REYNAUD

*Biological Agriculture and Horticulture*, 1988, Vol. 5, pp. 197–208
0144-8765/88 $5

Printed in Great Britain

# Colonization Potential of Cyanobacteria on Temperate Irrigated Soils in Washington State, U.S.A.

**Pierre A. Reynaud[1] and Blaine Metting[2]**

*[1]French Institute for Cooperative Research & Development (ORSTOM), Centre de Dakar, B.P. 1386, Dakar, Senegal.[2]R & A Plant-Soil, Inc., 24 Pasco-Kahlotus Road, Pasco, WA 99301, U.S.A.*

## ABSTRACT

Cyanobacteria (blue-green algae) have been studied as possible biofertilizers for rice cultivation because of the ability of heterocystous species to fix atmospheric N under aerobic conditions. The aim of this study was to investigate the potential for using cyanobacterial biofertilizers on irrigated temperate soil. Twenty-three morphological strains of heterocystous cyanobacteria originally isolated from African, Asian and North American soils were screened for their ability to colonize an agricultural soil in Washington State, U.S.A. Twelve of the 23 morphological strains could be recovered from 0.5 $m^2$ plots following successive three-week experiments in which desiccation was frequent. *Nostoc* 79WA01 (a local isolate) invaded the entire experimental area and may have influenced population interactions in subsequent experiments.

After the initial screening experiments, *Nostoc* 79WA01 and *Calothrix* (strain 12 from Senegal) were selected for mass culture and inoculation through centre pivot sprinklers onto soil cropped to winter wheat. Standing biomass of the added cyanobacteria was estimated and compared to the presence and quantity of native eukaryotic and cyanobacterial taxa. *Nostoc* biomass increased 395-fold (to 79 dry kg $ha^{-1}$) on a 55 ha field after inoculation at 0.2 kg $ha^{-1}$ and represented 66% of total microalgal biomass. Following harvest and desiccation of the soil, *Nostoc* biomass was reduced to 3 kg $ha^{-1}$, but still represented 63.5% of the algal community. *Calothrix* failed to effectively colonize the 60 ha field to which it was applied. Although it could be recovered on each sampling date, *Calothrix* biomass peaked at but 0.3 kg $ha^{-1}$ following inoculation with 0.09 kg $ha^{-1}$ of material.

Reproduction of *Nostoc* to 79 kg $ha^{-1}$ in 66 days represented an average productivity of 0.12 g $m^2$ $day^{-1}$ demonstrating that cyanobacteria can colonize a temperate soil on an agricultural scale. However, much greater standing crops would have to be achieved for provision of a significant quantity of N for crop use.

## INTRODUCTION

Cyanobacteria, or blue-green algae, include edaphic, filamentous species capable of biological nitrogen fixation. Because they derive energy for growth and nitrogen fixation from sunlight and can synthesize and operate the

nitrogenase complex in oxygenated surroundings, heterocystous cyanobacteria are of interest as biofertilizers (Metting *et al.*, 1988). To date, fundamental and applied research with cyanobacterial biofertilizer technologies has almost exclusively focused on flooded rice cultivation for which there is some evidence that agronomically significant quantities of crop available N are provided. These technologies include green manuring with or companion cultivation of the water fern *Azolla,* which harbours symbiotic cyanobacteria, and inoculation with single or mixed species of free-living cyanobacteria, the latter technology referred to as algalization (Roger & Watanabe, 1986). Cyanobacteria are, however, common constituents of microalgal communities on temperate soils, particularly on moist neutral to alkaline soils (Metting, 1981).

Recent studies have shown that eukaryotic single-celled microalgae under development as biological soil conditioners will colonize moist temperate soils when large inocula can be applied (Metting, 1986 & 1987). With the advent of a microalgal biomass production technology in recent years, it is of interest to investigate whether cyanobacterial biofertilizers are feasible for use with crops on irrigated, but non-flooded soils. To begin this work, we have undertaken an initial screening of 23 African, Asian and American strains and selected for those able to colonize an intermittently wet soil. Two candidate strains were then mass cultured and subsequently inoculated onto centre pivot-irrigated soil cropped to wheat in eastern Washington state. This study reports results of these experiments.

## MATERIALS AND METHODS

Cyanobacteria screened for their ability to colonize soil are listed in Tables 1–3 along with the results of the screening experiments. The term strain is used throughout in the context of the microscopic morphology of individual filaments and colony forming units (cfu); genetic markers were not employed. Inocula for the screening experiments were axenic cultures grown in BG-11 medium (Rippka *et al.*, 1979) under cool white lights on a 16:8 (light:dark) photoregime at $30 \pm 3°C$. Cultures were harvested by centrifugation and inoculation rates onto 0.5 $m^2$ plots calculated on a dry weight basis equivalent to $\frac{1}{2}$ dry kg $ha^{-1}$. The soil on which the initial screening experiments took place was a Quincy sandy loam; a mixed, mesic, Xeric toripsamment according to unpublished data for Franklin County, Washington State. Selected properties of the soil included: texture (71.2% sand, 20.8% silt, 8.0% clay), 7.9 meq 100 $g^{-1}$ cation exchange capacity, 0.55 mmho $cm^{-1}$ electrolytic conductivity, pH of 7.0, and 0.03% total (Kjeldahl) N. Soil nutrients estimated one week before the first experiment were (meq 100 $g^{-1}$) $Ca^{+2}$ – 4.9, $Mg^{+2}$ – 1.8; (ppm) $K^{+}$ – 133,

$Na^+ - 0.2$, $P_2O_5 - 28$, $SO_4^- - 10$; and (kg $ha^{-1}$) $NO_3^- - 28$, and $NH_4^+ - 20$. Moisture content at –0.1 bars is 18.3% (w/w).

Successive screening experiments were initiated on March 28, April 25, and May 17, 1986 and sampled on April 23, May 16 and June 7. The plots were watered daily for one week following inoculation and then permitted to dry prior to collecting ten 1 $cm^2$ samples for qualitative and quantitative enumeration of cyanobacteria and eukaryotic microalgae by the dilution method of Reynaud & Laloe (1985). Values were recorded as cfu $cm^{-2}$ on BG-11 medium with or without $NaNO_3$. Environmental conditions were not monitored.

*Nostoc* 79WA01 and *Calothrix* 12 were mass cultured for ten days to two weeks in 8000 l (20 $m^2$) semi-continuous mode under constant illumination from 1000 W high-intensity Sylvania Metalarc lamps. Mass cultures were unialgal but not axenic in a proprietary mass culture medium at 20 ± 2°C. Though these cyanobacteria have higher temperature optima, provision for control in mass culture was lacking. The mass cultures were harvested but not concentrated and applied independently via irrigation systems onto two circular fields (55 and 60 ha) from May 5 to May 25. Injection pumps were calibrated to permit even dispersal of the biomass over the soil surfaces. A total of 90 g (dry wt.) of *Calothrix* 12 was applied per ha to the 60 ha field while the 55 ha field received a total of 210 g (dry wt.) $ha^{-1}$ of *Nostoc* 79WA01. The small and discrepant inoculation rates were the consequence of having to utilize mass culture facilities and apply the cultures in such a manner as not to disrupt ongoing commercial and farming activities.

Soils making up the two fields were similar according to unpublished survey results and included sandy loams, loamy fine sands, loamy sands, and irregularly spaced pockets of nearly 100% sand. Twenty kg $ha^{-1}$ of P as solubilized phosphate was applied to the soil prior to planting in fall of 1985. From mid-March through June, 1986 the fields received a total of approximately 180 kg $ha^{-1}$ of N applied with the irrigation water as an aqueous $NH_3$ solution. The selective post-emergence herbicide 2,4 dichlorophenoxy-acetic acid was applied in late February, 1986. The fields were cropped to soft white winter wheat (*Triticum aestivum* L. 'Basin') which was planted in October, 1985 and harvested on July 20, 1986. Climatic variables were not monitored.

Cyanobacterial and eukaryotic microalgal population dynamics were estimated by taking 50 core samples (1 $cm^2$ × 1 cm) per field along a circular path 50 m (radius) from the centre of the field and combining the samples for dilution and plating on BG-11 medium with and without N as described above. Whenever morphologically distinct colonies formed on the agar surfaces, samples were removed for subsequent preparation of unialgal cultures and quantification of specific cell volume to biomass relationships

permitting estimation of *in situ* biomass by the method of Reynaud & Laloe (1985). Time limitations and logistical considerations prevented having performed similar measurements for the earlier screening experiments which are reported only as cfu $cm^{-2}$.

## RESULTS

Of the eight cyanobacteria inoculated onto the 0.5 $m^2$ plots on March 28, it was possible to reisolate (recover) six, on the basis of morphological similarity, from agar surfaces one week after sampling on April 23 (Table 1). In contrast, only two of five cyanobacteria were reisolated from the April 25 experiment (Table 2) and but four of ten from the May 17 experiment (Table 3). The cyanobacterium demonstrating the greatest colonization potential was *Nostoc* 79WA01. Even though *Calothrix* 12 achieved a greater population size on a cfu basis than any other of the added cyanobacteria in any of the

TABLE 1

Microalgal and cyanobacterial colony-forming units per $cm^2$ from the first screening and selection experiment (March 28 to April 23, 1986).

| Treatment[a] | Added strain | *Pseudanabaena* sp. | *Nostoc* spp. | LPP[b] spp. | Green/ Y-green[c] | Diatoms |
|---|---|---|---|---|---|---|
| Control | — | $< 10^3$ | $< 10^3$ | 0 | $5 \times 10^3$ | 0 |
| *Anabaena* 77WA165 | $1 \times 10^4$ | 0 | 0 | $5 \times 10^3$ | $6 \times 10^3$ | $< 10^3$ |
| *Calothrix* 8 (S) | $4 \times 10^3$ | 0 | $< 10^3$ | 0 | $10^3$ | $10^3$ |
| *Calothrix* 12 (S) | $3 \times 10^4$ | 0 | $< 10^4$ | $2 \times 10^3$ | $3 \times 10^4$ | 0 |
| *Cylindrospermum* 79WA02 | 0 | 0 | $< 10^3$ | 0 | $3 \times 10^4$ | 0 |
| *Nodularia implexa* (S) | $3 \times 10^3$ | 0 | 0 | 0 | $5 \times 10^3$ | $10^3$ |
| *Nostoc* 79WA01 | $6 \times 10^3$ | 0 | $< 10^3$ | $< 10^3$ | $4 \times 10^3$ | 0 |
| *Nostoc* 77WA160 | 0 | $10^3$ | $2 \times 10^3$ | 0 | $1 \times 10^4$ | 0 |
| *Nostoc* 79SO5 (S) | $1 \times 10^4$ | 0 | 0 | $2 \times 10^3$ | $2 \times 10^4$ | 0 |

(a) Strains designated with 'WA' were isolated from Washington state soils while those followed by (S) were isolated in Senegal.
(b) LPP designates the homocystous filamentous cyanobacteria in the *Lyngbya-Plectonema-Phormidium* complex (Rippka *et al.*, 1979).
(c) Eukaryotic green (Chlorophyceae) and yellow-green (Xanthophyceae) microalgae.

TABLE 2

Microalgal and cyanobacterial colony-forming units per $cm^2$ from the second screening and selection experiment (April 25 to May 16, 1986).

| Treatment[a] | Added strain | *Nostoc* 79WA01 | *Nostoc* spp. | LPP[b] spp. | Green/ Y-green[c] | Diatoms |
|---|---|---|---|---|---|---|
| Control | — | $5 \times 10^4$ | $< 10^3$ | $< 10^3$ | 0 | $< 10^3$ |
| *Anabaena* 75S03 (S) | $< 10^3$ | $6 \times 10^3$ | 0 | 0 | $4 \times 10^3$ | $1 \times 10^3$ |
| *Calothrix scopulorum* (S) | 0 | $4 \times 10^3$ | 0 | 0 | $2 \times 10^3$ | $< 10^3$ |
| *Nodularia* 39 (S) | 0 | $5 \times 10^4$ | $< 10^3$ | 0 | $2 \times 10^3$ | 0 |
| *Nostoc* 74SO7 (S) | $< 10^3$ | $1 \times 10^3$ | 0 | 0 | $< 10^3$ | 0 |
| *Scytonema* 79S11 (S) | 0 | $8 \times 10^3$ | 0 | $< 10^3$ | $< 10^3$ | 0 |

(a) All strains in this second experiment were isolated from Senegalese soils.
(b) LPP designates the homocystous filamentous cyanobacteria in the *Lyngbya-Plectonema-Phormidium* complex (Rippka *et al.*, 1979).
(c) Eukaryotic green (Chlorophyceae) and yellow-green (Xanthophyceae) microalgae.

three trials ($3 \times 10^4$ cfu $cm^{-2}$), *Nostoc* 79WA01 succeeded in becoming established over the entire $3 \times 4$ m area within which all three experiments were conducted. In all likelihood, this took place as a consequence of mechanical tillage and manual raking of the surface following the first experiment because it was not observed on dilution plates representing the uninoculated control after the first experiment. However, it might have been the case that *Nostoc* 79WA01 was native to the site—it was originally isolated from a soil located 100 km to the north—but by the April 23 sampling data was not present in sufficient quantities to be observed at the dilution rates employed.

Three of the five temperate (Washington State) cyanobacteria could be recovered following the three week duration of each of the 0.5 $m^2$ screening experiments. Likewise, nine of 15 Senegalese strains, but neither the Philippine nor *T. tenuis* from India were recovered. Reisolation was not related to whether the strain was originally isolated from a semi-dry or moist environment. For example, *Nostoc* 79WA01 originated in an irrigated agricultural soil collected when wet while *Calothrix* 12 was isolated from a dry tropical soil in Senegal. Strains for which less than $10^3$ cfu are reported represent one or two colonies only and in most cases these colonies did not appear on all three replicate plates.

*Calothrix* 12 and *Nostoc* 79WA01 were chosen for mass culture and inoculation onto the pivot-irrigated fields because they were the most effective colonizers during the first screening experiment and because there was success

TABLE 3

Microalgal and cyanobacterial colony-forming units per $cm^2$ from the third screening and selection experiment (May 17 to June 7, 1986).

| Treatment[a] | Added strain | *Nostoc* 79WA01 | *Nostoc* 81WA03 | *Nostoc* spp. | *Pseudanabaena* sp. | Green/ Y-green[b] | Diatoms |
|---|---|---|---|---|---|---|---|
| Control | — | $9 \times 10^3$ | 0 | 0 | $7 \times 10^3$ | $2 \times 10^4$ | 0 |
| *Anabaena* 74SO8 (S) | $3 \times 10^3$ | $4 \times 10^3$ | 0 | 0 | $2 \times 10^3$ | $3 \times 10^4$ | 0 |
| *Aulosira* ae (S) | 0 | $6 \times 10^3$ | 0 | 0 | 0 | $3 \times 10^4$ | 0 |
| *Calothrix* 10 (S) | $2 \times 10^3$ | $3 \times 10^3$ | 0 | 0 | 0 | $1 \times 10^4$ | 0 |
| *Cylindrospermum* (S) | $10^3$ | $8 \times 10^3$ | 0 | 0 | $< 10^3$ | 0 | $< 10^3$ |
| *Fischerella* F2B6 (P) | 0 | $3 \times 10^3$ | 0 | $< 10^3$ | 0 | $2 \times 10^3$ | $3 \times 10^3$ |
| *Fischerella* FB10 (P) | 0 | $4 \times 10^3$ | $10^3$ | $< 10^3$ | 0 | $2 \times 10^3$ | $< 10^3$ |
| *Gloeotrichia* 77SO3 (S) | 0 | $4 \times 10^3$ | 0 | 0 | $10^3$ | $1 \times 10^3$ | 0 |
| *Nostoc* 77S17 (S) | 0 | $6 \times 10^3$ | $10^3$ | 0 | $2 \times 10^3$ | $2 \times 10^3$ | 0 |
| *Nostoc* 81WA03 | $10^3$ | $10^4$ | — | 0 | $2 \times 10^3$ | $2 \times 10^5$ | 0 |
| *Tolypothrix tenuis* (I) | 0 | $3 \times 10^3$ | 0 | 0 | 0 | $< 10^3$ | 0 |

(a) Strains designated with 'WA' were isolated from Washington state soils. Those with (S) were isolated in Senegal. *T. tenuis* (I) is from India courtesy of G.S. Venkataraman.
(b) Eukaryotic green (Chlorophyceae) and yellow-green (Xanthophyceae) microalgae.

in maintaining viable 10, 500, and 8000 l cultures. Also, the intention was to be able to inoculate the agricultural fields over as long a period as possible and with as great an inoculum as feasible. Results from periodic enumeration of cyanobacterial/microalgal populations on the 55 and 60 ha circles are collected in Tables 4 and 5. The 55 ha circle inoculated with *Nostoc* 79WA01 was sampled five times. On three of those dates, the 60 ha field inoculated with *Calothrix* 12 was sampled as well. The soil-conditioning microalga *Chlamydomonas sajao* was co-inoculated onto both fields at a rate of 0.13 kg (dry wt) $ha^{-1}$ and had been inoculated onto the 55 ha field the previous year as well. Tables 4 and 5 also include results of quantitation of *C. sajao.*

*Calothrix* 12 failed to effectively colonize the soil to an important extent, never representing more than 0.8% of the total microalgal biomass. In contrast, *Nostoc* 79AW01 inoculation was successful to the extent that it

TABLE 4

Algal biomass at three dates on two inoculated wheat fields and a control.

| Days after cyanobacterial inoculation: | 28 | | | 51 | | | 66 | | |
|---|---|---|---|---|---|---|---|---|---|
| Alga (or algal group) Field: | 55ha | 60ha | Cont. | 55ha | 60ha | Cont. | 55ha | 60ha | Cont. |
| | kg dry weight·hectare$^{-1}$ | | | | | | | | |
| *Nostoc* 79WA01 | 0.17 | — | — | 25 | — | — | 79 | — | — |
| *Calothrix* 12 | — | 0.32 | — | — | 0.32 | — | — | 0.15 | — |
| *Anabaena-Nostoc* spp. | 0 | 0 | 0 | 0 | 0.68 | 6.3 | 3.8 | 1.1 | 0.01 |
| *Chlamydomonas sajao* | 11 | 3.7 | 0.3 | 29 | 43 | 0.8 | 7.4 | 0.8 | 0.8 |
| Diatoms | 9.8 | 1.2 | 0 | 20 | 3.5 | 0.01 | 0.3 | 0 | 0.01 |
| Filamentous green algae | 26 | 10 | 0 | 95 | 24 | 8.4 | 24 | 41 | 36 |
| *Pseudanabaena* sp. | 13 | 0.36 | 0.7 | 7.3 | 2.5 | 3.1 | 0.6 | 0.2 | 7.2 |
| LPP taxa | 78 | 24 | 0 | 92 | 2.9 | 15 | 3.6 | 9.7 | 11 |
| Total algal biomass | 138 | 40 | 1 | 268 | 77 | 34 | 119 | 53 | 55 |
| % Inoculated cyanobacteria | 0.1% | 0.8% | — | 9.3% | 0.4% | — | 66% | 0.28% | — |
| % *Chlamydomonas sajao* | 8% | 9% | 30% | 11% | 56% | 2.3% | 6% | 1.5% | 1.5% |

TABLE 5

Algal community development on the fifty-five hectare wheat field.

| Days after inoculation with: *Nostoc* 79WA01 | 4 | 28 | 51 | 66 | 93 | (re-wetted) 160 |
|---|---|---|---|---|---|---|
| *Chlamydomonas sajao* | –26 | –2 | 21 | 36 | 63 | 130 |
| Alga (or algal group) | kg dry weight·hectare$^{-1}$ | | | | | |
| *Nostoc* 79WA01 | 0 | 0.2 | 25 | 79 | 2.9 | 0.15 |
| *Chlamydomonas sajao** | 8.9 | 11 | 29 | 7.4 | 1.5 | 0.47 |
| Diatoms | 2.5 | 9.8 | 20 | 0.3 | 0.004 | 0.36 |
| Filamentous green algae | 0 | 26 | 95 | 24 | 0 | 3.9 |
| *Pseudanabaena* sp. | 0.6 | 13 | 7.3 | 0.6 | 0.06 | 1.26 |
| LPP taxa | 81 | 78 | 92 | 3.6 | 0.12 | 4.10 |
| *Anabaena-Nostoc* spp. | 0 | 0 | 0 | 3.8 | 0 | 0 |
| Total algal biomass | 93 | 138 | 268 | 119 | 4.6 | 10.3 |
| % *Nostoc* 79WA01 | 0% | 0.12% | 9.3% | 66% | 63.5% | 1.5% |
| % *Chlamydomonas sajao* | 9.6% | 8.0% | 10.8% | 6.4% | 32.5% | 4.5% |

**Chlamydomonas sajao* had been inoculated onto this field the previous year as well.

contributed 9.3% of the total biomass after 51 days and 66% after 66 days (Table 4). And although absolute *Nostoc* biomass dropped shortly after harvest and desiccation of the soil surface (from 79 dry kg $ha^{-1}$ to 2.9), it still represented nearly 64% of the total algal biomass on that field.

Patterns of development of native microalgal and cyanobacterial populations were highly variable among and within both the 0.5 $m^2$ experimental plots and the wheat fields. In particular, non-filamentous green and yellow-green microalgae were common on the small plots but not on the circles where only filamentous forms were abundant. Also, diatoms were scarce on the small plots but often present in large numbers on the wheat fields. These patterns are, however, somewhat reminiscent of population dynamics of filamentous eukaryotic, homocystous, and heterocystous cyanobacteria on rewetted soils of dry tropical West Africa (Reynaud, 1987).

## DISCUSSION

The common presence of heterocystous cyanobacteria on moist, semi-arid and dry temperate soils has long been known yet they have been subject to surprisingly little research relevant to their potential use in agriculture. In contrast to the many investigations of algalization and rice production that have been summarized by Roger and his colleagues (Roger & Kulasooriya, 1980; Roger & Watanabe, 1986), studies by Witty (1974, 1979) and Anderson *et al.* (1982) include the only research known by us to deal with inoculation of temperate soils with free-living, heterocystous cyanobacteria. Henriksson (1971) estimated cyanobacterial nitrogen fixation on 1000 Swedish soils to range from 0.4 to 5.1 g N $m^{-2}$ $yr^{-1}$, and so it might be possible to provide N to temperate crops with these microorganisms. Experience with eukaryotic microalgal soil conditioners and the ecology of cyanobacteria in natural plant communities suggest that provision of sufficient moisture is critical (Metting, 1986; Zimmerman *et al.*, 1980). Therefore, if a cyanobacterial biofertilizer technology is feasible for temperate agriculture, it will most probably be restricted to irrigitated soils and in particular to continuously sprinkler-irrigated surfaces.

The first step in exploring the potential for inoculation of temperate soils with cyanobacteria is to screen strains and select those capable of colonizing surfaces under climatic stress and competition from other microalgae. A related question is whether cyanobacteria will effectively colonize foreign soil or, if not, will it be necessary to utilize locally-adapted strains? Witty (1974, 1979) reported from research in England that both a native *Nostoc ellipsosporum* and alien strains of *N. punctiforme* and *Anabaena cylindrica* successfully colonized plots sown to wheat which were regularly irrigated. Anderson *et al.*

(1982) screened three temperate and four tropical cyanobacteria for colonization potential in the laboratory preliminary to successfully introducing a local *Anabaena* and a tropical *Tolypothrix tenuis* (from India) onto field plots cropped to irrigated corn in Washington State. However, in both studies the cyanobacterial populations did not persist. In the first study the *Anabaena* and *Nostoc* inoculants failed to recover following desiccation of the surface (Witty, 1974). In the latter study, *Anabaena* and *Tolypothrix* populations increased to 2–3 orders of magnitude greater than populations of native cyanobacteria on control plots before disappearing during a six day period without irrigation. In neither study did acetylene reduction measurements suggest an agronomically significant level of nitrogen fixation.

Relative population dynamics of added cyanobacteria and native cyanobacteria and microalgae on temperate soil has not been investigated prior to this study, yet competition might be the most important barrier to effective and consistent performance of biofertilizers on soils where abiotic factors are not limiting. In this study, community dynamics were complex. On the 0.5 $m^2$ plots, all of which were within a 12 $m^2$ area, native taxa were either very irregularly distributed or somehow influenced by competition from added inocula. Enumerations were based on ten pooled 1 $cm^2$ samples from each plot, yet taxa absent from one plot would be present on adjacent plots in numbers as large as $10^3$–$10^4$ cfu $cm^2$ (Tables 1, 2, & 3). Green and yellow-green microalgae were ubiquitous, but diatoms were not represented in numbers as large as might have been expected. This anomaly might be explained, however, as simply a consequence of poor growth of diatoms on the silicon-poor medium tailored for cyanobacteria. Native cyanobacteria (*Nostoc* spp., *Pseudanabaena* sp., LPP taxa) were always present, but varied in abundance among plots in an irregular manner as well. The irregular distribution of native taxa was evident for the wheat fields also, varying considerably over time and relative to the 0.5 $m^2$ plots which were less than 1 km distant. A notable difference was the apparent replacement of coccoid and sarcinoid green and yellow-green microalgae on the small plots by filamentous green algae and the added *Chlamydomonas sajao* on the circles.

Of the two cyanobacteria inoculated onto the circles with the irrigation water, only *Nostoc* 79WA01 reproduced to an appreciable extent, although both colonized the soils. Sixty-six days after inoculation at 0.2 kg (dry wt) $ha^{-1}$, *Nostoc* biomass increased 395-fold to 79 dry kg $ha^{-1}$. The population of the added cyanobacterium dropped dramatically following desiccation of the soil after harvest, consistent with the earlier studies cited above (Anderson *et al.*, 1982; Witty, 1974). *Calothrix* 12 inoculated at 90 dry g $ha^{-1}$ achieved a maximum standing biomass of but 0.32 kg $ha^{-1}$. The data do not reveal why *Nostoc* was more successful than *Calothrix* on the wheat fields (Table 4) when on the 0.5 $m^2$ plots, *Calothrix* reproduced to a greater extent (Table 1). Either

*Nostoc* was simply more suited to the conditions imposed on soil in the wheat field, or the 2-fold greater inoculation rate of *Nostoc* was an important factor. Reproduction of *Nostoc* to 79 dry kg $ha^{-1}$ represented an average growth rate over 66 days of 0.12 g $m^{-2}$ $day^{-1}$, a value substantially less than estimated for *C. sajao* (0.8 g $m^{-2}$ $day^{-1}$) in the field in the same area (Metting, 1986). The *Chlamydomonas* work also suggested threshold inoculation rates exist below which effective colonization is not probable in the face of competition.

In the light of the cropping cycle, certain community dynamic features are reminiscent of an earlier study of the drying of a rice soil (Reynaud & Roger, 1978). Figure 1 illustrates the changing proportions of major microalgal groups on that field (BGA refers to blue-green-algae). Most of the contribution to the total cyanobacterial biomass was by *Nostoc* 79WA01, whose growth was exponential, as shown. A similar decrease in homocystous cyanobacteria and eukaryotic filamentous green algae was correlated in the

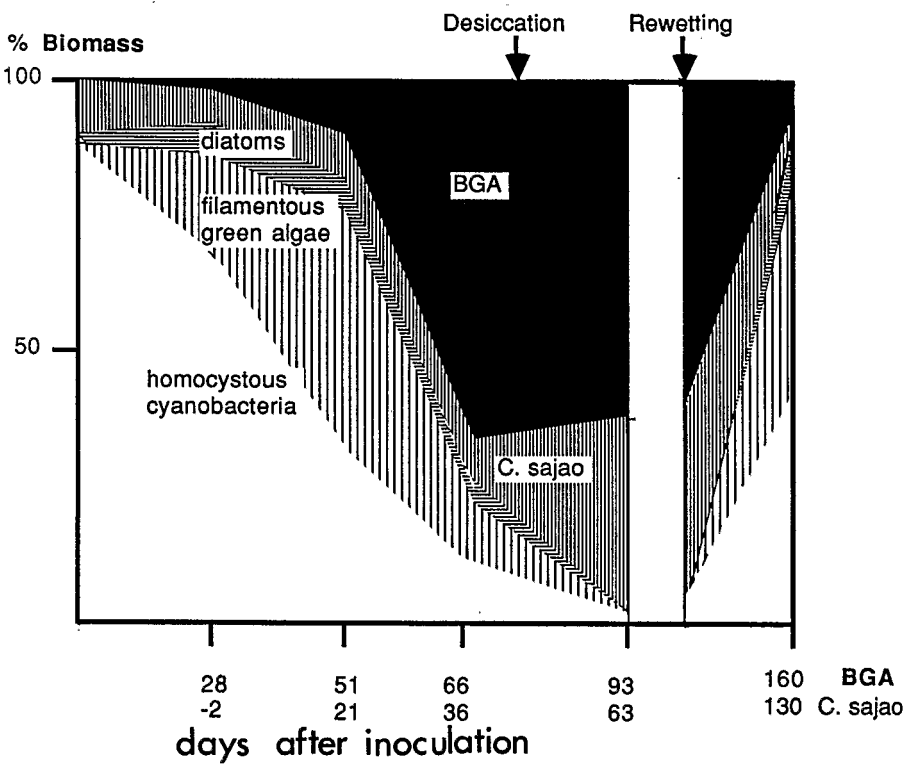


FIGURE 1. Algal community development on a wheat field (55 hectares) Pasco, Washington State. May–October 1986.

earlier African study to density of plant cover, use of N fertilizer, pH, and temperature.

Theoretical attractiveness of cyanobacterial biofertilizers for biological agriculture follows from 1) the fact that they need not compete for C and energy with the resident microflora, and 2) fixed N would be made available to crops slowly as a combination of leached N from living filaments and mineralization of dead biomass. The first suggests the possibility of consistent performance whenever competition from native microalgae can be overcome by the correct combination of proper strains with proven competitive ability and judicious timing and rate of application. The second has implications for reduction of groundwater pollution following applications of large amounts of synthetic N fertilizers at single points in time should cyanobacteria be able to supplement this practice to an agronomically significant extent. Though *in situ* biomass of the cyanobacteria in this study was never sufficient for provision of significant N to crops, the data show that heterocystous cyanobacteria will colonize an irrigated temperate soil on an agricultural scale and that preliminary screening for colonization potential could be an important tool.

## References


Anderson, D.B., Hanson, K.R., Metting, B. & Molton, P.M. (1982). *Assessment of Blue-Green Nitrogen-Fixing Algae as Living Sources of Nitrogen Fertilizer.* Pacific Northwest Laboratory (Department of Energy) Technical Report 4187. Richland, Washington (U.S.A.)

Henriksson, E. (1971). Algal nitrogen fixation in temperate regions. In *Biological nitrogen fixation in natural and agricultural habitats* (T.A. Lie & E.G. Mulder, eds.), *Plant & Soil* Special Volume, pp. 415–419.

Metting, B. (1981). The systematics and ecology of soil algae. *Botanical Review,* **47**, 196–312.

Metting, B. (1986). Population dynamics of *Chlamydomonas sajao* and its influence on soil aggregate stabilization in the field. *Applied & Environmental Microbiology* **51**, 1161–1164.

Metting, B. (1987). Dynamics of wet and dry aggregate stability from a three-year microalgal soil conditioning experiment in the field. *Soil Science,* **143**, 139–143.

Metting, B., Rayburn, W.R. & Reynaud, P.A. (1988). Algae and agriculture. In *Algae and Human Affairs* (C.A. Lembi & R.A. Waaland, eds.), pp. 335-370 Cambridge University Press; Cambridge.

Reynaud, P.A. (1987). Ecology of nitrogen-fixing cyanobacteria in dry tropical habitats of West-Africa: a multivariate analysis. *Plant & Soil,* **98**, 203–220.

Reynaud, P.A. & Laloe, F. (1985). La methode des suspensions-dilutions adaptee a l'estimation des populations algales dans une riziere. *Revue D'Ecologie et de Biologie du Sol,* **22**, 161–192.

Reynaud, P.A. & Roger, P.A. (1978). $N_2$-fixing algal biomass in Senegal rice fields. *Ecological Bulletin (Stockholm),* **26**, 148–157.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology,* **111**, 1–61.

Roger, P.A. & Kulasooriya, S.A. (1980). *Blue-Green Algae and Rice.* International Rice Research Institute; Los Banos, Philippines.

Roger, P.A. & Watanabe, I. (1986). Technologies for utilizing biological nitrogen fixation in wetland rice: potentialities, current useage, and limiting factors. *Fertilizer Research,* **9**, 39–77.

Witty, J.F. (1974). *Algal Nitrogen Fixation on Solid Surfaces and Temperate Agricultural Soils.*

Ph.D. Thesis; University of London.
Witty, J.F. (1979). Algal nitrogen fixation on temperate arable fields. Algal inoculation experiments. *Plant & Soil*, **52**, 165–183.
Zimmerman, W.L., Metting, B. & Rayburn, W.R. (1980). The occurrence of blue-green algae in Whitman County, Washington soils. *Soil Science*, **130**, 11–18.

*(Received 21st May, 1987; accepted 19th August, 1987)*