effect (grain yield) of an agronomic practice (algalization) is observed and where the intermediary effects are not studied. The significance of small-scale and short-term experiments must also be examined.

5.1.1. Comparison between pot and field experiments

There are direct and indirect evidence indicating that BGA develop better in flask or pot experiments than in the field. Soil samples removed from the Apg
horizon and incubated under laboratory conditions gave surface-soil ARA values higher than those in the field because of the much higher N₂-fixing BGA growth in the laboratory (193). In Japan, *Aulosira fertilissima* developed profusely in pot experiments with soil pH of 5.6 (283), but the same alga failed to grow after inoculation in field experiments where the pH was the same (350). Comparing pot and field experiments where *Anabaena cylindrica* was inoculated, Huang (94) observed an increase of 34 to 41% in grain yield in the pots, depending upon the rice cultivar; in the field, no enhancement effect was observed.

From reports of simultaneous pot and field inoculations (Table 16), the relative increase in grain yield over the treatment is an average 28% in pot experiments and 15% in field experiments. Taking into account all the data listed in Table 17, these values become, respectively, 42% in pot experiments and 15% in field experiments. Standard deviations are higher than the mean in pot experiments and lower than in field experiments, also indicating a greater variability of the results in pot experiments (Table 16).

The better growth of BGA in pot experiments than in the field is probably due to less of climatic or mechanical disturbances than in the field (rain, wind, water movement), better control of the experimental conditions, and better care than that in the field. It may also be a mechanical effect of the wall of the pot where, frequently, algae seem to grow preferentially and profusely.

Dawson pointed out that small-scale experiments in greenhouses or laboratories would hardly be representative of a paddy field (48). From the preceding results it appears that pot experiments may be suitable for qualitative studies but may overestimate the effects of algal inoculation.

### 5.1.2. Duration of the experiments
Although flask experiments have shown that BGA liberate portions of their fixed nitrogen, the principal manner in which N is made available to the crop appears to be the release through microbial decomposition after the death of the algae, which is frequently associated with soil desiccation at the end of the growth cycle.

#### Table 16. Relative increase in grain yield over the treatment in pot and field experiments.

<table>
<thead>
<tr>
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<th>Field experiments</th>
</tr>
</thead>
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<tr>
<td><strong>Experiments comparing both methods</strong></td>
<td>28.1</td>
<td>15.2</td>
</tr>
<tr>
<td>Mean</td>
<td>33.2</td>
<td>12.3</td>
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<tr>
<td>Standard deviation</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Number of data</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All experiments listed in Table 17*

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<thead>
<tr>
<th></th>
<th>Pot experiments</th>
<th>Field experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>42.0</td>
<td>14.5</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>59.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Number of data</td>
<td>64</td>
<td>102</td>
</tr>
</tbody>
</table>

*Reference no. 94, 112, 143, 212, 213, 286, 287.*
This results in a delayed effect of BGA on the rice crop and a gradual buildup of soil fertility that have been observed in long-term experiments when algalization was effective (see sections 4.1 and 5.2.1.5). Therefore, experiments carried out over only one growing season may underestimate the effects of algalization; the advantages of a slow N release might not be apparent in the first crop following algal inoculation.

5.1.3. Assessment of the effects of algalization
On a practical basis, grain yield is certainly the most important result for assessment of the effects of algalization. However, experiments conducted on this basis only will not explain the mode of action of BGA on rice nor allow improvements of the technology of algalization.

Unfortunately, most experiments have been conducted on a “black box” basis where only grain yield was measured; very little information is available on the qualitative and quantitative evolution of the N₂-fixing algal flora, the evolution of the phototrophic nitrogen-fixing activity, and the nitrogen balance in an inoculated paddy field. Moreover, basic information like physicochemical characteristics of the soil and climatic data are generally not given.

According to Stewart (281) the most satisfactory method of determining the importance of N₂-fixing BGA is to measure rice yield in the presence and absence of added BGA in long-term field experiments and to compare that yield with that obtained when fertilizer nitrogen is added. However, experiments conducted with ecological and physiological bases, relating the qualitative and quantitative evolution of the N₂-fixing algal biomass and the fixed nitrogen to the environmental parameters, are badly needed to determine:

- the limiting factors for algalization;
- the relative importance of growth-promoting substances, compared to fixed N, in increasing grain yield;
- the availability of fixed nitrogen for rice; and
- the efficiency of algalization compared with that of agronomic practices that enhance the growth of the indigenous N₂-fixing algae (Is it always necessary to inoculate?).

5.2. EFFECTS OF ALGALIZATION ON RICE
Algalization, when effective, has reportedly increased the size of the plant; its nitrogen content; and the number of tillers, ears, spikelets, and filled grains per panicle (see section 5.2.2). The result is better grain yield that has been the most frequently used criterion in assessing the effects of algalization.

5.2.1. Effect on grain yield
5.2.1.1. Global effect
The available reports on the effect of algalization on grain yield in the presence or absence of additional treatments are summarized in Table 17. There are dif-
Table 17. Summarization of the reports on the effects of algalization on grain yield.

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Experimental</th>
<th>Grain yield in the control (kg·ha⁻¹)</th>
<th>Number of Crops</th>
<th>Variation in grain yield due to Algalization Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Over the treatment Relative (%)</td>
<td>Absolute (kg·ha⁻¹)</td>
<td>Over the treatment Relative (%)</td>
</tr>
<tr>
<td>122ᵃ (43)</td>
<td>Example</td>
<td>2000</td>
<td>15</td>
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The values used for this example are as follows:

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Grain yield (kg·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without algalization</td>
<td>With algalization</td>
</tr>
<tr>
<td>Control</td>
<td>2000</td>
</tr>
<tr>
<td>+ Urea, 50 kg N·ha⁻¹</td>
<td>3000</td>
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</tbody>
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ᵃ122ᵃ (43) = results from reference no. 122ᵃ (nonavailable), cited in reference no. 43. ᵇ_f = field experiment, p = pot experiment, N = nitrogen, U = urea, AS = ammonium sulfate, P = phosphorus, K = potassium, L = lime, Mo = molybdenum, R = rabbing. ᶜThe control can be either a "no addition" control or a control receiving a fertilizer application or a cultural practice common to the other treatments. ᵈU (50): urea applied at a rate of 50 kg N·ha⁻¹; the rate of fertilizer application is given as N, K₂O, P₂O₅, and sodium molybdate in kg·ha⁻¹. ᵉIn this study we refer to "treatment" as any fertilizer or cultural practice, except algalization, applied over the control. ᶠAll the values have been rounded.

Continued on opposite page
<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Experimental</th>
<th>Grain yield in the control (kg·ha⁻¹)</th>
<th>Number of Crops</th>
<th>Variations in grain yield due to Algalization (kg·ha⁻¹)</th>
<th>Treatment (kg·ha⁻¹)</th>
<th>Geographical location</th>
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<td>Average on a 66 ha area</td>
<td>2966 4</td>
<td>10 27 27 815</td>
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<td>-</td>
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<tr>
<td>7 f</td>
<td>AS (30) P (80) K (80) control</td>
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<td>10 13 16 453</td>
<td>11 340</td>
<td>16 485</td>
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<th>Sites</th>
<th>Variation in grain yield due to Algalization</th>
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<td>Absolute (kg·ha⁻¹)</td>
<td>Relative (%)</td>
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Table 17 continued

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<th>Number of</th>
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<td>Sites</td>
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<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>10.4</td>
<td>11.6</td>
<td>20.0</td>
<td>436</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td>39</td>
<td>31</td>
<td>17.3</td>
<td>13</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of data</td>
<td></td>
<td></td>
<td>25</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average in the field experiments in presence of nitrogen fertilizer</td>
<td></td>
<td></td>
<td>14.3</td>
<td>14.9</td>
<td>32.2</td>
<td>1038</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>11.8</td>
<td>8.4</td>
<td>23.8</td>
<td>766</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td>44</td>
<td>36</td>
<td>32.3</td>
<td>32</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of data</td>
<td></td>
<td></td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td>37.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Geographical location:
- India: 30
- Japan: 5
- China: 3
- Egypt: 3
- Phil.: 1
- USSR: 1
different ways of expressing such results (absolute or relative variation from a control or an additional treatment), including some open to criticism, like comparing the effect of algalization in combination with an additional treatment with a control where the additional treatment was not applied. It must also be pointed out that the relative increase in yield due to algalization is not a convenient measure as it varies with the yield in the reference. In fact, the best figure is the absolute grain yield, in kilograms per hectare, that on one hand is the only one the farmer is interested in and, on the other hand, permits all the other calculations.

Unfortunately results have been reported in diverse ways, and in order to permit a comparison and an analysis, we have expressed the given data in terms of:

1. Relative variation from the treatment due to algalization,
2. Relative variation from the control due to algalization,
3. Absolute variation from the treatment due to algalization,
4. Relative variation from the control due to the treatment, and
5. Absolute variation from the control due to the treatment.

These different variables, the terminology, and the abbreviations used are defined by an example at the beginning of Table 17. From the results of field experiments, it appears that average algal inoculation, when effective, causes a relative increase in grain yield of about 14% over the treatments and 16% over the control, corresponding to about 450 kg grain per hectare and per crop (Table 17). No significant difference was observed between average yields in fields receiving nitrogen fertilizers and fields where none was applied. When additional treatments were used, their effect was more pronounced than that of algalization. An average relative increase in grain yield over the control was 28% with non-nitrogen fertilizers and 32% with nitrogen fertilizers (Table 17).

5.2.1.2. Algalization in the presence of fertilizers other than nitrogen

Phosphorus, lime, and sometimes molybdenum application have been demonstrated to frequently have a beneficial effect on the establishment and growth of the \( \text{N}_2 \)-fixing algal flora (see section 2.5.2). In field experiments, algalization in combination with lime, phosphorus, and molybdenum application was more efficient than algalization alone (112, 126, 212, 213, 216, 242).

From the reported data (Table 18), however, it appears that the increase in yield due solely to algalization does not significantly differ in the presence or absence of non-nitrogen fertilizers and that the increase in yield due to application of non-nitrogen fertilizers is generally higher than that due to algalization. As these experiments did not study the evolution of the algal flora and photosynthetic NFA, it is not possible to know the relative importance of the direct effect of non-nitrogen fertilizers on rice and that of the indirect one of promoting algal growth. It would be useful to check if lime, P, and Mo application in combination with algalization promote preferentially the indigenous algal flora or the inoculated algae.

5.2.1.3. Algalization in the presence of nitrogen fertilizers

Biological nitrogen fixation is known to be suppressed in the presence of in-
Table 18. Effect of lime, phosphorus, and molybdenum application, in combination with algalization, on grain yield in field experiments.

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Treatment</th>
<th>Yield in the control (kg grain·ha⁻¹)</th>
<th>Increase in yield (kg grain·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Over the control when inoculated</td>
</tr>
<tr>
<td>112</td>
<td>P (?)</td>
<td>2008</td>
<td>743</td>
</tr>
<tr>
<td>126</td>
<td>L (?)</td>
<td>3372</td>
<td>49</td>
</tr>
<tr>
<td>212</td>
<td>P (67)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>P (67) Mo (0.28)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>L (2242)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>L (2242) Mo (0.28)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>P (67) L (2242) Mo (0.28)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td></td>
<td>Mo (0.28)</td>
<td>2916</td>
<td>561</td>
</tr>
<tr>
<td>213</td>
<td>P (20) L (1000) Mo (0.28)</td>
<td>2379</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>R + P (20) L (1000) Mo (0.28)</td>
<td>2379</td>
<td>199</td>
</tr>
<tr>
<td>216</td>
<td>P (20) L (500) Mo (0.28)</td>
<td>2587</td>
<td>-</td>
</tr>
<tr>
<td>242</td>
<td>P (20) L (1000) Mo (0.28)</td>
<td>1536</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>R + P (20) L (1000) Mo (0.28)</td>
<td>1537</td>
<td>274</td>
</tr>
</tbody>
</table>

Number of data | 13 | 12 | 12 | 13
Mean           | 2561 | 425 | 714 | 358
Standard deviation | 569 | 213 | 470 | 262

organic nitrogen, and failure of algalization in the presence of fertilizer N can be expected. In Japan, Watanabe pointed out that inoculation with algae was generally fruitless where nitrogen fertilizers had been applied to the land (350). A similar observation was made in India by Sankaran (245, 246), who indicated that "any supply of nitrogen by way of nitrogenous fertilizer (except urea as foliar spray) seems to inactivate the algae in nitrogen fixation." In plot experiments comparing the effect of phosphorus, potassium, lime, ammonium sulfate, and organic manure on the growth of BGA, the lowest yield of algae was obtained in ammonium sulfate plots (278, 280, 310).

Algalization with *Tolypothrix tenuis* in the presence of ammonium sulfate had a depressive effect on rice yield that was explained by a competition between algae and rice for nutrients (5). In Egypt, conditions for growth of inoculated *Tolypothrix* and rice yield were optimal when the application of ammonium sulfate was postponed to 3-4 weeks after transplanting (52).

There are, however, numerous reports on a beneficial effect of algalization in the presence of fertilizer N. Under field conditions, supplementation of urea at 60 kg N·ha⁻¹ with algal inoculum resulted in a grain yield comparable to that obtained with 120 kg N as urea (255). In a plot experiment involving 4 levels of nitrogen fertilizer (0, 50, 100, and 150 kg N as urea), a statistically nonsignificant interaction was observed between nitrogen and algalization, indicating a uniform beneficial effect of inoculation at every level of nitrogen (7). Similar observations were made in pot and field experiments with ammonium sulfate, even at N levels...
Table 19. Absolute variation in grain yield over the treatments due to algalization at different levels of fertilizer N in field experiments.

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Yield (kg grain·ha⁻¹)</th>
<th>25–30 kg N·ha⁻¹</th>
<th>50–60 kg N·ha⁻¹</th>
<th>75–100 kg N·ha⁻¹</th>
<th>120–150 kg N·ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7</td>
<td>815</td>
<td>453</td>
<td>597</td>
<td>715</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>636</td>
<td>743</td>
<td>353</td>
<td>696</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>489</td>
<td>333</td>
<td>216</td>
<td>155</td>
</tr>
<tr>
<td>109</td>
<td>7</td>
<td>103</td>
<td>227</td>
<td>730</td>
<td>678</td>
</tr>
<tr>
<td>111</td>
<td>7</td>
<td>798</td>
<td>1004</td>
<td>770</td>
<td>530</td>
</tr>
<tr>
<td>111</td>
<td>7</td>
<td>656</td>
<td>49</td>
<td>7</td>
<td>690</td>
</tr>
<tr>
<td>243</td>
<td>7</td>
<td>472</td>
<td>856</td>
<td>498</td>
<td></td>
</tr>
<tr>
<td>255</td>
<td>7</td>
<td>831</td>
<td>814</td>
<td>498</td>
<td></td>
</tr>
<tr>
<td>271</td>
<td>7</td>
<td>676</td>
<td>93</td>
<td>393</td>
<td>440</td>
</tr>
<tr>
<td>271</td>
<td>7</td>
<td>332</td>
<td>626</td>
<td>440</td>
<td></td>
</tr>
<tr>
<td>275</td>
<td>7</td>
<td>442</td>
<td>652</td>
<td></td>
<td></td>
</tr>
<tr>
<td>324</td>
<td>7</td>
<td>610</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>331</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

as high as 120 kg N·ha⁻¹ (77, 314, 315, 324). In a large number of Indian field trials, recommended high levels of nitrogen fertilizers complemented with algal inoculation resulted in yield significantly higher than that without algalization. Yield increases ranged from 2.2 to 28.9% and averaged 7.2% (331).

A comparison of different times of fertilizer N application (basal, 10 days after transplanting, and at tillering and panicle initiation) showed that grain yield was better with algalization, irrespective of the method of fertilizer application (275). From the recorded data (Table 19), it appears that response to algalization was positive at any level of nitrogen; however, this response was generally lower at high levels.

The beneficial effect of BGA inoculation in the presence of N fertilizers has been most frequently interpreted as an action of biologically potent substances (growth-promoting substances) produced by the algae. The validity of this interpretation is discussed in section 4.2. As production of growth-promoting substances is not confined to N₂-fixing BGA, field inoculation of non-N₂-fixing strains would permit assessment of the auxinic effect of BGA on rice; but, to our knowledge, such experiments have not been performed.

As BGA are generally inoculated 1-2 weeks after fertilizer N application, a decrease in mineral nitrogen concentration in floodwater can be expected. Venkataraman reported that nitrogenase activity was not depressed in a soil-rice-algae system in the presence of less than 40 ppm NH₄⁺·N (331). He pointed out the importance of critical evaluation of the relative contribution by algae of N or other substances, or both, at high levels of N. There is a dearth of information on this aspect.
Table 20. Effects of partial soil sterilization in conjunction with fertilizer application and algalization on grain yield (data from references no. 200, 227, 228, 230, 231, 266, 269, 270).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variation from the control due to the treatment (%)</th>
<th>Variation from the control due to algalization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pot experiments</td>
<td>Field experiments</td>
</tr>
<tr>
<td>Control (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial sterilization (2)</td>
<td>160</td>
<td>5</td>
</tr>
<tr>
<td>Partial sterilization + lime, P, Mo (5)</td>
<td>126</td>
<td>46</td>
</tr>
<tr>
<td>Control (2)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Partial sterilization (3)</td>
<td>19 373</td>
<td>3 43</td>
</tr>
<tr>
<td>Partial sterilization + lime, P, Mo (2)</td>
<td>55 1069</td>
<td>14 231</td>
</tr>
</tbody>
</table>

The numbers in parentheses represent number of data.

5.2.1.4. Rabbing

Algalization in conjunction with partial soil sterilization by rabbing (heating top-soil by burning straw) was demonstrated to have a beneficial effect on grain yield. This effect was enhanced in the presence of phosphorus, lime, and molybdenum (233, 242, 243, 245, 246, 283, 284, 287). In pot experiments, soil sterilization by autoclave, formaldehyde, or rabbing enhanced algal growth, but in the field the beneficial effect on rice yield was observed only with rabbing (283).

From the data in Table 20, it appears that rabbing, alone or in conjunction with fertilizers, increases grain yield but does not enhance the effects of algalization in the field. In contrast, partial sterilization in the absence of fertilizers seems to have a detrimental effect on the inoculated algae.

The beneficial effect of soil sterilization on grain yield was thought to be due to improvement in the physical properties of the soil; death of undesirable pathogens, parasites, and weeds; and to increased production of ammonia, nitrates, and other available mineral plant nutrients (287). Surface sterilization of the soil by burning straw affects the indigenous algal flora by killing the spores in the soil and on the surface of the straw. Theoretically this practice would reduce the competition between algal strains and favor the establishment of the inoculated algae, but the few reported results are not in agreement with such a hypothesis.

5.2.1.5. Cumulative and residual effect of algal inoculation

There is evidence that the favorable effect of algal inoculation increased year after year (Table 21). This was attributed to the accumulation of algae that would...
decompose partially, while the remaining cells would proliferate in the following years to increase the nitrogen fertility of the soil (344).

Repeated inoculations for several cropping seasons were also observed to have a residual effect (248). After 4 crops in an experiment comparing inoculated and noninoculated plots, a fifth crop to which no algae were added indicated a positive residual effect of algalization even in the treatments receiving N fertilizers (273). In a similar experiment, increases in yield over the control ranged from 3 to 14%. It was concluded that the residual effects of application of BGA for a continuous period of four seasons will provide sufficient inoculum for subsequent crops (110). Long-term experiments also indicated that after a 2-year period of cropping with algal inoculation, a residual effect was observed for the 3 following crops in the treatment receiving algae, lime, phosphorus, and molybdenum (243, 245, 246).

It is clear that the cumulative and residual effects of algal inoculation are due to a buildup of both the organic N content and the number of BGA propagules in the soil that will facilitate the reestablishment of the algal biomass. But no data are available to qualify the relative importance of these two phenomena in the residual effect of inoculation.

5.2.1.6. Effect on grain yield and nitrogen economy

Venkataraman (331) reported results of extensive field trials conducted in many parts of India where the effect of algalization on grain yield was compared to that of nitrogen fertilizers. From the results (Table 22, Fig. 3) he concluded that:

- In areas where chemical N fertilizers are not used, algal inoculation can give the farmers the benefits of applying 25-30 kg N-ha⁻¹.
- Where N fertilizers are used, the dose can be reduced by about one-third through algal supplementation.
- Even at high levels, algal complementation has a beneficial effect (311).
Table 22. Effect of algal inoculation at reduced levels of N fertilizer on the grain yield of rice (figures in parentheses indicate number of field trials) (reproduced from Venkataraman, 331).

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Orissa (1)</th>
<th>Bihar (1)</th>
<th>Madhya Pradesh (56)</th>
<th>Maharashtra (1)</th>
<th>Tamil Nadu (111)</th>
<th>Kerala (4)</th>
<th>Uttar Pradesh (2)</th>
<th>Andhra Pradesh (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 N</td>
<td>2.98</td>
<td>2.30</td>
<td>2.42</td>
<td>2.07</td>
<td></td>
<td></td>
<td>3.52</td>
<td>3.64</td>
</tr>
<tr>
<td>0 N + BGA</td>
<td>3.71</td>
<td>3.06</td>
<td>2.82</td>
<td>2.55</td>
<td></td>
<td>4.36</td>
<td>4.43</td>
<td></td>
</tr>
<tr>
<td>60 N</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 N + BGA</td>
<td>3.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 N</td>
<td>3.44</td>
<td></td>
<td></td>
<td>5.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 N + BGA</td>
<td>3.44</td>
<td></td>
<td></td>
<td>5.11</td>
<td></td>
<td>3.56</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>90 N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 N + BGA</td>
<td>3.73</td>
<td></td>
<td></td>
<td>4.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 N</td>
<td>3.66</td>
<td></td>
<td></td>
<td>5.21</td>
<td></td>
<td></td>
<td>5.83</td>
<td>5.76</td>
</tr>
<tr>
<td>100 N + BGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 N + BGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.04</td>
</tr>
<tr>
<td>100 N + BGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Figures preceding N indicate rate of application in kilograms per hectare. BGA = blue-green algae inoculation.
3. Effect of algal inoculation on the grain yield of rice (ADT 31) in the presence of different levels of nitrogen fertilizer (Paddy Experiment Station, Aduthurai, Tamil Nadu) (reproduced from Venkataraman - 331).

5.2.2. Effects of algalization on rice other than grain yield

Besides an increase in grain and straw yield, algalization when effective has increased the nitrogen content of both grain (93, 292, 315) and straw (93, 292). The relative increase of nitrogen content of the grain (3.2%) was lower than that of the straw (10.9%) (93). In pot experiments, symptoms of nitrogen deficiency such as coloration of the leaves, poor tillering, and bad development of seeds disappeared in treatments inoculated with *Tolypothrix tenuis*, although there was no other source of nitrogen (62). In the absence of N fertilizer, the same alga increased yield and nitrogen content of the grain and the straw; but in the presence of ammonium sulfate, it gave a significant increase in yield only, and not in nitrogen content. This result was considered evidence of the effects of growth-promoting substances on rice yield (292).

Algalization was also reported to increase the height of plants (266), leaf length (334, 335), number of tillers (7, 14, 111, 266, 289, 344), number of ears (266, 289, 334, 335), number of spikelets per panicle (9, 111), and the number of filled grains per panicle (111). Better tillering in the presence of algae was attributed in part to a higher water temperature in the plots colonized by algae (14).
Soaking the rice seeds with BGA cultures or extracts has enhanced germination and growth, prolonged the period of tillering, promoted the growth of roots, and increased the weight and protein content of the grain (see section 4.2).

5.3. EFFECTS OF ALGALIZATION ON SOIL PROPERTIES AND SOIL MICROFLORA

Algalization seems to have little effect on the physical properties of the soil; however, it may improve soil aggregation (244). Several reports indicate an increase of the nitrogen content of inoculated soil in both pot and field experiments (5, 9, 37, 52, 145, 344). Algalization with P or NP application reportedly caused a 50% rise in organic N; there was no N addition in control soils. A characteristic feature of algal inoculation in this experiment was the sustenance of both total and organic N beyond the tillering stage (37). Algalization also increased the available nitrogen as expressed by the amount of ammonification measured by the method of Shioiri and Aomine (344).

In a long-term experiment, there was a gradual increase in organic carbon due to algal inoculation, but the amount remained steady at the end of 3 years (244). An increase of organic matter (68.7%), water holding capacity (34.7%), and exchangeable Ca (58.3%) was also reported in algal plots compared with the control in “Usar” soil (266).

These results agree with the reported cumulative effect of algal inoculation that was attributed in part to the accumulation of algal material and a buildup of the organic N content of the soil (see section 5.2.1.5). In Kerala State, however, after four successive crops, algal inoculation had no appreciable effect on the level of organic matter and nitrogen content of the soil but had greatly lowered the content of reduced compounds. In the soils of this region, iron and sulfide toxicity is a common phenomenon, and a significant decrease of oxidizable organic matter, total sulfide, and ferrous iron was observed (7).

Algalization was also reported to increase the available phosphorus in the soil, because of excretion of organic acids by algae. This effect was not specific to BGA and was also observed with a eukaryotic alga (24).

Laboratory experiments conducted in beakers (237) showed that algal growth initially caused an increase in the soil pH, which later declined to the original value in some of the soils. The available phosphorus content decreased up to 90 days of algal growth and began to increase toward the later period of incubation. A drastic fall of water-soluble plus exchangeable manganese, due to algal growth, was accompanied by an increase in reducible manganese content. No appreciable change in water-soluble plus exchangeable ferrous iron was observed, but the N-NH₂OHC (pH 3) extractable iron due to algal growth progressively decreased with the progress of the incubation period (237).

Very little is known about the changes in the soil microflora after algal inoculation. Preliminary observations done by Venkataraman indicated that the quantitative incidence of other microorganisms may be affected by algalization (318).
In a pot experiment with *Tolypothrix tenuis*, inoculation increased the soil N and the total microbial count, and encouraged the development of *Azotobacter*, *Clostridia*, and nitrifiers (96).

### 5.4. SIGNIFICANCE OF ALGALIZATION AND STRAIN SELECTION

As pointed out by Stewart (281) management practices enhancing growth and activity of indigenous natural populations of N\textsubscript{2}-fixing BGA (liming, P application, etc.) can provide an immediate source of nitrogen for exploitation. Such practices (see sections 2.5.2 and 5.2.1.2) were observed to generally increase the grain yield more efficiently than algalization does. Algal inoculation in a field where management practices like liming, and P and Mo application have been conducted for the first time will provide an extra inoculum that can induce a better growth of BGA and a better grain yield than in an uninoculated field. But in a long-term treatment, the buildup of the indigenous flora may make algalization unnecessary. Unfortunately, there is a dearth of information on this aspect.

On the other hand, algal inoculation becomes a necessity where N\textsubscript{2}-fixing strains are not present in the soil. According to Venkataraman (13, 328), this seems to be frequent in India where only about 33% of 2,213 soil samples from rice fields were found to harbor nitrogen-fixing forms. Okuda and Yamaguchi (186) also observed that in certain soils, inoculation is needed to permit algal growth and an increase in nitrogen fixation.

Even when N\textsubscript{2}-fixing indigenous strains are present in the soil, their efficiency may be poor and the introduction of new strains can be beneficial. This was true in Egypt where inoculation with nonindigenous strains was more efficient than with indigenous ones (64). There are large differences in the amount of N fixed by various genera and sometimes by the same species from different localities (316). Watanabe (349) reported the quantity of N fixed by 46 strains tested in flask cultures; among these *Tolypothrix tenuis*, *Calothrix brevissima*, and *Cylindrospermum* sp. were the most efficient. This result may be due to variations in the cultural conditions. However, the influence of genetic constitution might also play a vital part in determining the capacity to fix nitrogen (311), and the large variations in doubling time observed among different species (see 281) are most probably related to strain abilities rather than to cultural conditions.

The desirable characteristics of BGA strains suitable for field inoculation have been summarized by Stewart (281) as follows:

"Strains selected for use in the field should be fast-growing and capable of fixing N\textsubscript{2} under aerobic, microaerobic, and anaerobic conditions. They should also be able to grow photo-autotrophically and chemoheterotrophically, and store endogenous carbohydrate reserves. They should evolve little H\textsubscript{2} and liberate nitrogen excess of their requirements for optimum growth... Cyanobacteria differ in whether they liberate extracellular NH\textsubscript{4}\textsuperscript{+} on inhibition of glutamine synthetase. The ways in which glutamine synthetase of Cyanobacteria is regulated could be of importance in strain selection."
Likewise, it is possible that the antagonistic effect of other organisms may affect the survival of a strain and the successful introduction of an effective strain may depend on its ability to survive and compete with the native flora for establishment growth and effective N$_2$-fixation (311). It is also clear that the climatic and physicochemical properties of the environment and the resistance to pesticides will influence the efficiency of the inoculated BGA. These parameters have to be taken into account when selecting strains.

According to Venkataraman (308), induction of mutations to enhance nitrogen-fixing power does not seem to be a profitable approach to improve strain efficiency, as most of the mutants seem to have lost their nitrogen-fixing ability.

It now appears that the search for highly efficient strains is still at a theoretical level; therefore the recommended inoculum is a soil-based mixture of *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema* (13) (see section 5.6.1.3).

### 5.5. LIMITING FACTORS FOR ALGALIZATION

Negative results of algal inoculation on rice yield have been reported only in pot experiments in the presence of ammonium sulfate (59, 60). On the basis of chemical analysis of crop N, it was concluded that this depressive effect was due to a competition for nitrogen between the inoculated alga (*Tolypothrix tenuis*) and the crop (59).

Several experiments indicated no significant response to algal application. Watanabe (350) reported that *Aulosira fertilissima* widely used as an inoculum in India failed to achieve the desired effects in Japanese soils. Yamaguchi (365) conducted a field experiment to investigate the effect of algalization for 5 years. A statistical analysis of the results revealed that inoculation had no significant effect except for a slight increase of N uptake by the rice plant. Algalization was reported to be ineffective under widely different agroclimatic conditions (8, 9, 10, 13, 40, 94, 126, 180, 186, 271, 285, 345), and the possible limiting factors are discussed thereafter.

#### 5.5.1. Soil properties

There are only a few studies on the relationship between the physicochemical properties of soils and their response to algalization (181*, 186, 285). In most of the reports on the effect of algalization and soil treatments on grain yield, the physicochemical properties of the soil (even the pH) are not indicated.

Studying the relation between the growth of BGA and physical or chemical properties of soil by incubating different soil samples in the laboratory, Okuda and Yamaguchi (186) observed no significant relation between soil texture or soil organic matter content and algal growth. The growth of algae seemed to relate closely to pH value and available phosphorus content. Examining the effect of soil treatments and algal inoculation on nitrogen fixation, the same authors (186)
distinguished four soil types:

1. Soils in which N\textsubscript{2}-fixing BGA grew naturally with considerable fixation without any treatment;
2. Soils in which algae grew and fixation increased only when they are inoculated with these organisms;
3. Soils in which inoculation was effective only when supplemented with lime and phosphate; and
4. Soils in which nitrogen fixation was poor in spite of all these treatments.

The existence of soils of the fourth type indicated that a low pH and a low P content are not only limiting factors for algalization. In a preliminary study on the reaction of different rice soil types to algalization conducted in pots, Subrahmanian et al (285) concluded that from the analysis of pH, ECe of saturated extracts, texture, organic C content, total N, and loss by ignition it was not possible to single out any one factor or factors acting as a limiting factor. However, it can be pointed out that when no lime was added, the best response was observed in the most alkaline soil.

A low pH is certainly a frequent limiting factor for algalization that has been reported to be unsuccessful in acidic soils of Japan (126, 350, 364), of India (261), and Sri Lanka (133). In acidic soils of Japan, algalization was effective only when soil was supplemented with calcium carbonate (345); the magnitude of inoculation effects tended to correspond to the amount of lime added (350). However, pH was not the only limiting factor as indicated by failure of algalization in the lime plots of a long-term experiment (126).

Algalization has also been ineffective in saline soils where inoculated algae did not survive (180). However, a positive effect and a residual effect were observed in moderately salt-affected soils (90).

Among the other soil factors that inhibit algalization a high level of fertility has been indicated (8, 9). However, a beneficial effect of algalization has been demonstrated in the presence of high levels of nitrogen (see section 5.2.1.3).

5.5.2. Climatic factors
Low temperature in temperate regimes may be a limiting factor for BGA growth (40) and, as pointed out by Watanabe (350), the soils to be inoculated should preferably be situated in warm regions since the optimum temperature for the growth of N\textsubscript{2}-fixing BGA is between 25\textdegree{} and 35\textdegree{}C. In India (Aduthurai Exper. Stn.), algalization was effective for the autumn crop but not for the winter crop (271). Among the other climatic factors, water regime may affect the inoculated algae. Heavy rains and cloudy weather have been reported to have a disturbing effect on *Aulosira fertilissima* inoculated in the field (261). In areas without assured water supply, the minimum moisture level that can support the growth and activity of BGA needs critical examination (328).

5.5.3. Biotic factors
Among the biotic factors able to suppress the growth of the algal inoculum, grazing by the zooplankton has been observed in the field (90, 180, 336) (see also sec-
tion 2.3.3). Applying the insecticide at the same time as the algal inoculum was therefore recommended in both the algal multiplication plots and the inoculated fields (see sections 2.5.4.4 and 5.6). Some other limiting biotic factors like antagonisms and competitions have been cited (311, 328). In the Punjab area (India), the examination of soils where no significant response to algal application was observed showed an aggressive indigenous non-nitrogen-fixing algal flora that might have been due to a continuous application of heavy levels of chemical nitrogen. The authors suggested that application of N2-fixing BGA over a longer period of time may be required for these areas to achieve an effective population buildup (13).

From the results it appears that little is known on the limiting factors for algalization. Knowledge of the relations between soil properties and the establishment of the algal inoculum is certainly a major gap. As pointed out by Yamaguchi (365) further studies on soil ecology are needed to ensure success in algalization.

5.6. ALGALIZATION TECHNOLOGY

Algalization in rice fields has proceeded a little beyond the stage of fundamental research and attempts have been made to popularize this technology, mostly among Indian farmers. Brief reports highlight the beneficial effect of algal inoculation on rice yield (3, 17, 18, 19, 20, 41, 138, 165, 209, 247, 313). Comprehensive treatments of the subject have been published in India with the primary purpose of transferring this technology to nonscientists. Some of the reports laid more emphasis on the practical aspects (99, 330), and others included more complete surveys of research findings (13, 326).

5.6.1. Inoculum production and conservation

The methodology of BGA mass production has been reviewed by Watanabe and Yamamoto (348) and Venkataraman (322, 326). Two types of production are distinguished: that under controlled conditions and that in open-air soil culture.

5.6.1.1. Production under artificially controlled conditions

Production under controlled conditions uses two main methods. In the first, algae are initially grown in liquid culture, then mixed with an inert material (support) and dried. In the second, algae are grown directly on the support.

A mass-culture procedure described by Watanabe (340) adopted three consecutive steps:

1. Preliminary culture of the unialgal strain (*Tolypothrix tenuis*) in ordinary flasks in the laboratory.
2. Stirring the culture in a large aseptic tank.
3. Outdoor culture in a closed circulation system using a large flat bag made of polyvinyl sheeting, and with bubbling of 5% CO₂ in air.

The maximum growth rate in the outdoor culture was 7.9 g dry weight-m²-day⁻¹ (340). To diminish the cost of algal mass production, hot spring water
and combustion of natural gas (methane) were used to provide CO₂ and warm an open bubbling system, permitting a yield of 6.4 g *Tolypothrix tenuis* (dry wt)-m⁻²-day⁻¹ (341). Different types of tank cultures permitting a growth rate of about 0.2 g (dry wt)-day⁻¹ have been described (318, 326, 340).

To conserve algae and facilitate their transportation, various inert supports have been tested. Pumice stone proved to be an efficient material (93). Mixing algal suspensions with sand and drying under the sun also permitted the alga to retain its capacity for growth unimpaired for about 2 years (312). This method, however, is disadvantageous in that the sand particles, being heavier, sink into the mud and thus hamper the rapid growth of the adhering algae (318). Growing the algae on porous gravel made of volcanic earth and soaked in nitrogen-free medium gave a material suitable for direct use in the field. This material remained efficient even after storage of 2 years (338). Synthetic sponge cut into blocks (2-4 cm) was also used as support for algae growth and conservation (326).

### 5.6.1.2. Open-air soil culture

Algae can be grown either in galvanized iron trays (326) and shallow tanks made of bricks and mortar (165) to which a few kilograms of soil is added, or in small field plots enclosed by earth embankments (13, 116, 195, 279, 318, 326). In China BGA were also grown in the rice nursery bed and in the field in between two crops (2).

The practices described for the open-air production of BGA are similar. We reproduce as an example the recommendations by the All India Coordinated Project on Algae in its short-term training course “Algal biofertilizers for rice” (13):

> “The starter culture used for multiplication is a soil-based mixture of *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena,* and *Plectonema.*
>
> The details of multiplication from the “starter culture” are as follows (the dimensions mentioned can be scaled up if desired and waste waters can also be used).
> 1. Prepare shallow trays (6' × 3' × 9") of galvanized iron sheet or brick and mortar structure, if permanent units are desired. The size can be increased if more materials is to be produced.
> 2. Place 8-10 kg soil (loamy) in the tray and mix it well with 200 g superphosphate.
> 3. Fill the trays with 2"-6" water, depending upon the local conditions and rate of evaporation (the pH of the soil should be around neutral (7-7.5); if acidic, correct it with lime.
> 4. After the soil settles down in the tray, sprinkle the starter culture on the surface of the standing water. Keep the trays in the open air completely exposed to the sun.
> 5. In hot summer months, the growth of the algae in the trays will be rapid and in about 7 days they form a thick mat on the soil surface and sometimes float up. If the daily rate of evaporation is high, add water to the trays intermittently. When the algal growth becomes sufficiently thick, stop watering.
> 6. Allow the water in the trays to dry up in the sun.
> 7. Collect the dry algal flakes from the surface or scrape them off and store them in bags for use in the fields.
> 8. Fill the trays again with water and add a small amount of the dry algal flakes to the trays (handful) as further inoculum. Continue the process as above round the year. Once the soil in the trays is exhausted (usually 3 to 4 harvests), put fresh soil in the trays, mix it with superphosphate and continue as before. A single harvest of surface algae from one tray of the above-dimension (6' × 3' × 9") will give about 1.5-2 kg material.
> 9. To prevent mosquito breeding and other insects, add Folidol (0.001 ppm) or Parathion (0.00075 ppm) or Carbofuran (3% granules) (25 g/tray) or any other insecticide.”
The recorded rate of production of algal flakes in the open-air soil culture ranges from 0.4 to 1.0 kg·m\(^{-2}\) in 15 days (13, 116, 165, 279) indicating that a 2-m\(^2\) tray can produce in 2-3 months enough algal material to inoculate a 1-ha rice field (165). The main advantage of this method is its simplicity and low cost, making it easily adoptable by the farmers. The limitations are essentially climatic: production is affected by low temperatures in winter and by washing out of the algae during the rainy season.

5.6.1.3. Strains

Unialgal cultures — mainly *Aulosira fertilissima*, *Tolypothrix tenuis*, and *Nostoc* sp. — have been used most frequently in fundamental research, but multistrain soil cultures are recommended for field application (13, 326, 330). As pointed out by Venkataraman (326) "the idea of using a mixture of algae is to offset the ecological or edaphic dangers to any one particular strain in a given locality. The only shortcoming is that when several forms are used as inocula, the ultimate proportion of individual strains in the soil tends to be unpredictable. Under field conditions this variation is less important, since the establishment of any efficient form will be enough."

The "starter culture" recommended by the All India Coordinated Project on Algae (13) is a soil-based mixture of *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema*.

5.6.2. Methods of inoculation

The methods of field application have been reviewed by Venkataraman (326). When rice is transplanted, the algal inoculum is generally applied 1 week after transplanting either as a liquid algal suspension (112) that can be supplemented with sodium molybdate (0.5 kg·ha\(^{-1}\)) (326) or as powdered material mixed with lime (266) or sand (348, 349). When rice is sown, seeds can be coated with a mixture of the algal suspension and 2.3 kg calcium carbonate per 10-20 kg seed, and air-dried in the shade (326). The efficiency of the different methods has been tested by Venkataraman (Table 23) who concluded that soil application and seed inoculation are preferable (326).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grain yield (Kg · 110 m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.66</td>
</tr>
<tr>
<td>Soil application 1 week before transplanting</td>
<td>67.36</td>
</tr>
<tr>
<td>Soil application 1 week after transplanting</td>
<td>67.02</td>
</tr>
<tr>
<td>Seed inoculation and direct sowing</td>
<td>64.19</td>
</tr>
<tr>
<td>Soaking the seedling roots in algal suspension</td>
<td>56.34</td>
</tr>
<tr>
<td>CD 5%</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Table 23. Efficiency of different methods of algal application (reproduced from Venkataraman, 306, 326).
Recommendations for field application of dried algal inoculum given by the All India Coordinated Project on Algae (330) are the following:

1. When nitrogenous fertilizers are not used, use algae to get the benefit of 20-30 kg N/ha.
2. When commercial nitrogen is used, reduce the dose by one third and supplement with algae.
3. Algae can also be used along with high levels of commercial nitrogenous fertilizer.
4. The sun dried algal material can be stored for long periods in a dry state without any loss in their viability.
5. Do not store the algal material in direct contact with chemical fertilizer or other agricultural chemicals.
6. Broadcast the dried algal material over the standing water in the field at the rate of 8-10 kg (soil based material from the production units) per hectare, one week after transplantation. Addition of excess algal material is not harmful; instead it will accelerate the multiplication and establishment in the fields.
7. Apply the algae at least for three consecutive seasons.
8. Recommended pest control measures and other management practices do not interfere with the establishment and activity of these algae in the fields.”

5.6.3. Economics
5.6.3.1. Inoculum production
In 1979 the cost of inoculum production in a field plot of 40 m² was calculated to range from US$0.16 to 0.02·kg⁻¹, depending on the number of harvests and productivity (279). The cost of production in BGA factories was reported in 1978 by Rao (209):

“To produce 400 kg of algal material per month it would require an investment of 6,000 to 7,000 rupees (Rs) and a running expenditure of about Rs 500-600 per month. If the material is sold at Rs 3 per kilo a net profit of Rs 600-700 a month can be expected and in less than one year the investment of the unit can be recovered (8 rupees = 1 US$)”. A more detailed budget sheet on the economics of inoculum production in a state farm in Tamil Nadu (India) was reported by Venkataraman in 1979 (331) and is reproduced below:

“Algae are produced in bulk using a portion of the threshing floor (8.5 × 7.1 × 0.23 m), the cost ratio is as follows:

<table>
<thead>
<tr>
<th>Raw Material required</th>
<th>Amount (kg)</th>
<th>Cost (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-green culture</td>
<td>8.25</td>
<td>10.06</td>
</tr>
<tr>
<td>Superphosphate</td>
<td>8.25</td>
<td>0.61</td>
</tr>
<tr>
<td>Sawdust</td>
<td>6.5</td>
<td>0.095</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.812</td>
<td>1.32</td>
</tr>
<tr>
<td>Soil transport</td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>Labor</td>
<td></td>
<td>1.22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10.96</strong></td>
<td><strong>0.61</strong></td>
</tr>
</tbody>
</table>

Harvest (US$)

<table>
<thead>
<tr>
<th>Produce obtained per 100 kg harvest</th>
<th>Cost (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net profit per 100 kg harvest</td>
<td>36.58</td>
</tr>
<tr>
<td>In a year of normal conditions, the farm can have 27 harvests with a net profit of</td>
<td>23.15</td>
</tr>
<tr>
<td>Cost of production per kilogram of algal material</td>
<td>625.05</td>
</tr>
<tr>
<td></td>
<td>0.134</td>
</tr>
</tbody>
</table>

In terms of chemical N input, 25 kg N/ha will cost about US$12.20. The algal material required for field inoculation to provide this amount of N will cost about US$3.65, if the material is produced commercially. However, if farmers produce their own material, the cost could be negligible (331)."
5.6.3.2. Payoff of algal technology

In trials conducted in 5 stations in India (1978), it was shown that by adding 10 kg algal culture-ha\(^{-1}\), costing about Rs 30, extra yields of paddy worth Rs 500 to 700 can be obtained on the average (209). On the basis of a large number of field experiments, the payoff from algal technology was discussed by the All India Coordinated Project on Algae (13) as follows:

"In terms of nitrogen input, an amount of 35 kg nitrogen per hectare will cost around Rs 100. The algal material required for field inoculation (10 kg-ha\(^{-1}\)) to provide this amount of nitrogen will cost around Rs 30, if the material is produced commercially. However, if the farmers produce their own material the cost will be negligible. If algal technology is introduced into even 50\% of the rice area in our country, it will result in a saving of about 0.38 \times 10^6 t of nitrogen worth about Rs 1.4 \times 10^6. In a large number of trials where the recommended high level of nitrogen fertilizer was complemented with algal application (10 kg-ha\(^{-1}\)), an average increase of about 300 kg was observed in the grain yield. This means an additional income of about Rs 300 for an investment of Rs 30 towards algal material. The cost-benefit ratio thus works out to 1:10."

The above report indicates that an algalization technology easily adoptable by farmers has been elaborated in India. This technology, tested in field trials in experimental farms, has given positive results in both grain yield and economics. To our knowledge such trials are still confined to India, and no information on the use of this technique is available in other countries.

CONCLUSION

The paddy field ecosystem provides an environment favorable for the growth of N\(_2\)-fixing BGA; however, the relative occurrence of BGA varies within large limits. From two extensive studies (328-349) it appears that they are not invariably present in rice soils. Reasons for their heterogeneous and sometimes limited distribution are still not well known as no systematic analysis has correlated their presence or absence with environmental factors.

Ecological studies of BGA in submerged soils are limited by problems in methodology primarily in estimating algal biomasses quantitatively. Fragmentary quantitative measurements indicate that N\(_2\)-fixing BGA population densities vary from a few to 10\(^5\) g\(^{-1}\) dry soil; biomasses vary from a few kilograms to 24 t (fresh wt)-ha\(^{-1}\). Due to the very variable water content of the strains, fresh weight measurements are not reliable in evaluating the potential contribution of N by BGA in the field. From the highest biomass recorded in terms of dry weight (480 kg-ha\(^{-1}\)), it appears that under favorable conditions a N\(_2\)-fixing algal bloom may contribute 30-40 kg N-ha\(^{-1}\) in the ecosystem.

From reports concerning the variations of the algal flora along the cultivation cycle, it appears that BGA could develop at any time; however, a frequent observation is that N\(_2\)-fixing BGA rarely become dominant at the beginning of the cultivation cycle.

Among the physical factors affecting the seasonal fluctuations of the phytoplankton, the light factor is certainly the most important, affecting the algal biomass qualitatively and quantitatively. In contrast with the green algae, BGA may be regarded as low-light species; under high incident light intensities, BGA
develop only when a sufficiently dense rice canopy has been established. On the other hand, during the rainy or cloudy season and under a dense rice canopy, light deficiency may also act as a limiting factor for BGA growth. To a lower extent, temperature and water regime may also influence BGA growth in the paddy field.

Among the biotic factors capable of limiting BGA growth in rice paddies, only the grazing by invertebrate populations has been documented. Evidence exists that pathogenicity and antagonisms may be operative in the fields, but these have not yet been demonstrated.

Among soil properties, pH is certainly the most important factor determining the algal flora composition. Under natural conditions, BGA grow preferentially in environments that are neutral to alkaline. A common observation is a positive correlation between pH and occurrence of BGA. Next to pH, the most decisive factor favoring BGA growth is the available phosphorus content of the soil. Very little information is available on the effect of other soil properties on BGA.

The various agronomic practices adopted along the cultivation cycle also influence growth of BGA. Land preparation and management seem to have only incidental effects. Pesticides — depending upon their nature, their concentration, and the algal strains — could have inhibitory selective or stimulatory effects on BGA. Experiments done mainly with flask cultures suggest that BGA were generally more resistant to pesticides than were other algae and tolerated pesticide levels recommended for field application. Insecticides are generally less toxic to BGA than other pesticides and have the secondary beneficial effect of suppressing the grazer population. As toxicity seems to be higher in flask cultures than in the field, experiments are needed to demonstrate more precisely the effects of pesticides and establish the in situ levels of toxicity.

Among chemical fertilization practices, phosphorus application and liming of acidic soils have demonstrated a beneficial effect on BGA growth. The effect of nitrogenous fertilizers is not well known, and the observed inhibition of algal ARA by mineral nitrogen in flask cultures may not be effective to the same extent under natural conditions. As pointed out by Lowendorf (144) the effect of N fertilizers in the field has received little attention. This is surprising in view of the observation by Venkataraman (331) that BGA inoculation produces an increase in grain yield, even at high levels of fertilizer N. From experiments conducted without algal inoculation, a depressive effect of N fertilizer on algal NFA has been established.

Other nutrients (Mo, Fe, Mg, K, etc.) are required for optimal growth of BGA, but their ecological implications as limiting factors or as factors affecting the composition of the algal community in paddy fields have not been documented.

Depending on their nature and mode of application, organic manure may favor or depress BGA growth. Plant residue incorporation, which produces anaerobic decomposition by-products toxic to algae, seems to be less beneficial to BGA than surface application.

In physiological studies on BGA in paddy fields, much emphasis has been laid on the NFA, whereas the study of in situ productivity and photosynthetic activi-
ty has been neglected. A conceivable role of CO₂ depletion as a limiting factor for ARA when large biomasses occur and the influence of the concurrent pH increase on nitrogen losses by volatilization need to be documented.

Algal NFA has most frequently been studied by ARA measurement. This method is certainly liable to misinterpretation of quantitative results, but it is very convenient and reliable for qualitative studies when the measurements are brief, the problems of gas diffusion and greenhouse effects are minimized, and statistically valid sampling methods are adopted.

Diurnal variations in ARA are related mainly to the variations of light intensity. Depending on the maximal value of the light intensity during the day, the curve will exhibit one or two maxima; the second pattern corresponds to an inhibitory effect of high light during the middle of the day. The studies reporting variations of algal ARA along the cultivation cycle indicate that a peak of activity may occur anytime. A predominant effect of light intensity in relation to the season and the plant cover seems to be well established.

The estimated amounts of fixed nitrogen vary from a few to 80 kilograms per crop. The average value of the reported estimates (30 kg-crop⁻¹) seems to constitute a satisfactory reference value when environmental factors favor BGA growth. The relative contribution of BGA as a percentage of the total nitrogen fixed in the paddy field varies within large limits and seems to be more affected by nitrogen fertilizers than the heterotrophic N₂ fixation.

BGA epiphytism makes a limited contribution to the nitrogen input in shallow-water rice, but this contribution has agronomic significance in deepwater rice.

As pointed out by Lowendorf (144), the process involved in transfer of fixed N to rice is largely a mystery. No information is available on the exudation of fixed nitrogen by BGA under field conditions; however, nutrient releases through microbial decomposition after the death of algae appear to be the principal means by which N is made available to the crop. The resulting delayed effect of algae on rice is confirmed by a cumulative effect of successive algal inoculations.

Besides increasing nitrogen fertility, BGA have benefited rice plants by the production of growth-promoting substances. The additive effect of algalization in the presence of a high level of fertilizer N was interpreted as an index of this growth-promoting effect, but such an interpretation has still not been demonstrated in the field and has to be treated with caution. Some other beneficial effects of BGA on rice — increasing phosphorus availability, decreasing sulfide injury, preventing the growth of weeds — have also been reported.

Among the algae detrimental to rice — because of their mechanical effect on the young plants — BGA can be considered as incidental. Even where they produced a bloom at the beginning of the cultivation cycle, their effect on yield was rarely negative.

Since BGA were recognized to be one, if not the most, important N₂-fixing agent in flooded rice soils, many trials have been conducted to increase rice yield by algal inoculation (algalization). Unfortunately most of the experiments have been conducted on a “black-box” basis where only the last indirect effect (grain
yield) of an agronomic practice (algalization) was observed and the intermediate effects were not studied. Very little information is available on the qualitative and quantitative evolution of the N₂-fixing algal flora, the evolution of the phototrophic NFA, and the nitrogen balance in inoculated paddy soils. Pot experiments may be suitable for qualitative studies, but they overestimate the effects of algal inoculation. On the other hand, most of the field experiments have been conducted over one growing season only and may underestimate the effects of algalization. The advantages of a slow N release might not be apparent in the first crop after algal inoculation.

Algalization has been reported to have a beneficial effect on grain yield in several countries; however, there are also reports indicating a failure of algalization under widely different agroclimatic conditions. Little is known about the limiting factors for algalization. Among the soil properties, a low pH and a low available phosphorus content are the only well-documented ones. Knowledge of the relation between soil properties and the establishment of the algal inoculum is certainly a major gap. Among the detrimental biotic factors, only grazing by zooplanktons has been studied. Low temperatures, heavy rains, and cloudy weather have also been reported to limit the establishment of the algal inoculum.

Algalization, when effective, has been reported to increase the size of the plant; its nitrogen content; and the number of tillers, ears, spikelets, and filled grains per panicle. The better grain yield has been used to assess the effect of algal inoculation. From the reports on field experiments, conducted mainly in India, it appears that on the average, algal inoculation, where effective, causes about 14% relative increase in yield, corresponding to about 450 kg grain·ha⁻¹·crop⁻¹.

A higher increase in grain yield was observed when algalization was done in combination with lime, phosphorus, and sometimes molybdenum application. It appears, however, that the increase in yield strictly due to algalization does not significantly differ in the presence or absence of non-nitrogen fertilizers and that the increase in yield due to non-nitrogenous fertilizers is higher than that due to algalization.

Results concerning the effects of algalization in the presence of nitrogen fertilizers are controversial. Several reports indicate a failure of algalization in the presence of fertilizer N. On the other hand, large-scale experiments conducted in India indicate a beneficial effect of algalization even at very high levels of nitrogen, however, this response was generally lower at high levels.

There is evidence that algalization produces both a cumulative and residual effect attributed to a buildup of the soil nitrogen, organic matter, and the algal flora. However, little is known about the effects of algalization on soil properties and soil microflora.

Another gap concerns the comparison between algalization and management practices enhancing growth and activity of indigenous natural populations of N₂-fixing BGA. In some cases, the latter can make algalization unnecessary. Algalization is necessary where efficient strains are absent in the soil. The search for highly efficient strains is still at a theoretical level, therefore, the recommended inoculum is a soil-based mixture of strains.
Algalization in rice fields has proceeded a little beyond the stage of fundamental research, and attempts have been made to popularize this technology among Indian farmers. A method for producing algal inoculum, easily adoptable by farmers, has been developed and recommendation for field inoculation given. The economics of inoculum production and payoff from the algalization technology have indicated a cost-benefit ratio working out to 1 to 10 and an additional income of about 300 Indian rupees per hectare and per crop (1979). To our knowledge, such trials are still confined to India.

As a general conclusion, a beneficial role of BGA in paddy fields appears to be true. The abundance of a sometimes repetitive literature on this subject clearly indicates that researchers have felt the importance and the potentialities of BGA in rice cultivation. Unfortunately the ecology of BGA in rice fields and their modes of action on the plant are still poorly understood. Long-term field experiments on an ecological basis — relating the occurrence of N₂-fixing strains, the qualitative and quantitative evolution of the algal flora, and the variations in phototrophic N₂-fixing activity to the environmental parameters — are badly needed to determine the following under inoculated and non-inoculated conditions:

- the mechanisms of algal successions,
- the limiting factors for the presence and growth of BGA, and their NFA, and
- the cultural practices favoring growth of N₂-fixing BGA.

Another important gap concerns the different ways in which BGA favor rice growth. Physiological and chemical field studies, including ¹⁵N and nitrogen balance studies, are needed to:

- quantify the nitrogen fixed by algae and determine how it becomes available to rice;
- determine the relative importance of growth-promoting substances in increasing grain yield compared to fixed nitrogen; and
- study the influence of BGA on soil properties and the effect of changes in soil properties on rice growth.

Despite all these gaps in our knowledge, observations and experiments done now enable us to conclude that increasing the efficiency of indigenous or inoculated N₂-fixing BGA by cultural practices is certainly an efficient way of providing an alternative source of nitrogen for rice cultivation.
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