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Ecosystem Manipulation for Increasing Biological N_2 Fixation by Blue-Green Algae (Cyanobacteria) in Lowland Rice Fields

I.F. Grant[1], P.A. Roger[2] and I. Watanabe

The International Rice Research Institute, Los Banos, Laguna, Philippines

INTRODUCTION

Lowland rice is grown under flooded conditions. Submergence is of considerable importance for the maintenance of soil N fertility while regularly cropping rice without nitrogenous fertiliser. In these conditions the natural supply of N derived from irrigation water and rain is generally small (Yamaguchi, 1979) in comparison with that supplied by biological N_2-fixation (BNF). The latter, which is estimated from N_2 balance studies as 15–50 kg N crop^{-1} (Koyama & App, 1979) has therefore been designated responsible for the continued maintenance of the crop yields. De (1936), attributed much of this natural fertility to N_2-fixing blue-green algae (BGA) and Okuda (1948) and Konishi & Seino (1961) found a correlation between the growth of algae and soil N gain. Increasing BNF is clearly desirable for resource neutral agricultural systems, particularly as the cost of commercial fertiliser is unpredictable.

Methods to increase biological N_2-fixation by algae in rice fields have included the use of P_2O_5, $NaMoO_4$ and lime as soil amendments, and these were measurably successful (De & Mandal, 1958, Amma *et al.*, 1966). Encouraging results were also obtained using the algal inoculation technique (Watanabe *et al.*, 1951; Singh, 1961; Subrahmanyan *et al.*, 1965; Aboul-Fadl *et al.*, 1967; Venkataraman, 1972) that relies upon the success of introduced strains of blue-green algae to fix atmospheric N_2. The technique is often referred to as a small-scale village biotechnology. However, many failures are

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[1] Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 15853, U.S.A.
[2] Maître de Recherches ORSTOM (France), visiting scientist at IRRI.

reported from different geographical locations; Japan (Watanabe, 1973); Taiwan (Huang, 1978); Philippines (Alimagno & Yoshida, 1975) and India (All India Coordinated Project on Algae, 1979). Moreover, nurturing inoculum growth was often difficult (Konishi & Seino, 1961). Competition from indigenous algae (Yamaguchi, 1979) and grazing of inoculum by invertebrates (Hirano *et al.*, 1955; Watanabe *et al.*, 1955) were detrimental to inoculum establishment. The role of some invertebrate grazers in restricting the growth and nitrogen fixing activities of BGA has been evaluated by Wilson *et al.* (1980), and Osa-Afiana & Alexander (1981); and consumption of BGA, quantified by Grant & Alexander (1981) and Grant *et al.* (1983a). These results collectively support the notion that primary consumers that proliferate at the expense of BGA are a major limiting factor to algal N_2 fixation in lowland rice fields. This paper will introduce the rice field ecosystem with emphasis on the grazing components and then will focus on the recent attempts made to increase the contribution of N_2 fixed by both indigenous and inoculated BGA through the manipulation of the rice field ecosystem.

LOWLAND RICE ECOSYSTEM

In general, the flooded rice field is an uncompromising habitat. With few exceptions, lowland rice fields are temporary aquatic environments that are subject to physical and chemical extremes of insolation, temperature, pH, O_2 concentration and nutrient status. Furthermore, ploughing, transplanting and weeding are cultural practices disruptive to the establishment of community stability. A rather specialised rice field fauna and community structure might therefore be expected to result. Indeed, rice cultivation freezes ecosystem development as a secondary succession, and thereby prevents the ecosystem from reaching any natural conclusion or climax. Presumably, if cultivation were suspended, rice fields would revert back to a marshland community.

Although continuous, lowland rice field habitats may be broadly distinguished into 4 major zones:

Floodwater

This is the photosynthetic (photic) zone when light penetration is not impaired by turbidity caused by rice cultural practices (land preparation, transplanting and weeding) heavy rain or the activity of benthic invertebrates which stir up clay and silt particles. Floodwater supports the photosynthetic and chemosynthetic producers (algae and bacteria), invertebrate and vertebrate primary consumers (grazing zooplankton) and the nektonic secondary and tertiary consumers (carnivorous insects and fish). The distribution of aquatic flora and fauna is probably influenced by the chemistry of the soils, so that biocoenoces characteristic of neutral, acid sulphate, alkaline, saline and peat soils should be evident. As yet, qualitative and quantitative observations are too scarce to permit comparisons.

Data on oxygen, temperature and even major limiting nutrients, e.g., N, P, Ca, are also of isolated occurrence, and data collection over an ecologically useful period of time, such as a cropping season, has not been maintained. Some diurnal curves are available for rainfed (Heckman, 1979) and irrigated fields (Saito & Watanabe, 1978) which show, at least in the presence of submerged weeds, dissolved oxygen to range between daytime supersaturation and anoxic conditions at night. Partial pressures of CO_2 are inversely proportional to pO_2 and consequently diurnal pH change from a normally neutral floodwater to pH 9.5 is not uncommon at times of algal blooms or luxuriant weed growth. Floodwater temperature is frequently subjected to a 10°C change diurnally and maxima during mid-afternoon, measured at the soil/floodwater interface, often reach 36–40°C.

Except for nutrient flux calculations following fertiliser applications (De Datta *et al.*, 1983), measurements of mineral nutrients in floodwater have not been attempted routinely. Release of nutrients into the floodwater after land preparation, particularly following dry fallowing, is certainly rapid for mineralised N (Shiga & Ventura, 1976) and probably accounts for the initial bloom of algae frequently observed about 2 weeks after puddling (Kurasawa, 1956; Saito & Watanabe, 1978).

Surface soil

By virtue of the maintenance of shallow floodwater (2–10 cm), the surface soil has a redox potential higher than 300 mV. The actual depth of this oxidised layer is dependent upon the dissolved oxygen concentration of the floodwater, the reducing capacity of the soil, and the activities of the benthos and infauna. It is usually between 2 and 20 mm (Watanabe & Roger, 1984) and distinguishable visually by colour and chemically by the predominance of ferric over ferrous ions. After land preparation, the surface soil is a site of algal growth, which supports a large grazing population. Later in the rice growing season, decomposable organic matter accumulates in this zone from which benthic filter and deposit feeders (e.g. rotifers and molluscs) acquire their energy. The activities of benthic invertebrates affect nutrient recycling either directly by excretion or indirectly by release of native minerals through soil perturbation. The surface-oxidised zone is microbiologically active. Aerobic and microaerobic conditions favour microbial processes which may affect the efficiency of N cycling (nitrification and N_2 fixation).

Reduced zone

The anaerobic reduced zone soil underlying the oxidised layer is frequently called the reduced layer. The Eh range is 300 mV to -300 mV. Reduction processes predominate and NH_4-N, sulphide and methane and organic acids are liberated. Decomposing organic matter in the reduced zone sustains populations of tubificid worms and chironomid larvae whose burrowing activities aid ammonia diffusion (Fenchel & Blackburn, 1979; Kikuchi & Kurihara, 1982; Gardner *et al.*, 1983; Grant & Seegers (in press)) and PO_4 release into the floodwater (Gardner *et al.*, 1981). Animals inhabiting this zone frequently contain haemoglobin or possess air sacs as adaptions to low oxygen concentrations.

Rice plant

The rice plant creates conditions which modify the soil/floodwater environment. Shading of the floodwater and surface soil increases as the rice canopy enlarges. The ensuing changes in light intensity affects the growth of photodependent organisms. In Senegalese rice fields, BGA growth was favoured by lowered light intensities (Reynaud & Roger, 1978) but not all BGA are distributed in relation to high or low light intensities. Rice also indirectly affects the floodwater/soil communities by lowering the temperature and CO_2 concentrations under the canopy. Reduction of solar radiation coupled with low CO_2 levels on calm days will affect growth rates, succession and perhaps distribution of autotrophic organisms. Rice plants act as substrate for epiphytic growths (Roger *et al.*, 1981) and provide mechanical support for many animal species. Pulmonate molluscs may escape high floodwater temperatures by resting at the air/floodwater interphase.

Rice roots remove nutrients from the floodwater and soil and exude organic substrates utilisable by bacteria as carbon and energy sources. Oxygen translocated from the aerial parts of the rice plant to the roots oxidises the rhizosphere and may create conditions favourable for the growth of N_2-fixing organisms (Watanabe & Roger, 1984).

GRAZING COMPONENT

Animals that obtain all or part of their energy requirements by filtering, browsing or shredding primary production are called grazers. As there is a close relation between algae and grazers early in the rice cultivation cycle (autotrophic succession), the possibility exists to relieve grazing pressure and encourage growth of N_2-fixing algae. We shall therefore concentrate on this part of the ecosystem.

Copepods, cladocerans and rotifers are typical planktonic grazers which filter phytoplankton and bacteria from the floodwater. At the floodwater/soil interphase, ostracods, chironomid larvae and molluscs browse the algal growths which luxuriate early in the rice cropping cycle. Quantitative studies of zooplankton and benthic invertebrates have almost exclusively been made in fertilised fields and the units of density are not usually comparable. However, Kurasawa (1956) recorded, for example, 198 *Daphnia*, 15 *Bosmina*, 42 *Cyclops*, 42 *Branchionus* and 56 *Keratella* (per litre) about 6 weeks after planting. Crustacean zooplankton densities ranged between 200–800 l^{-1} in another Japanese study (Kikuchi *et al.*, 1975). Many of these small filter feeders are not able to feed upon the large filamentous BGA, which are rejected by their feeding apparatus. The larger benthic invertebrates can readily ingest the N_2-fixing algae and large populations of ostracods (10–20,000 m^{-2}) chironomid larvae (8000 m^{-2}) and molluscs (up to 1000 m^{-2}) have been observed in Philippine rice fields (unpublished).

By combining the data of Pantastico & Suayan (1973), Watanabe *et al.* (1978), and Saito & Watanabe (1978) with our own observations, a generalised succession of the predominant dry season flora and fauna from unfertilised rice fields of the Philippines is presented in Figure 1. Colonisation of the prepared and puddled rice field is rapid unless the land was not previously flooded. Propagating bodies (such as spores, akinetes etc.) quickly produce unicellular eukaryotic algae which are soon succeeded, albeit briefly, by fast-growing heterocystous BGA about 2–3 weeks after transplanting. The chlorophyll a concentration of the floodwater and floodwater/soil interphase reaches a peak as a result of this BGA bloom, but rapidly subsides to a basal level (0–3 mg Chl.a m^{-2}) thereafter. Fields of higher natural fertility will possess greater concentrations of chlorophyll a or chlorophyll-like substances (Wada *et al.*, 1982). About 4 weeks after transplanting the population of the floating and slower growing mucilagenous colonial BGA develop, and these are observed to reach a maximum biomass just before the rice harvest. A cause of the decline in the initial bloom of BGA is thought to be grazing by ostracods and molluscs. Flooding induces rapid ostracod development from eggs resistant to desiccation. The young increase their population density at the expense of bacteria and later, when their size allows it, the BGA. The collapse of the ostracod population quickly follows that of the BGA population, i.e., about 4 weeks after transplanting.

The recruitment rates of molluscs are dependent upon the previous condition of the rice field. When flood fallowed or wet, molluscs may swiftly attain population densities detrimental to BGA growth, but these populations quickly subside with the disappearance of algae, which also signals the change from an autotrophic to an indicated heterotrophic succession.

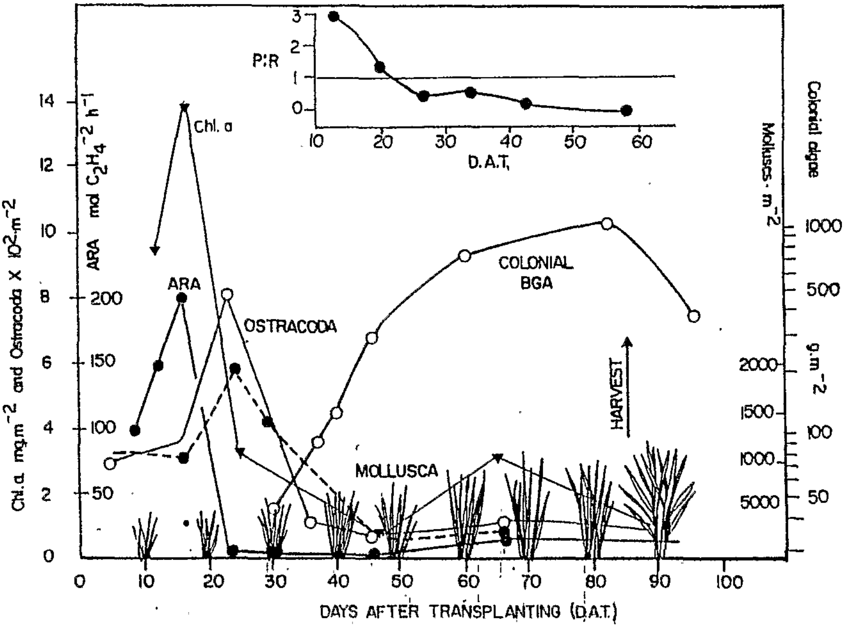


FIGURE 1 Generalised fluctuations of algae, ARA and grazers in floodwater of unfertilised lowland rice fields. Production: respiration (P:R) calculated from Ichimura (1954).

A heterotrophic succession is only indicated from data using O_2 measurements of productivity. Underestimates of O_2 concentrations due to non-biological O_2 consumption (Saito & Watanabe, 1978) and cultural practices such as removal of filamentous algae or submerged weed may cloud the real picture. Bacterial numbers are greatest following algal growth.

Primary production typically exceeds community respiration ($P:R > 1$) over the first month of rice cultivation (inset, Fig. 1) which leads to the build up of organic matter and a decrease of the ratio to below 0.1. The instability of the rice field ecosystems prevents maturity, which would lead to reduced grazing through increased food chain complexity.

Coincident with the initial BGA bloom, which is not usually resistant to grazing, is a peak of nitrogen fixation activity. Measured by the acetylene reduction assay (ARA), which usually underestimates the actual fixation rate, this was equivalent to about 2–3 kg N ha^{-1} over its duration (18 days). Arriving at a meaningful figure of N_2-fixation by the floating colonies of BGA later in the season is difficult but, Watanabe *et al.* (1978) present data from which it might be extrapolated. However, even if fixation of the late season BGA is considerable, the efficiency of N transfer to the succeeding rice crop must be low but there is no data in support of this. For this reason, regulation of a grazing population to increase initial growth and N fixation of BGA should be attempted. Fixed nitrogen released from algal cells would then become available at times of the rice N requirements, i.e., tillering and panicle initiation of the planted crop. Moreover, rice inoculation techniques are usually implemented immediately after land preparation, so techniques to improve survival of inocula such as a reduction of grazer pressure, need implementation at the same time.

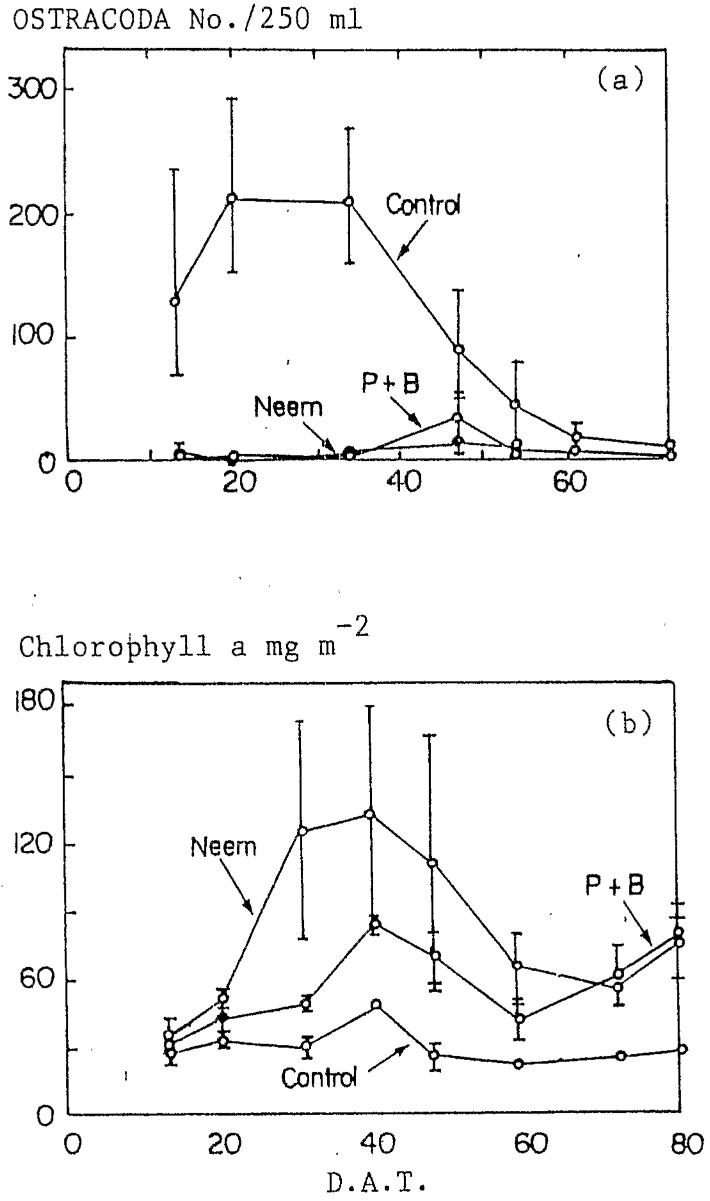
EFFECTS OF CONTROLLING GRAZING

In field experiments conducted at the International Rice Research Institute (Grant *et al.*, 1983b), grazing by Ostracoda was arrested using Perthane or crushed neem seeds (*Azadirachta indica*). The active principals in neem seeds reduce ostracod feeding and growth (Grant *et al.*, 1984). Molluscs, the other dominant grazer of BGA at this site, were controlled with Bayluscide. Four applications of these control agents were made at two week intervals from transplanting. Populations of ostracods (Fig. 2a) and molluscs were effectively controlled until ripening stage by neem and Perthane + Bayluscide (P + B) applications. Population densities of ostracods rapidly increased in control plots.

Heterocystous BGA were dominant in all plots from transplanting to 59 days after transplanting (DAT) when chlorophytes succeeded. Throughout the cultivation cycle, chlorophyll a concentrations were significantly higher in those treatments which reduced ostracod populations (Fig. 2b). *Nostoc* sp. and *Anabaenopsis* sp. were characteristic of the plots with lowered grazer pressure and blooms of these algae lasted about 30 days. From 50 DAT onwards the dominant BGA were mucilagenous colonial strains of *Gloeotrichia*, *Nostoc* and *Aphanothece*. Early in the cultivation cycle, the blooms of algae in neem and P + B treatments fixed significantly higher amounts of N (as measured by ARA, Fig. 2c), than the control (10 times less). As a result of higher N_2-fixation rates attributed to ostracod population control, grain N was significantly increased by 37% and 24% compared with the controls using neem and P + B respectively. It was noted in the case of neem treatments, where *Anabaenopsis* bloomed at 31, 59 and 72 DAT in replicates, that grain yield and grain N was highest in that replicate which bloomed 31 DAT. It was speculated that algal N available at this stage contributed to yield increases whereas later blooms could only contribute to successive crops.

The application of neem seeds or Perthane + Bayluscide therefore extended the length and increased the intensity of the BGA blooms and hence the N_2-fixation capacity. (cf with natural conditions in Fig. 1).

These results, particularly with neem, which is a cheap alternative to


OSTRACODA No./250 ml
(a)
300
200
100
0
0
20
40
60
Control
P + B
Neem
Chlorophyll a mg m^{-2}
(b)
180
120
60
0
0
20
40
60
80
Neem
P + B
Control
D.A.T.

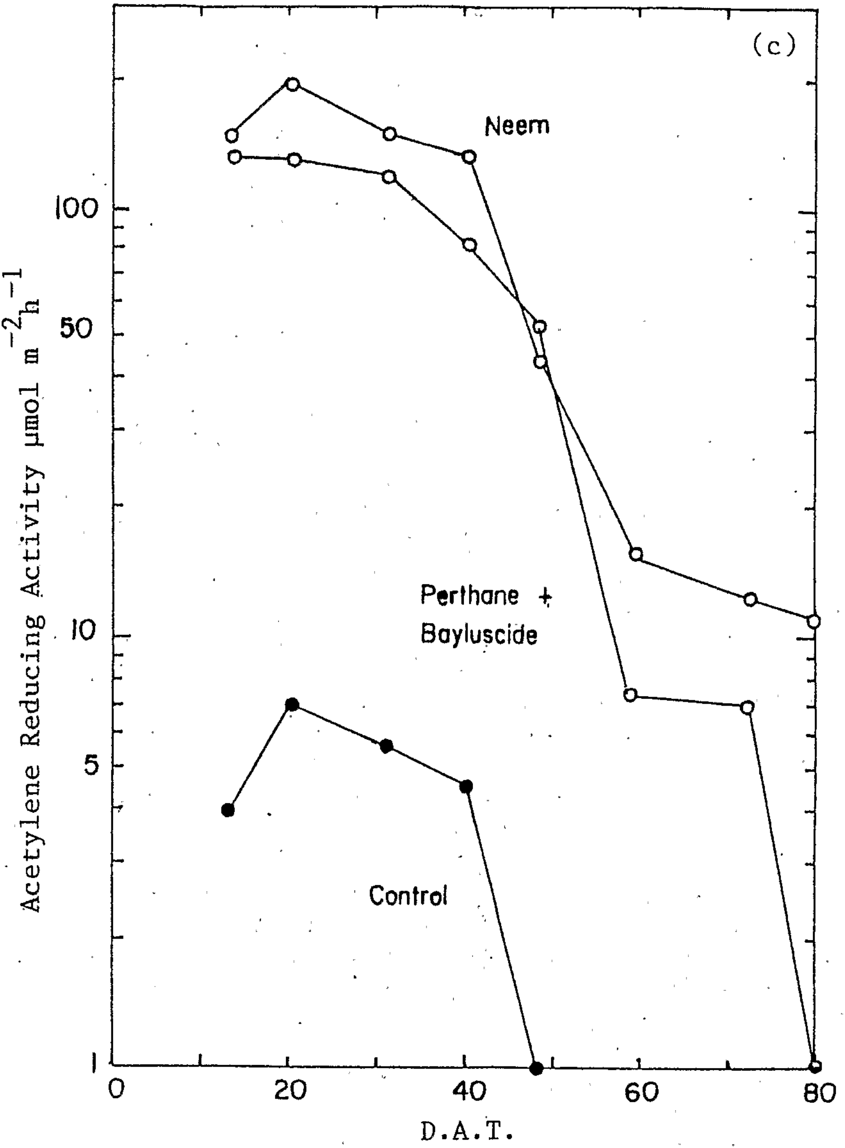


FIGURE 2 a) Mean density of ostracods (±S.E.), b) Mean chlorophyll concentrations and c) sliding averages of total ARA. (Floodwater+soil surface ARA) in three treatments. DAT=Days after transplanting.

TABLE 1

Nitrogen balance after five crops

Treatment	mg N					
	Crop	Initial soil	Final soil	N inputs	N balance[a]	N gain as % of crop N
Neem seed	421±39	4215± 65	4444±49	40	609^{a}	144
Neem cake	403±48	4257± 43	4218±82	82	280^{bc}	69
Control	382±13	4369±138	4238±89	0	251^{bc}	65
Neem seed, black cloth	249±21*	4219± 64	3988±46**	40	22^{c}	7.4

[a] Mean±S.E. Means followed by the same letter are not significantly different at 5% level
*, ***Significant at 5 and 1% level*

conventional pesticides, were encouraging, but it was not certain that reduction of grazing and thus increased N_2-fixation by BGA was responsible for the increases in rice protein. A N balance study by Grant *et al.* (1984) quantified the amount of N_2 fixed biologically by BGA as a result of one application of neem. Pots containing flooded soil were inoculated with 20 ostracods and a soil slurry containing BGA. Crushed neem seed and powdered neem cake (at 100 and 200 kg ha^{-1} respectively) were administered to control ostracod growth. A neem seed treatment with a black cloth which covered the pot prevented photodependent N_2 fixation. The difference between initial and final soil N content plus rice crop N uptake is the N_2 balance, any N inputs such as N in neem, having been subtracted. After 5 crops a large and significant N balance was obtained in neem seed treated plots (Table 1). This was ascribed to increased N_2 fixation by BGA as seen from that small balance obtained in the absence of light (i.e. heterotrophically). Crop N uptake was similar between treatments except when BGA were excluded. Thus the large N surplus accrued in neem seed treated soils was either unavailable to the rice (immobilised) or was due in part, to less N_2 loss. The latter is possible as neem is a known nitrification inhibitor (Bains *et al.*, 1971). Neem cake was not effective in producing an N surplus.

GRAZER CONTROL WITH INOCULATION

A field experiment in the Philippines again employed neem to control ostracod grazing while an algal inoculum, composed of 2 fast-growing and efficient N_2-fixing strains, were broadcast onto experimental plots. In order to allow the algae to establish while the grazers were suppressed, an application of neem was made at the beginning of the experiment only a few days before

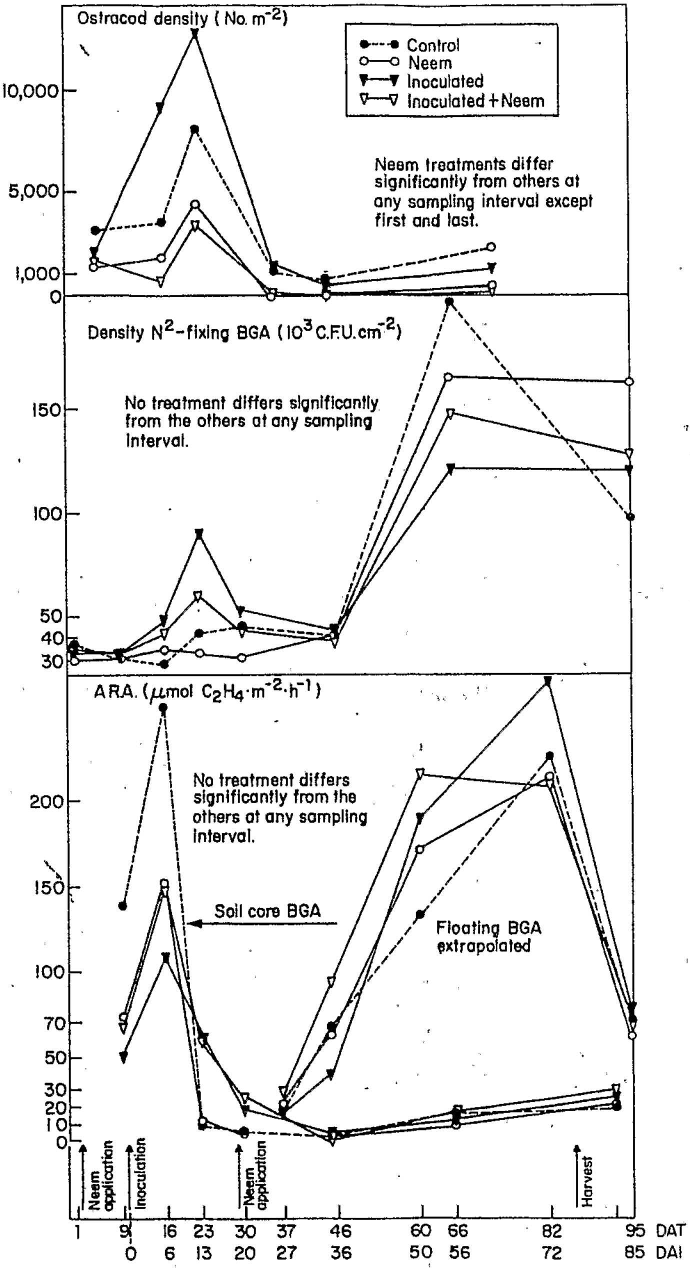


FIGURE 3 Ostracod density, density of BGA and ARA in algal inoculation trial. DAT = days after transplanting. DAI = days after inoculation.

the powdered algal inoculant was administered. Plots were treated with 1) a combined *Tolypothrix tenuis* and *Nostoc* sp. inoculum; 2.5 kg dry wt. ha^{-1}, 2) crushed neem seeds, 3) inoculum + neem seeds and 4) a control receiving no treatment. All received P as 5 kg KH_2PO_4 ha^{-1}.

Ostracods reached their maximum density in inoculated plots (Fig. 3). Both neem treatments contained significantly less dense populations of ostracods than the control and inoculated plots. All ostracod populations crashed 4 weeks after inoculation, presumably as food became exhausted.

Floodwater and soil samples were plated upon a N-free agar growth medium. Figure 3 shows the density of colony-forming units of N_2 fixing BGA (CFU cm^{-2}) from transplanting to post harvest drying of the field. There were no significant differences of CFU between treatments and the tendency towards more CFU in inoculated and inoculated + neem treatments just 2 weeks after inoculation was not due to inoculated strains. Inoculated strains were not recorded. Coinciding with the ostracod population crash one month after inoculation was an increase in CFU of *Nostoc* spp. evidently derived from floating mucilagenous strains which are highly resistant to grazing. This floating biomass consisted of *Gloeotrichia*, *Nostoc* and *Aphanothece* and no differences in their weight between treatments were evident. Twelve to 15 t ha^{-1} were recorded (fresh wt), but this was only equivalent to 2.7 kg N ha^{-1}.

The characteristic early burst of N_2-fixing activity corresponding to the fast growth of indigenous strains of *Anabaena* (Fig. 3) occurred in all treatments. Significant differences in ARA were not apparent. The second peak (Fig. 3) is extrapolated from laboratory assays of harvested mucilagenous BGA and, as such, is difficult to interpret. There were no differences in grain yield or grain N between treatments.

In the second crop, neem seeds of different origin were used. As a result, better control of ostracods was achieved and ARA was doubled, but again the inocula were not responsible for the increased fixation. Rather, blooms of indigenous *Anabaena* sp. were identified as responsible.

GRAZER CONTROL AND ALGAL INOCULATION IN SUSTAINABLE AGRICULTURE

Sustainable agriculture usually requires low cost inputs and practices. Application of the seeds of *Azadirachta indica* provided control of ostracod grazing and permitted increases in algal biomass, N_2 fixation and grain N. However, variation in the effectiveness of neem seeds according to origin and storage conditions has been reported (Schumtterer & Zebitz, 1984), and this was considered responsible for a less than satisfactory control of ostracods in the first inoculation experiment. The efficiency and economy of neem in increasing BNF may be maximised by proper timing of applications to coincide with transplanting or broadcasting of rice and phosphorus application. In the field, neem will adequately control ostracod populations for up to 18 days.

Grazing of BGA by molluscs assumes importance if land preparation is done much in advance of transplanting and P application, or if floodwater is obtained from irrigation canals. As much sustainable rice agriculture relies upon rain, simple cultural procedures such as planting and P application soon after ploughing can alleviate the problem of grazing from molluscs. Alternatively, the seeds of berries of *Phytolacca dodecandra*, *Croton tiglium*, *Jatropha curcas* (McCullough *et al.*, 1980) and crushed cashew nut shells (*Anacardium occidentalis*) are also molluscicidal (Webbe & Lambert, 1983) and offer economical substitutes for expensive commercial preparations.

Plants having pesticidal properties are frequently cheap and may be free if grown by the farmer. Many plants such as neem and those listed with molluscicidal properties are frequently available in rice growing areas. Extracts of seeds and leaves are often more efficient and effective than the addition of whole plant parts, and they may be made with simple equipment. When grazer control measures were extended over 6 weeks from transplanting the short-lived BGA population normally observed early in the rice cultivation cycle was extended over a 40 day period. Repetition of treatments to gain longer control of grazers and more intense BGA growth was costly but resulted in grain N increases unlike simple applications of neem. Control measures administered only once (inoculation trials) at transplanting showed a positive effect upon N_2-fixation but the increase was only a small proportion (5%) of the total N_2-fixing activity over the whole rice cropping cycle (e.g. 15–50 kg N ha^{-1}, Koyama & App, 1979). Control measures maintained over 6 weeks increased the proportion of N_2-fixed to approximately 10% of the total N fixed per crop. Under stable conditions of the greenhouse, the N balance experiment showed that N fixed as a result of a short period of grazer control was considerable (144% N gain as % of crop N), but much of the N which accrued in the soil was not used by the crop. The N balance experiment, clearly showed the potential of BGA in submerged soil but the reasons for N immobilisation need elucidation. Furthermore, any effects of neem upon prevention of N losses by nitrification cannot be contrasted against the N gains by N_2 fixation. It is too early to make decisions using economics as the criterion for length of grazer control. Only further field trials can establish how long treatments should be and much variation is to be expected between rice fields. Whether such manipulative practices are economically feasible may depend upon the region.

Another approach to grazer control is the development of grazer-resistant algae. It has been noted already that many mucilagenous BGA strains have extensive resistance to invertebrate grazers. However, the mucilagenous strains are slow growing and thus characterise the later stages of rice cultivation. Their biomass may be high; up to 2.4 kg m^{-2} (wet weight) has been reported in the Philippines, but this is exceptional and moreover only represents a small amount of N (viz. 2 g N and 0.7 g P m^{-2} as N = 3% and P = 1% of dry wt. of alga: IRRI, 1983). Mucilagenous strains generally show much less ARA than the fast-growing non colonial BGA typical of the early part of rice cultivation. Strains which have doubling time of 8 h are on record (Chen, 1983) but very few of the faster growing and efficient N_2 fixing strains have shown resistance to ostracod grazing, and those which have were of temperate origin (Grant, unpublished). Screening for resistance is continuing because a successful candidate would be a better alternative economically, than natural pesticide control methods. Nevertheless, the problems associated with nurturing algae to establishment are apparently great and maintenance of a bloom difficult because other grazers gradually exploit the available energy source. A better knowledge of the soil/floodwater ecosystem would increase the chances of successful inoculation, but there has been little advance in our understanding of the ecosystem since attention was drawn to this scarcity of information by Roger & Kulsaooriya (1980).

Inoculation of non-indigenous strains of BGA was unsuccessful. As ostracods rapidly attain high densities soon after land preparation, they threaten the use of an algal inoculation technology. It was not established whether ostracods were responsible for the growth failure of the algal inoculant administered; for although ostracod densities were greater in inoculated plots these may have been sustained by the increased growth of indigenous flora as indicated by the number of CFU in these plots. Decomposition of inocula liberates nutrients which are probably utilised by indigenous flora. Survival of an inoculum is dependent upon many environmental factors and as such, successful introduction of non-native strains of BGA is troublesome if not impractical. It may be easier to encourage growth of indigenous N_2-fixing BGA which, by all accounts, are widespread geographically and present in many soil types.

The practices of algal inoculation and enhancement of indigenous BGA, are both in the experiment stage of development. Although many inoculation trials have been reported, none have shown conclusively that the algae inoculated was responsible for either the ensuing bloom (if present) or measured yield increases (if any). According to Venkataraman (1972) the cost of producing inoculum in plots is low but the cost of nurturing the inoculum once in the field will be additional if soil amendments and grazer control is necessary. Encouraging indigenous strains to grow or increase their biomass in the field dispenses with inoculum production. Still, the greatest obstacles to both techniques are not knowing what factors influence bloom formation and what makes a certain inoculum competitive in a foreign environment. Nevertheless, BGA already constitute a N source in neutral and alkaline soils and efforts to increase the range of soil types in which BGA activity can be intensified should be pursued.

SUMMARY

An introduction to the soil/floodwater ecosystem of lowland rice fields is given. Two primary consumers are particularly important in limiting the growth and N_2-fixing activities of blue-green algae in irrigated rice; the Ostracoda (Class Crustacea) and the Pulmonata (Mollusca). Control of grazing by neem seeds *Azadirachta indica* A. Juss and cultural practices enhanced BGA biomass and increased N_2-fixation ten fold. Significant increases in rice grain protein occur if heterocystous algae bloom early in the rice cultivation cycle and grazing control is maintained over 40 days. A large positive N balance was obtained over 3 rice crops by using neem seeds to control grazing of BGA. Algal inoculants used in conjunction with grazer control failed to establish themselves, and factors other than grazing were considered responsible. Plant-derived pesticides showed great promise for sustainable agriculture.

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