EPIPHYTIC NITROGEN FIXATION ON WEEDS IN A RICE FIELD ECOSYSTEM

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ABSTRACT

Epiphytic N_2 fixation on submerged (Chara *sp.*, Najas *sp.) and non-submerged* (Monochoria *sp.*, Cyperus *sp.) weeds in a paddy field was studied by:*

- *evaluating the weed biomass in planted and fallow fields;*
- *measuring specific dark and light-dependent acetylene reducing activity (ARA);*
- *enumerating and identifying epiphytic N_2-fixing micro-organisms.*

Submerged weeds produced a mean biomasses of 1 t ha^{-1} at rice tillering and 3 t ha^{-1} at rice harvest stage; under fallow they reached 7.5 t ha^{-1}. Corresponding biomasses of non-submerged weeds were 1.7 t ha^{-1} under rice and 7.7 t ha^{-1} under fallow at rice harvest stage.

Dominant N_2-fixing Cyanobacteria were Gloeotrichia sp., Nostoc *spp. and* Calothrix *spp. Epiphytism by* Gloeotrichia *was predominantly on* Chara *whereas that by other Cyanobacteria did not exhibit any host selectivity. Submerged weeds harboured both aerobic and micro-aerophilic N_2-fixing bacteria. Growth on glucose medium showed the presence of acid-gas-producing organisms (probably Enterobacteriaceae), while growth on malate revealed* Azospirillum*-like organisms. Light ARA on the submerged weeds (29-35 nmoleC_2H_4 (g fresh weight)$^{-1}$ h^{-1}) was about ten times higher than that on the non-submerged ones (1.8-4.4 nmoleC_2H_4 (g fresh weight)$^{-1}$ h^{-1}). Dark activity was about the same for all the weed types studied (0.9-2.5 nmoleC_2H_4 (g fresh weight)$^{-1}$ h^{-1}). Relating specific ARA to weed biomass measurements it was found that the non-submerged weeds exhibit a very low activity (0.4-2.3 g N ha^{-1} d^{-1}) while the activity on submerged weeds (5-34 g N ha^{-1} d^{-1}) makes an appreciable N_2 input into this ecosystem.*

INTRODUCTION

Biological nitrogen fixation contributes significantly to the fertility of rice soils. Microorganisms operative in this process and their spatial distribution within a rice field ecosystem are illustrated in Fig. 1. Studies have been conducted in most of the components depicted in this Figure and have been recently reviewed by Dommergues & Rinaudo (1979) on the rhizosphere; by Matsuguchi (1979) on heterotrophic bacteria; by Roger & Reynaud (1979) and Venkataraman (1979) on the blue-green algae (BGA), and by Watanabe (1978) and Becking (1979) on *Azolla*. There are a few reports on nitrogen-fixing bacteria associated with rice stems (Watanabe *et al.*, 1979; Watanabe & Barraquio, 1979) and nitrogen fixation by blue-green algae epiphytic on fresh water macrophytes (Finke & Seeley, 1978), but we are unaware of any studies on nitrogen fixation by epiphytic BGA in rice fields.

The epiphytic microflora appears to occupy an ecological niche with certain distinctive features. Being attached in a somewhat permanent submerged position, BGA are protected from dessication and inhibitory effects of high solar radiation (Reynaud & Roger, 1979). This epiphytic habit is advantageous to the heterotrophic bacteria, which may obtain nourishment from their hosts, but no nutritive association between the algae and the host plants has yet been found.

The submerged weed population in a rice field can develop into a considerable biomass (Saito & Watanabe, 1978) and the nitrogen fixed by their epiphytic microflora could then make a significant contribution to the total nitrogen input. Studies were therefore undertaken to investigate the epiphytic N_2 fixation on weeds in a rice field.

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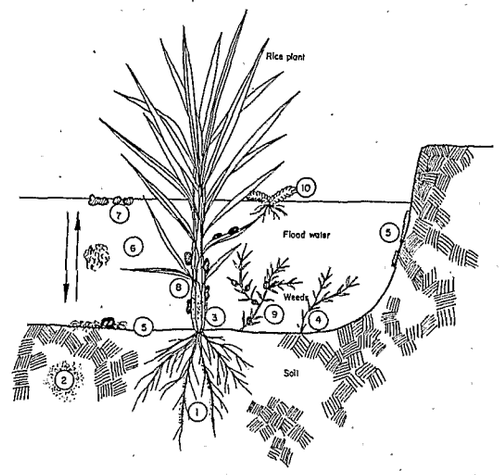

Fig. 1. Diagram of N_2-fixing components in a rice field ecosystem.

Bacteria	Cyanobacteria
1) *rhizosphere*	5) *Soil water interface*
2) *soil*	
3) *epiphytic on rice*	6) *free floating*
4) *epiphytic on weeds*	7) *water air interface*
10) Azolla	8) *epiphytic on rice*
	9) *epiphytic on weeds*

MATERIALS AND METHODS

Experimental

An experiment was conducted in 1.5 m^2 plots with four treatments in triplicate, distributed on a randomized block design. Each plot was planted separately with either submerged weeds (*Chara* sp. or *Najas* sp.) or non-submerged weeds (*Monochoria vaginalis* or *Cyperus iria*). The plots with submerged weeds were sampled by harvesting the total plant material in a plot, mixing them together and removing random triplicate 10-g samples for subsequent analysis. In the case of non-submerged weeds, the root system and the aerial parts above the flood water level were first cut off, and the remaining material was mixed together before sampling. The samples were studied in regard to their specific acetylene reducing activity (ARA) and their epiphytic algal and bacterial flora.

As it was observed that more algae were present on the older parts of submerged weeds, old and young parts were separated and aliquot samples were analyzed for ARA and N_2-fixing microflora. To extrapolate to the field from the specific ARA measurements (expressed in terms of activity per gram of host), an assessment of the weed biomass and its variability in the field was done at two stages of the growth of rice and in fallow plots.

Acetylene reducing activity

ARA measurements were conducted in 250-ml Erlenmeyer flasks, under an atmosphere of 10% acetylene in air. Incubation was done either under 800 lx provided by fluorescent lights, or in the dark by wrapping the flask with aluminium foil. Plant material destined for the dark incubation was covered *in situ* with a black cloth the day before harvesting, in order to eliminate any residual algal activity. Gas samples were removed after 0.5, 1, 2, 4, and 6 hours of incubation and analysed by gas chromatography.

Algal counts

Algae were enumerated by plating on BG II medium (Allen & Stanier, 1968) with and without combined nitrogen to estimate respectively the total and the N_2-fixing algal populations. After incubating for three weeks at 30 C under continuous fluorescent light (800 lx) the plates were observed under a stereoscopic microscope, algal colonies were identified and separately counted.

Bacterial counts

Aerobic heterotrophic N_2-fixing bacteria were enumerated by the most probable number (MPN) technique as described by Watanabe *et al.* (1979). Inoculation was done into semi-solid glucose-yeast extract medium, which usually gives higher counts than malate medium (Watanabe *et al.*, 1979) and into malate-yeast-extract medium to detect the presence of *Azospirillum* (Day & Döbereiner, 1976). After incubating for two days at 30 C, the tubes were exposed to

10% acetylene in air for 24 hours and the ethylene formed was measured. The tubes with ethylene values twice that of the uninoculated controls were considered as positive.

Total aerobic heterotrophic bacteria were enumerated by spreading on tryptic-soy (0.1%) agar (1.5%) plates (Watanabe & Barraquio, 1979). After one week of incubation at 30 C, the colonies were counted on the plates containing 30 to 300 colonies.

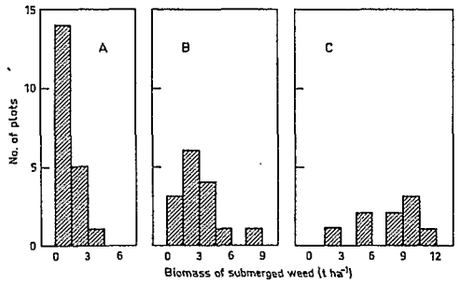

Fig. 2. Distribution of submerged weeds biomass (t ha^{-1} fresh weight) among
A: 20 plots at end of tillering; the plots had been handweeded four weeks before the measurement,
B: 15 plots at harvesting stage; no weeding was performed,
C: 9 fallow plots at harvesting stage of rice.

RESULTS

Biomass of weeds

The distribution of the biomass of submerged weeds (*Chara* and *Najas*) is shown in Fig. 2 at tillering stage, harvesting stage and in a fallow plot at harvesting stage of rice. This Figure shows that the submerged weed population under a rice crop at the end of tillering had a mean biomass of about 1 t ha^{-1} within a range of 0.4 to 3 t ha^{-1} and that it had increased at maturity to a mean of 3 t ha^{-1} within a range of 0.2 to 4.5 t ha^{-1}. The highest values, which ranged from 2.7 to 12 t ha^{-1} with a mean of 7.5 t ha^{-1}, were recorded in the fallow plots. Twenty field measurements of non-submerged weed biomass under rice cropping gave a mean value of 1.7 t ha^{-1} and a maximum of 4.1 t ha^{-1}. In fallow plots, completely covered either with *M. vaginalis* or *C. iria*, the values obtained were 7.7 t ha^{-1} and 2.8 t ha^{-1} respectively, of which about 10% was found to remain submerged. These figures give an idea of the weed biomass that is available for colonization by epiphytic microorganisms in a rice field.

Epiphytic microorganisms and their distribution

Two types of algal epiphytism, (1) visible to the naked eye and (2) observable only under the microscope, were noticed, specially in the case of the submerged weeds. In the first type, globose gelatinous colonies of *Gloeotrichia* (2-10 mm in diameter) were attached to the *Chara* filaments (Plate 1a). The distribution of the colonies on the host was frequently unequal, the older parts being more heavily colonized (Plate 1a, 1b). The second epiphytic habit, which could be seen only under the microscope, was predominantly due to *Nostoc*, *Calothrix* and *Anabaena* sp., whose filaments grew firmly attached to the host surface. Even in this case, colonization by the epiphytes became progressively higher from apex to base of the host and this was quite apparent among the young, intermediate and old leaves of *Najas*. These observations were confirmed by algal enumerations done separately on old and young parts of *Chara* and *Najas*. Table 1 shows that the total algal population on the old parts was four times that on the young parts.

Submerged weeds harboured both aerobic and micro-aerophilic N_2-fixing bacteria. Growth on glucose medium showed the presence of acid-gas-producing organisms (probably Enterobacteriaceae), while growth on malate revealed *Azospirillum*-like organisms.

Based on the MPN method the number of N_2-fixing bacteria was in the order of 10^5 cell (g fresh weight)$^{-1}$ of host. There was very little difference in the cell numbers on the different weeds and between old and young parts (Table 1) except, on old parts of *Chara*, where the N_2-fixing bacterial population was approximately three times that on the young parts.

ARA measurements in the light, carried out separately (Table 2), showed that the activity on young parts of *Chara* was much higher than on old parts, while on *Najas* both old and young parts had the same activity. ARA measurements in the dark (Table 2) also showed higher

activities on young parts, specially on *Chara*, despite the fact that the populations of N_2-fixing bacteria on the older parts were higher (Table 1).

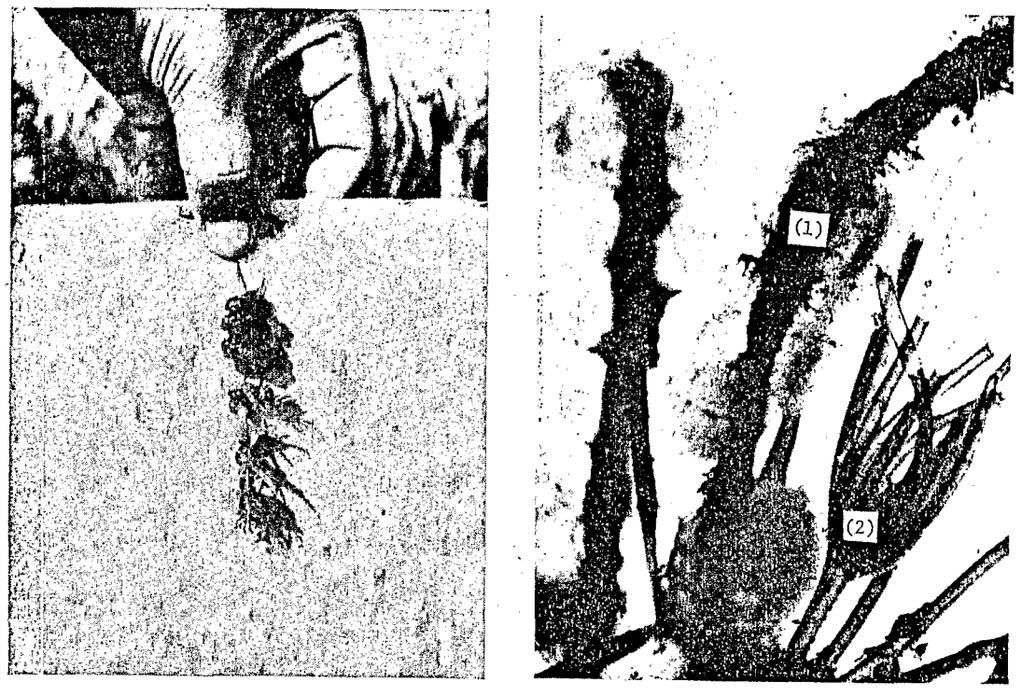

Plate 1

a) Gloeotrichia *colonies epiphytic on* Chara.

b) Gloeotrichia *epiphytism on* Chara *(x 20).*
- present on old parts: (1)
- absent on young parts: (2)

Table 1. Enumeration of epiphytic microorganisms on submerged weeds

	Chara		*Najas*	
	Old parts	Young parts	Old parts	Young parts
	(Number g^{-1} fresh weight of host)			
N_2-fixing blue-green algae	$78 \cdot 10^4$	$19 \cdot 10^4$	$13 \cdot 10^4$	$4 \cdot 10^4$
Bacteria on:				
glucose[a]	$70 \cdot 10^4$	$16 \cdot 10^4$	$6 \cdot 10^4$	$5 \cdot 10^4$
malate[b]	$40 \cdot 10^4$	$25 \cdot 10^4$	$25 \cdot 10^4$	$11 \cdot 10^4$
tryptic soy agar[c]	$140 \cdot 10^6$	$210 \cdot 10^6$	$32 \cdot 10^6$	$99 \cdot 10^6$

[a] N_2-fixing Enterobacteriaceae.
[b] N_2-fixing *Azospirillum*-like.
[c] Total aerobic heterotrophs.

Table 2. ARA[a] by epiphytes on old and young parts of submerged weeds, in the light and in the dark

	Chara		*Najas*	
	Light	Dark	Light	Dark
	(nmole C_2H_2 h^{-1} g^{-1} fresh weight)			
Old parts	23.6	1.51	27.2	1.97
Young parts	49.6	5.05	26.2	1.57

[a] Difference between 60 at 30 minutes measurements.

Acetylene-reducing activity

Time course of incubation. Cumulative production of ethylene by epiphytes on *Chara* and *Najas* incubated in the light exhibited a non-linear increase after one hour of incubation, as reported by David & Fay (1977). Therefore, specific activities were calculated using the difference between 60 and 30 minutes measurements.

Specific activities and extrapolation to the field scale. Results presented in Table 3 show that the activity per unit fresh weight in the light on the submerged weeds is much higher than that on the non-submerged ones. Compared to the light activities, the dark activities are very low and are of the same order among the different weeds. The light activities measured under laboratory conditions were multiplied by 1.8 to extrapolate them to the field, as it was found that the ARA measured in the laboratory was, on an average, 55% of the outdoor activity. Relating the specific ARA to the biomasses of the weeds, it was found that epiphytic N_2 fixation on the submerged weeds could contribute 11 to 24 g N ha^{-1} d^{-1} under rice and 41 to 63 g N ha^{-1} d^{-1} under fallow, whereas the activity on the non-submerged weeds contributes only negligible quantities of nitrogen to this ecosystem.

Table 3. Specific ARA on weeds and extrapolation of NFA to field level using mean and maximum (in parentheses) values of weed biomass recorded

Habitat	Weed type	ARA under lab. conditions (nmole C_2H_4 h^{-1} (g^{-1} fresh weight)		Biomass of submerged host material (t ha^{-1})		ARA values extrapolated to field condition (g N ha^{-1} d^{-1})[a]	
		Light	Dark	Under rice crop	Fallow	Under rice crop	Fallow
Submerged	*Chara*	35	0.9	2.0 (4.5)	7.5 (11.8)	7 (13)	22 (34)
	Najas	29	1.7			5 (11)	19 (29)
Non-submerged	*Monochoria vaginalis*	1.8	1.3	1.7 (4.1)	7.7 (n.d.)[b]	0.4 (1.0)	2 (n.d.)
	Cyperus iria	4.4	2.5		2.8 (n.d.)	1.0 (2.3)	1.6 (n.d.)

[a] Assumes C_2H_2 : N_2 = 3:1, [b] not determined.

DISCUSSION AND CONCLUSION

Results indicate that both N_2-fixing algae and bacteria were present on weeds but most of the activity was due to blue-green algae. Although higher densities of these organisms were observed on old parts of submerged weeds, ARA measurements showed that the activity on young parts was either higher (*Chara*) or of the same order (*Najas*). This perhaps indicates a higher concentration of quiescent or less active populations on the old parts.

Among the different weeds studied, only submerged ones exhibited a significant activity, approximating to an input of 2 kg N ha^{-1} $crop^{-1}$ under rice and 4 kg N ha^{-1} under fallow (C_2H_2:N_2 = 3).

Another important role of the submerged weeds, mainly *Chara*, is to offer a substratum

suitable for the attachment of *Gloeotrichia* sp. This blue-green alga forms floating, flobose, colonies that could develop considerable biomasses of several t ha^{-1} (Watanabe *et al.*, 1978), but are frequently washed out of the field by heavy rains or bleached by high light intensities. Epiphytic *Gloeotrichia* are protected from these adverse conditions and provide an inoculum from which regeneration of the bloom is possible. It is therefore clear that in the nitrogen cycle of a rice field, the submerged weeds play a positive role in the N_2 fixation process.

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