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Dynamics of algal populations and acetylene-reducing activity in five rice soils inoculated with blue-green algae

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Summary. The dynamics of five inoculated strains of heterocystous blue-green algae (BGA) and indigenous algae were studied for 1 month in 1-m^2 microplots of five soils previously air-dried or oven-dried. The same soils were then dried and resubmerged for another 2 months to study the effect of controlling algal grazers with neem (*Azardirachta indica*) seeds on the revival and dynamics of indigenous and inoculated algae. During the month following inoculation, inoculated BGA multiplied to some extend in all soils but never dominated the total algal flora. They rarely dominated the indigeneous heterocystous BGA, and did so only when the growth of N_2-fixing BGA was poor or after the decline of blooms of indigenous strains. Once the soils were dried, two of the five inoculated strains did not reappear. During the 1st month following rewetting, the remaining inoculated strains again exhibited poor growth; however, after 2 months of submergence, inoculated *Aulosira fertilissima* developed an agronomically significant bloom in neem-treated plots of two soils. Correlations between acetylene-reducing activity and heterocystous BGA populations indicated a major contribution by indigenous BGA and a minor contribution by inoculated BGA to the N_2-fixing activity of the soils during the first experiment and the 1st month of the second experiment. The establishment of inoculated BGA exhibited clear differences among strains but was less affected by the nature of the soil and heat treatment. Neem application might have had a delayed positive effect on the late establishment of inoculated *A. fertilissima* and favored BGA growth and N_2 fixation by the total algal population.

Key words: Blue-green algae – Cyanobacteria – Rice – Inoculation – Neem seeds – *Azadirachta indica*

BGA are widely distributed in nature and form a prominent component of autotrophic microbial populations of wetland soils. A number of BGA fix atmospheric N_2 and contribute to the fertility of rice fields. In 1939, De suggested their use as biofertilizer in rice production. Since then, many investigations have been conducted to enhance N_2 fixation in wetland rice fields by inoculation with BGA (Venkataraman 1972; Roger and Kulasooriya 1980; Venkataraman 1981). BGA inoculation has not increased yields consistently. In most experiments, the only variable measured was grain yield; therefore, reasons for the presence or absence of a yield increase after algal inoculation are still poorly understood (Roger and Kulasooriya 1980). So far, no concerted attempt has been made to evaluate the fate of inoculated BGA and assess their influence on N_2 fixation in wetland rice soils.

We studied the fate of five strains of heterocystous BGA simultaneously inoculated into five soils. In the first experiment, we compared the dynamics of indigenous and inoculated algae in soils previously air-dried or oven-dried. Heat treatment was performed to reduce the indigenous algal population and to assess whether such a reduction favors the establishment of inoculated algae.

Because invertebrate populations that feed on algae have proved to be a major factor limiting BGA growth, in the second experiment we tested the effect of controlling algal grazers with a pesticide of plant origin (*A. indica*) on the dynamics of indigenous and inoculated algae in the soils used in the first experiment.

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Materials and methods

Soils. Five soils were chosen to represent a range of physicochemical properties prevailing in the Philippines (Table 1); an acidic peat

A mixture of dry soil-based inocula produced on Maahas soil and containing non-indigenous *Anabaena variabilis, Tolypothrix tenuis, A. fertilissima, Fischerella* spp. (IRRI strain B10) and

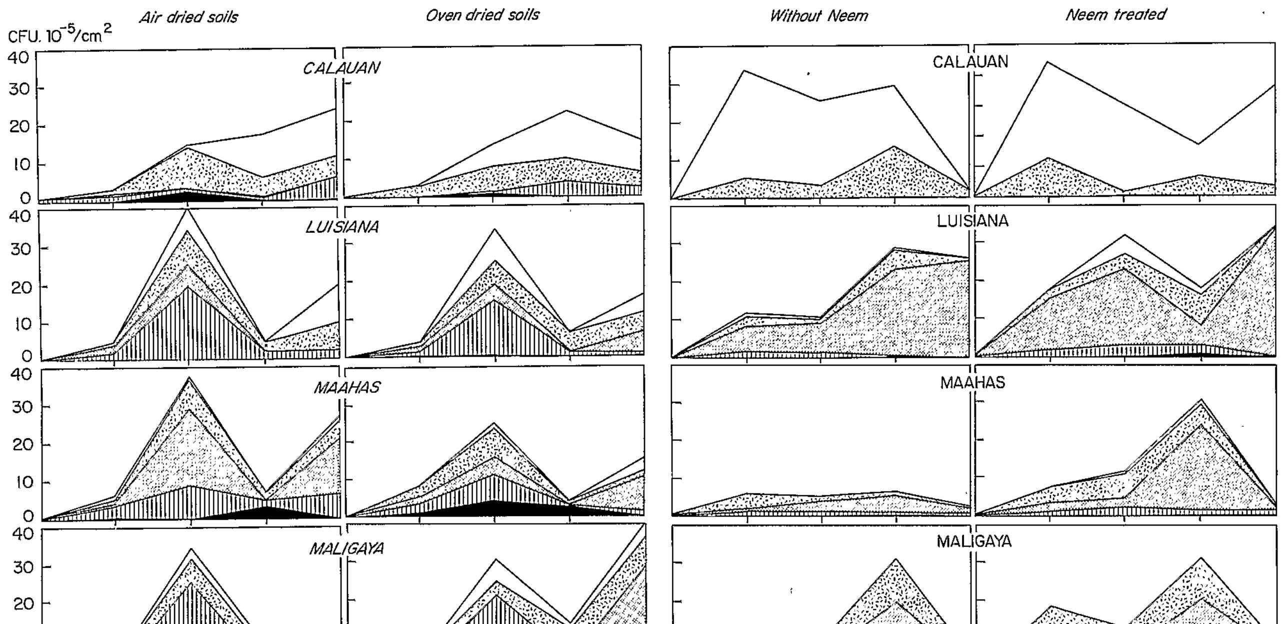
Air dried soils
Oven dried soils
Without Neem
Neem treated
CFU. 10^-5/cm^2
40
30
20
10
0
CALAUAN
LUISIANA
MAAHAS
MALIGAYA

Table 2. Counts of inoculated (Inoc.) and indigenous (Indi.) heterocystous BGA, and dominant strains, species or genera in air-dried (AD) and oven-dried (OD) soils)

Soil		Day 7		Day 14		Day 21		Day 28	
		Inoc.	Indi.	Inoc.	Indi.	Inoc.	Indi.	Inoc.	Indi.
		Counts (10^3 CFU/cm^2)							
Calauan	AD	2	107	*237*[a]	90	1	25	7	551
	OD	2	6	*105*	37	2	410	1	240
Luisiana	AD	10	163	6	1947	9	200	1	220
	OD	43	111	39	1467	6	116	3	85
Maahas	AD	43	293	22	860	*309*	150	1	617
	OD	107	234	429	710	*227*	59	7	118
Maligaya	AD	20	135	4	2543	*70*	5	7	24
	OD	10	79	3	2103	*337*	100	4	470
Tiaong	AD	4	200	6	399	37	138	10	33
	OD	3	34	3	272	1	43	*33*	10
		Dominant strains[a, b]							
Calauan	AD	Tt	N (g)	*N (SL)*	A	F	N (s)	Tt	A
	OD	Tt	N (g)	*Av*	A	Af	A, N (g)	F	A
Luisiana	AD	A, Tt, F	N (g)	Tt	N (g)	Tt	N (g, p)	Tt	N (p)
	OD	F	N (g)	Av	N (p)	Tt	N (p)	Tt	N (g)
Maahas	AD	Av	N (g)	Tt	N (g)	*Av*	N (g)	Af, Tt	N (g)
	OD	F	N (g)	N (SL)	N (g)	*Av*	N (g)	Tt/F	N (g)
Maligaya	AD	Tt	N (g)	F	N (p)	*N (SL) Tt*	N (g)	Tt	N (s)
	OD	Tt	N (g)	Tt	N (p)	*N (SL)*	N (s)	Tt	N (p)
Tiaong	AD	F	N (g)	Af	N (g)	Av	N (g, p)	*Tt*	N (s)
	OD	F	N (g)	Tt	N (g)	F	N (p)	*Tt*	N (s)

[a] Values and abbreviated names are in italics when inoculated strains are more numerous than indigenous ones; [b] Abbreviations: inoculated strains: Av, *Anabaena variabilis*; Af, *Aulosira fertilissima*; F, *Fischerella* spp. B10; N (SL), *Nostoc* spp. (Sri Lanka); Tt, *Tolypothrix tenuis*; indigenous strains: A, *Anabaena* spp.; N, *Nostoc* spp. Letters in parentheses refer to morphological features of colonies on GO medium: g, globose; p, pinhead; s, spread

Table 3. Counts of inoculated (Inoc.) and indigenous (Indi.) heterocystous BGA and dominant strains, species or genera in soils treated (n+) and non-treated (n−) with neem

Soil		Day 7		Day 14		Day 21		Day 28	
		Inoc.	Indi.	Inoc.	Indi.	Inoc.	Indi.	Inoc.	Indi.
		Counts (10^3 CFU/cm^2)							
Calauan	n−	1	7	1	1	1	2	1	1
	n+	2	2	3	38	1	5	*3*	1
Luisiana	n−	0	141	3	93	*33*	4	1	11
	n+	0	167	3	257	67	210	1	19
Maahas	n−	0	104	1	99	4	62	1	40
	n+	0	100	1	204	23	101	3	123
Maligaya	n−	2	23	34	76	*33*	2	2	6
	n+	0	107	37	93	*33*	7	3	34
Tiaong	n−	1	94	1	101	0	50	0	40
	n+	1	176	1	334	35	280	1	100
		Dominant strains[a]							
Calauan	n−	Tt	N (g)	Tt	N (g/s)	Tt	N (g)	Tt	N (g/s)
	n+	Tt	C	Tt	N (g)	Tt	N (s)	*Tt*	N (g)
Luisiana	n−	–	N (s)	Tt	N (g/s)	*Tt*	N (s)	Tt	N (g)
	n+	–	N (p)	Tt	G	Tt	N (s)	F	N (g)
Maahas	n−	–	N (s)	Af/F	N (g/s)/A	F	N (g)	Tt	N (s)
	n+	–	N (g)	Af/Tt	N (g)	Tt	N (s)	Tt	N (s)
Maligaya	n−	Tt	N (s)	Tt	N (g)	*Tt*	N (s)	Tt	N (s)
	n+	–	N (g)	Tt	N (g)	*Tt*	N (g/s)	Tt	N (s)
Tiaong	n−	F	N (g)	F	N (s)		N (g)		N (g)
	n+	F	N (p)	Tt/F	N (s)	F	N (g)	Tt/F	N (s)

[a] Abbreviations: C, *Cylindrospermum* spp.; G, *Gloeotrichia* spp.; others as in Table 2

richer in organic matter. In the other three soils, heterocystous BGA comprised a significant percentage of the total algal population. They became dominant by about the 14th day of submersion in the Maligaya and Luisiana soils.

In most cases, indigenous heterocystous BGA were more numerous than inoculated BGA. The CFU ratio of indigenous to inoculated heterocystous BGA ranged from 0.1 to 840 and averaged 104. Only in 7 out of 40 cases were inoculated heterocystous BGA more numerous than indigenous heterocystous BGA (Table 2). This was observed with no growth or with late growth of indigenous heterocystous BGA (Tiaong and Calauan soils), or after the decline of a bloom of indigenous heterocystous BGA (Maahas and Maligaya soils). The general trend in all soils was a low to moderate establishment of inoculated heterocystous strains which did, however, attain densities higher than in the initial inoculum. This indicated that inoculated strains multiplied in all soils. The maximum growth of inoculated strains was either similar (Calauan, Maahas and Tiaong) or better (Luisiana, Maligaya) in oven-dried soils than in air-dried soils (Table 2).

Among the 40 observations made, *T. tenuis* was the most abundant inoculated strain in 46% of cases, followed by *Fischerella* spp. (22%), *A. variabilis* (13%), *A. fertilissima* (9%) and *Nostoc* spp. SL (9%). However, where inoculated strains developed significantly, exhibiting densities higher than 5×10^4 CFU/cm^2, *Nostoc* spp. SL and *A. variabilis* were the dominant inoculated strains, as observed in Calauan, Maahas and Maligaya soils. Among indigenous heterocystous BGA, *Nostoc* spp. were dominant in 36 cases and *Anabaena* spp. in 4 cases.

Second experiment. Neem application reportedly favors algal growth in wetland soils (Grant et al. 1985). However, growth enhancement by neem was conspicuous only in the Maahas soil (Fig. 1). The dynamics of algal populations were similar after both treatments for the other soils. In the Tiaong soil, the relative importance of the algal groups differed.

As in the first experiment, eukaryotic algae developed better in the Calauan and Tiaong soils. Together with homocystous BGA, they dominated the algal populations in these two soils, while unicellular and homocystous BGA dominated in the other soils.

Homocystous BGA grew well in all soils, contributing a significant percentage to the total algal population. Their growth did not seem to be affected by the application of neem except in the Tiaong soil where homocystous BGA showed a delayed and enhanced growth in neem-treated plots. Unicellular BGA showed no or little relative growth in the Calauan and Tiaong soils but were dominant in other

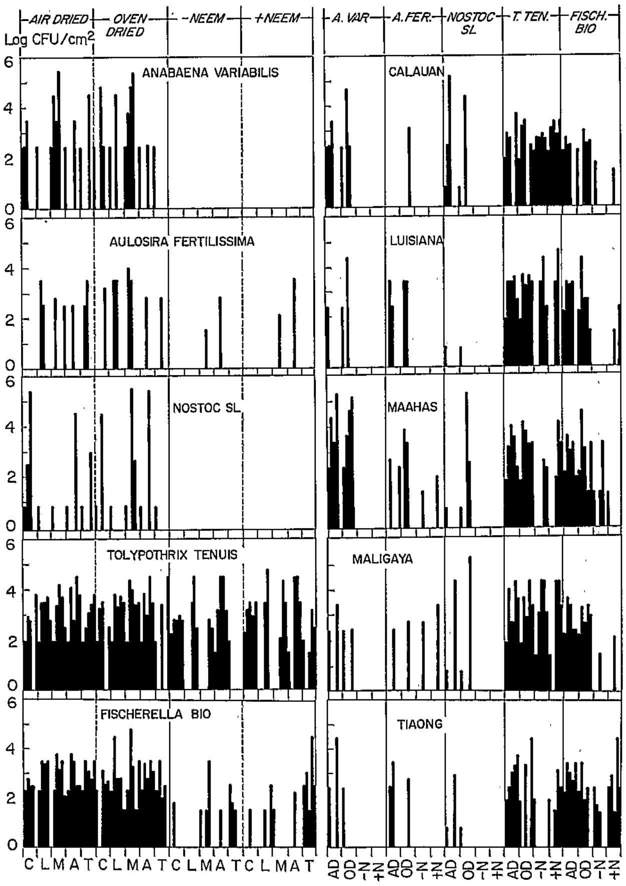

Fig. 2. Fate of inoculated blue-green algae in different soils during first and second growth cycle. Soils: *C*, Calauan; *L*, Luisiana; *M*, Maahas; *A,*, Maligaya; *I,*, Tiaong. Treatments: *AD,*, air-dried; *OD,*, oven-dried; $-N$, without neem; $+N$, with neem

soils. In the Maahas soil, their growth was better in the neem-treated plots than in the controls.

Heterocystous BGA never became dominant. Their growth was poor in all soils but better in neem-treated plots than in the controls (Fig. 1). Their growth was markedly lower than during the first experiment, but, as for the first experiment, indigenous strains were usually more abundant than inoculated strains (Table 3). *Nostoc* spp. were usually dominant among indigenous heterocystous BGA. However, *Cylindrospermum* spp. became dominant by the 7th day in neem-treated plots of Calauan soil and *Gloeotrichia* spp. by the 14th day in neem-treated plots of Luisiana soil. Only on 4 out of 40 occasions were inoculated heterocystous BGA more abundant than the indigenous heterocystous BGA, and only after the decline of the indigenous populations of heterocystous BGA. Among BGA inoculated in the soils during the first experiment, *T. tenuis*, *Fischerella* spp. B10 and *A. fertilissima* continued to appear while *A. variabilis* and *Nostoc* spp. SL disappeared (Table 3). *T. tenuis* was recorded as the most abundant inoculated strain in

65% of the cases and *Fischerella* spp. B10 in 17% of the cases. In 18% of the cases, no inoculated strain was recorded.

Establishment of inoculated algae

A summary of the counts of inoculated BGA in the two experiments (Fig. 2) according to strains, soils and treatments showed no clear effect of oven-drying of soil on the establishment of inoculated BGA. Soils

oculation, developed and persisted during the second experiment. *A. variabilis* and *Nostoc* spp. SL attained high densities during the first experiment but did not appear during the second experiment.

Acetylene-reducing activity

The dynamics of acetylene-reducing activity were similar in air-dried and oven-dried soils, with a maximum at 14 days, except in the Calauan soil (Fig. 3). On

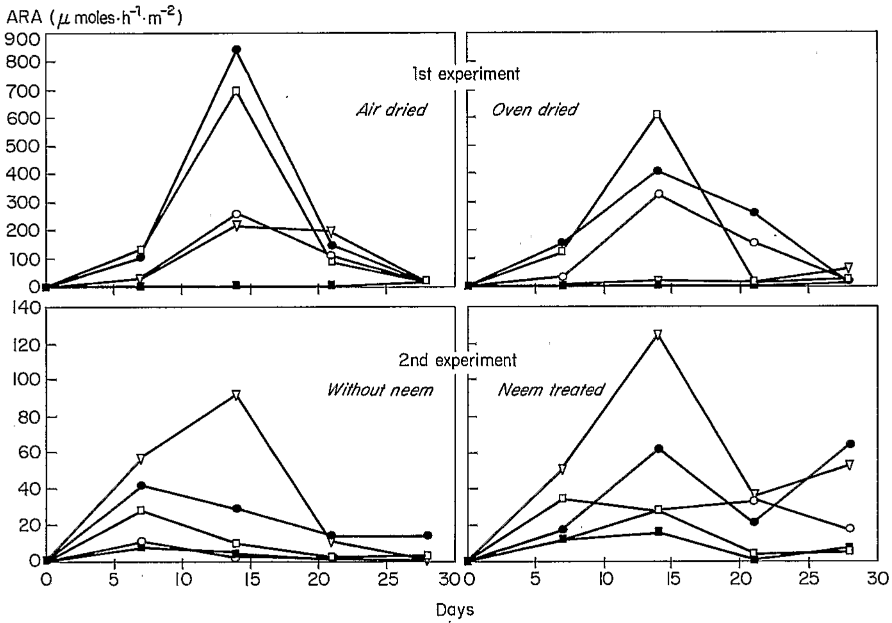

Fig. 3. Dynamics of acetylene-reducing activity (ARA) in air-dried, oven-dried, neem and non-neem treated soils. ■, Calauan; ○, Luisiana; ●, Maahas; □, Maligaya; ▽, Tiaong

Table 6. Identification and average biomass (g/m^2) of dominant blue-green algae (BGA) and green algae after 2 months of second growth cycle

Soil	Replication	N_2-fixing BGA		Green algae	
		Neem −	Neem +	Neem −	Neem +
Calauan	1, 2	None	None	Clado	Clado
	3	None	Cylin	Clado	Clado
	4	None	Anab	Clado	None
Aver. d.w.		0.0	17.7	20.9	21.3
Luisiana	1−3	Glo	Glo	Chara	Chara
	4	Glo	Glo	Chara	Chara/fil gr
Aver. d.w.		21.1	26.4	4.6	5.2
Maahas	1	Glo	Glo/Apha	Chara	Chara
	2	Glo	Glo/Apha	Chara	Chara
	3	Apha	None	Chara	Chara
	4	Glo/Apha	Glo/Apha	Chara	Chara/fil gr
Aver. d.w.		4.5	16.5	12.3	8.9
Maligaya	1	Apha/Glo	Aulo/Apha	Chara	Chara
	2	Apha	Aulo/Apha	Chara	Chara
	3	Apha	Aulo/Apha	Chara	Chara
	4	Apha/Glo	Aulo/Apha	Chara	Chara
Aver. d.w.		5.9	5.5	11.4	15.8
Tiaong	1−4	None	Aulo	Clado	Clado
Aver. d.w.		0.0	45.4	6.7	8.6
Average		6.3	22.3	11.2	12.0

Abbreviations: Anab, *Anabaena variabilis*; Apha, *Aphanotheae*

BGA but not with the counts of inoculated BGA. This indicated that acetylene-reducing activity arose principally from indigenous BGA. However, correlation coefficients were generally higher with total (indigenous+inoculated) heterocystous BGA than with indigenous heterocystous BGA (Table 5), which indicates a contribution by inoculated BGA.

Unicellular BGA grown in N-depleted BG11 medium are known to fix N_2 (Singh 1973). However, their counts did not correlate with acetylene-reducing activity. Taking them into account together with heterocystous BGA rendered correlations between acetylene-reducing activity and algal counts non-significant or less significant, which indicated very little or no contribution to acetylene-reducing activity which indicated very little or no contribution of acetylene-reducing activity by this algal group.

Algal biomass after 2 months of submersion

The study of the nature and biomass of algae after an additional month of submersion at the end of the second experiment showed a higher BGA productivity in neem-treated plots (Table 6). Visual observations indicated a lower incidence of grazers in neem-treated plots. This favored the growth of the BGA that were susceptible to grazing but had no effect on green algae, which were mostly macrophytic and not susceptible to grazing by the invertebrate populations present in the developed in neem-treated Maligaya and Tiaong soils. In the Tiaong soil it became dominant and produced blooms corresponding to an average biomass of 450 kg dry weight per ha (35 kg N/ha), which was the highest algal biomass recorded in a plot during this experiment.

Establishment of algae was generally consistent among replicated plots of Luisiana, Tiaong and Maligaya soils and, to a lesser degree, in the Maahas soil. By contrast, variability was high in the Calauan soil, with two plots showing no BGA growth, one with a bloom of *Cylindrospermum* spp. and one with a bloom of *Anabaena* spp.

Conclusion

Our results show that the establishment of inoculated non-indigenous strains was infrequent in the rice soils studied. This agrees with the results of Grant et al. (1985) indicating no significant difference in the number of N_2-fixing BGA in field plots inoculated with non-indigenous strains and in non-inoculated plots, and the failure of inoculated BGA to become established, even in plots where grazers were controlled. Our results also confirm that neem application has a beneficial effect on the photodependent N_2-fixing activity of soils, as reported earlier by Grant et al. (1985). However, the longer persistence of some of the strains and the late establishment of blooms of inoculated *A. fertilissima* in plots of two of the soils treated with neem, where this strain developed the highest BGA biomass recorded in the experiment, indicate some potential for inoculation of rice soils with foreign strains.

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