Biological control of rice nematodes using sulphate reducing bacteria

Vincent A. Jacq and Renaud Fortuner

Laboratoire de Microbiologie des Sols, ORSTOM, B.P. 1386, Dakar, Sénégal and Laboratoire de Nématologie, ORSTOM, B.P. V-51, Abidjan, Côte d'Ivoire.

SUMMARY

Population levels of nematodes in the soil of rice fields are lower when the activity of sulphate reducing bacteria is high. It is possible to artificially increase bacteria activity, after the harvest, to further reduce the number of viable nematodes present in the soil. However, some or all eggs, laid inside the roots, are not killed. This method is harmless to the rice, controls nematode population well and increases yield.

RÉSUMÉ

Lutte biologique contre les nématodes du riz au moyen des bactéries sulfato-réductrices

Les niveaux des populations de nématodes dans le sol des rizières sont plus faibles quand l'activité des bactéries sulfato-réductrices est élevée, produisant des concentrations létales de sulfures. Il est possible d'augmenter artificiellement l'activité de ces bactéries après la récolte en assurant une bonne anaérobiose par l'inondation du champ et en apportant aux bactéries composés minéraux soufrés et matière organique. Ceci permet de diminuer davantage le nombre de nématodes présents dans le sol. Cependant, les œufs pondus à l'intérieur des racines ne sont pas, ou pas tous, tués par les sulfures et leur éclosion permet la formation d'une nouvelle population de parasites qui reste cependant à un niveau inférieur à celui des témoins. Une culture de contrôle, faite après le traitement, montre que cette méthode n'est pas dangereuse pour le riz, qu'elle permet de lutter efficacement contre les nématodes et qu'elle augmente les rendements.

A survey of root parasitic nematodes of rice in Senegal indicated that fields, in which activity of sulphate reducing bacteria was very high, harboured practically no nematodes (Fortuner & Merny, 1973). Sulphate reducing bacteria are strictly anaerobic organisms which obtain their energy by reducing sulphates to sulphides ; these products are toxic to rice, producing physiological decay (Vámos, 1958). This suggested that the low nematode populations might be due to nematicidal properties of soluble sulphides. A similar observation was reported in Louisiana by Rodriguez-Kabana, Jordan and Hollis (1965), who also established *in vitro* that hydrogen sulphide was toxic to the nematode *Tylenchorhynchus martini*. A study made in Senegal proved that three other nematodes : *Hirschmanniella oryzae*, *H. spinicaudata* and *Tylenchorhynchus mashhoodi* were also killed by soluble sulphides. The letal dosages were the same for the three species (Fortuner & Jacq, 1976).

These observations and experiments suggest that soluble sulphides are able to limit nematode populations in paddy fields if the activity of sulphate reducing bacteria is sufficiently high.

Materials and Methods

To test this hypothesis, population levels of nematodes : *Hirschmanniella oryzae*, and sulphate reducing bacteria (= S.R.B.) : *Desulfovibrio* sp., were determined in fifteen microplots cultivated with rice cv. I.R. 8. Then two experiments were made : on the same microplots (experiment No. 1) and the other on twenty additional microplots (experiment No. 2).

Microplots were concrete pits (1×1×1 m), filled, except for the upper twenty centimeters, with soils from a rice field near Dakar (Niayes) which had been autoclaved before transfer. Certain physico-chemical properties of these soils are given in Table 1. Because these plots are routinely used by the ORSTOM laboratory in Dakar to study damage to rice caused by *H. oryzae*, they had been inoculated one or two crops prior to these experiments with individuals from *H. oryzae* cultures derived from soil samples collected in the Senegal River delta.

Preliminary observations and experiment No. 1 were made on a group of fifteen plots, cultivated with rice, cv. I.R. 8, and the second experiment was made in another group of twenty plots cultivated with cv. Moroberekan. Description of treatments are given in Tables 2 and 3.

Populations of sulphate reducing bacteria were estimated by the technique of Mouraret and Baldensperger (pers. comm.). The technique of Chaudhry and Cornfield (1966) was used to estimate the concentration of total sulphides, i.e. H_2S, solubles sulphides and insoluble iron monosulphide (preliminary observations) and that of Jacq (1978 *a*) for the concentration of soluble sulphides (experiments 1 and 2). Nematodes were extracted from soil by elutriation (Seinhorst, 1962).

Table 1

Certain physico-chemical properties of the soils

Observations on rice	*Total Sulphur* (‰, w/w)	*Organic Carbon* (%, w/w)	*Clay* (%, w/w)	*pH*
Experiment 1	0.4-1.4	1.14-1.34	44-48	7.8
Experiment 2	0.2-1.1	1.60-1.80	43-45	8.2

Table 2

Description of treatments during preliminary assay

Group	*Water retention*	*Fertilizer*	*Number of plots*
a	poor (craks on walls)	Urea	4
		S.C.U.	5
b	good (no craks)	Urea	3
		S.C.U.	3

Table 3

Description of treatments during experiments

	Group	*Number of plots*	*Fertilizer*	*Waterlogging*
Experiment 1	a : control	9	Urea	0-39th days
	b : treated	6	S.C.U.	0-39th days
Experiment 2	a : control	6	None	No water
	b_1 : treated	7	Sulphur	0-35, 56-70th days
	b_2 : treated	7	Sulphur	0-35, 70-85th days

Results

Observations during rice cultivation

An experiment was designed to evaluate the relationship between sulphate reducing bacteria activities and dynamics of *H. oryzae* populations. Higher sulphate reducing bacteria activity was expected to be obtained when "Sulfur Coated Urea" was used instead of non-coated urea. No significant response was obtained because craks had developed in the cement wall of some plots (Table 2 : group a : 9 plots, 4 of which having received non-coated urea, the other 5, S.C.U.) allowing drainage of irrigation water. In such plots, anaerobiosis was insufficient and production of sulphides reached only a low concentration. The other plots (Tabl. 2 : group b : 6 plots, half with non-coated urea, the other half with S.C.U.) retained water well, providing permanent anaerobiosis, and the production of sulphides was higher.

Thus an unsuspected fact (presence of craks in the walls of the plots) produced the expected results : a relationship was found between sulphate reducing activities and dynamics of *H. oryzae* populations.

Fluctuations of S.R.B. populations, concentrations of total sulphides and fluctuations of nematode populations are given in Figure 1.

In plots having high S.R.B. activity (group b), damage to rice occured, resulting in 15 to 35% plant mortality, but nematode populations remained at the same level throughout the cultivation of rice ; in plots in which S.R.B. activity was low (group a), no damage occured, but nematode populations increased 4 to 5 fold.

Toxicity of soluble sulphides to nematodes has been demonstrated previously by *in vitro* studies (Fortuner & Jacq, 1976). The same phenomenon was suspected, but not proven in the plots : unfortunately no method for determining only soluble sulphides concentrations in the field was available at this time. However, it is logical to assume that total sulphides (i.e. soluble sulphides and insolubilized iron sulphides) are correlated with soluble sulphides. Thus, field observations, *in vitro* tests and these observations suggested that, if the activity of sulphate reducing bacteria is sufficiently high, soluble sulphides produced can control the nematodes and prevent a population increase during the rice culture. However, even in those plots where sulphate reduction was very high, causing rice mortality, there was no decrease in the initial number of nematodes. Consequently, to obtain better nematode control, bacterial activity should be artificially increased at a time when there is no danger of damage to the rice, i.e. after harvest.

Experiment No. 1

It was made in the same plots. Sulphate reducing bacteria activity was increased by three means, simultaneously applied to treated plots :

1) *Flooding of the plots.* With normal rice production methods, water is usually drained

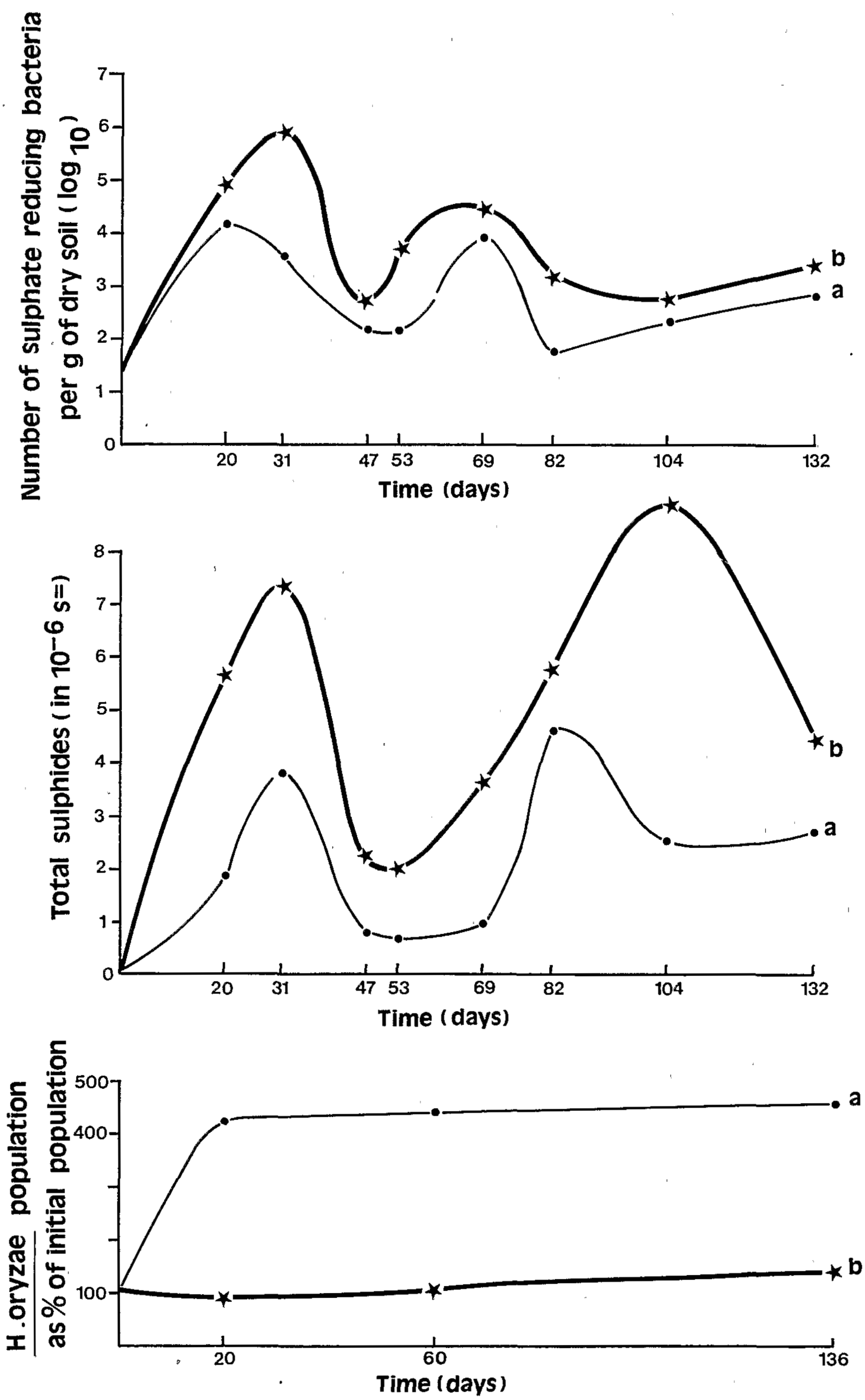

Fig. 1 : Preliminary observation : sulphate reducing bacteria populations, total sulphides concentrations and *Hirschmanniella oryzae* populations. a = aerobic plot, b = anaerobic plots.

after harvest and the soil allowed to dry until the next culture. Aeration greatly reduces the number of bacteria and prevents sulphides production ; the few sulphate reducing bacteria still living are inactive in aerated soil. Strict anaerobiosis provided by permanent flooding is a necessity for this control method.

2) *Incorporation of rice straw*, cut 10 cm above ground, into the soil, to provide bacteria with useful organic material.

3) *Application of Sulphur.* In this experiment, "Sulfur Coated Urea" was added to the test plots at the rate of 28 kg S and 40 kg N per hectare. The coating of the "Sulfur Coated Urea" is presumably oxidized to sulphate in a few days by another group of bacteria (*Thiobacillus* sp.) and, thus, sulphate becomes available to sulphate-reducing bacteria which, in turn, produce more sulphides. As will be seen later, some of the sulphur can be directly reduced into sulphides by sulpho-reducers.

In control plots, sulphate and sulphur reducing bacteria activity was increased only by flooding and incorporation of rice straw to soil; no sulphur was added but nitrogen was supplemented by the same amount as that provided by S.C.U. in treated plots.

Treatments are reported in Table 3. It should be noted that control plots (group a : 9 plots) are the same "a" plots of the preceding observation : walls were not repaired, and so, anaerobiosis was not pronounced.

Sulphate reduction was slow in the control plots and higher in the treated plots.

In Figure 2 are presented :

— evolution of the sulphate reducing bacteria populations ;

— soluble sulphide production expressed in Sulphide Time Units, or "STU" (a nematode is submitted to the toxic effect of 1 STU when it is placed for 1 day in a 1 ppm sulphide solution) ; and

— evolution of *H. oryzae* populations as a percentage of the initial population in the soil estimated the day before soil was saturated : in control plots : 100% = 750 nematodes ; in treated plots : 100% = 430 nematodes/l of soil.

During the initial phase (0-18 days), because of combined effect of rehydratation and the lag phase of microorganism growth, populations of *H. oryzae* in treated plots increased but remained static in control plots. Except for a brief period of "cool" weather between 29-32 days, when night temperature decreased from 22 °C to 15 °C, and day temperature from 28 to 21 °C, resulting in a decrease of number and activity of sulphate reducing bacteria, nematode populations decreased slowly in the control plots, as is normal in fallow soil. In the treated plots, decrease of *H. oryzae* populations was marked : after 39 days the population was only 9% of the initial population.

Irrigation of the plots was stopped after the 39 th day and the soil allowed to dry in order to reestablish aerobic conditions. The soluble sulphides were oxidized and their concentration diminished rapidly. When the water was removed, populations of *H. oryzae* increased in all plots, probably due to the hatching of eggs. It is assumed that not all eggs of *H. oryzae*, laid within rice roots, are killed by soluble sulphides.

Experiment No. 2

A second experiment was made on another group of 20 microplots in an attempt to kill newly hatched, second-stage juveniles observed in the first experiment. Two water management regimes were used (Tabl. 3) :

Plots flooded for 35 days, drained for 21 days (during which some eggs hatched) and reflooded for 15 days (group b_1).

Plots flooded for 35 days, drained for 35 days, and reflooded for 15 days (group b_2).

Control plots were not flooded (group a).

This experiment differed from the previous one in that : 1) *H. oryzae* populations were higher at the beginning of treatment : control plots : 890 (Exp. 2) against 750 (exp. 1) ; treated plots 1,200 (exp. 2) against 430 (exp. 1) nematodes per liter of soil ; and 2) elemental sulphur, rather than "Sulfur Coated Urea" was added at the same concentration, in treated plots.

The same parameters as in the previous experiment were measured (Fig. 3). No sulphate reduction occured in control plots (dry soil) and no soluble sulphides were produced. More STU (60 to 80) were needed in treated plots to decrease *H. oryzae* populations to a low level : 54% of the initial population in group b_1 plots at the 77th

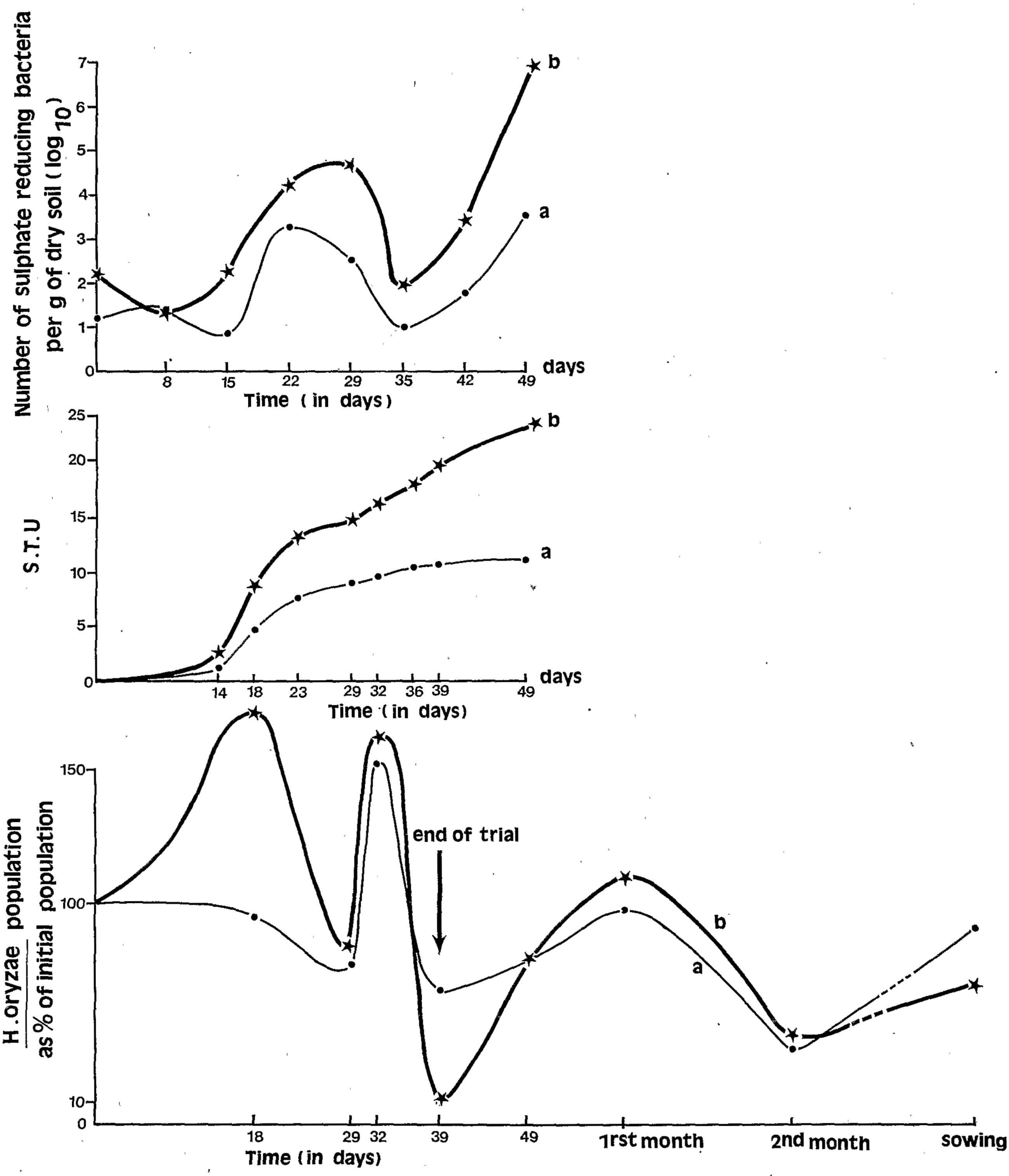

Fig. 2 : First experiment : sulphate reducing bacteria populations, soluble sulphide toxicity, expressed in S.T.U. (see text) and *Hirschmanniella oryzae* populations. a = control plots ; b = treated plots.

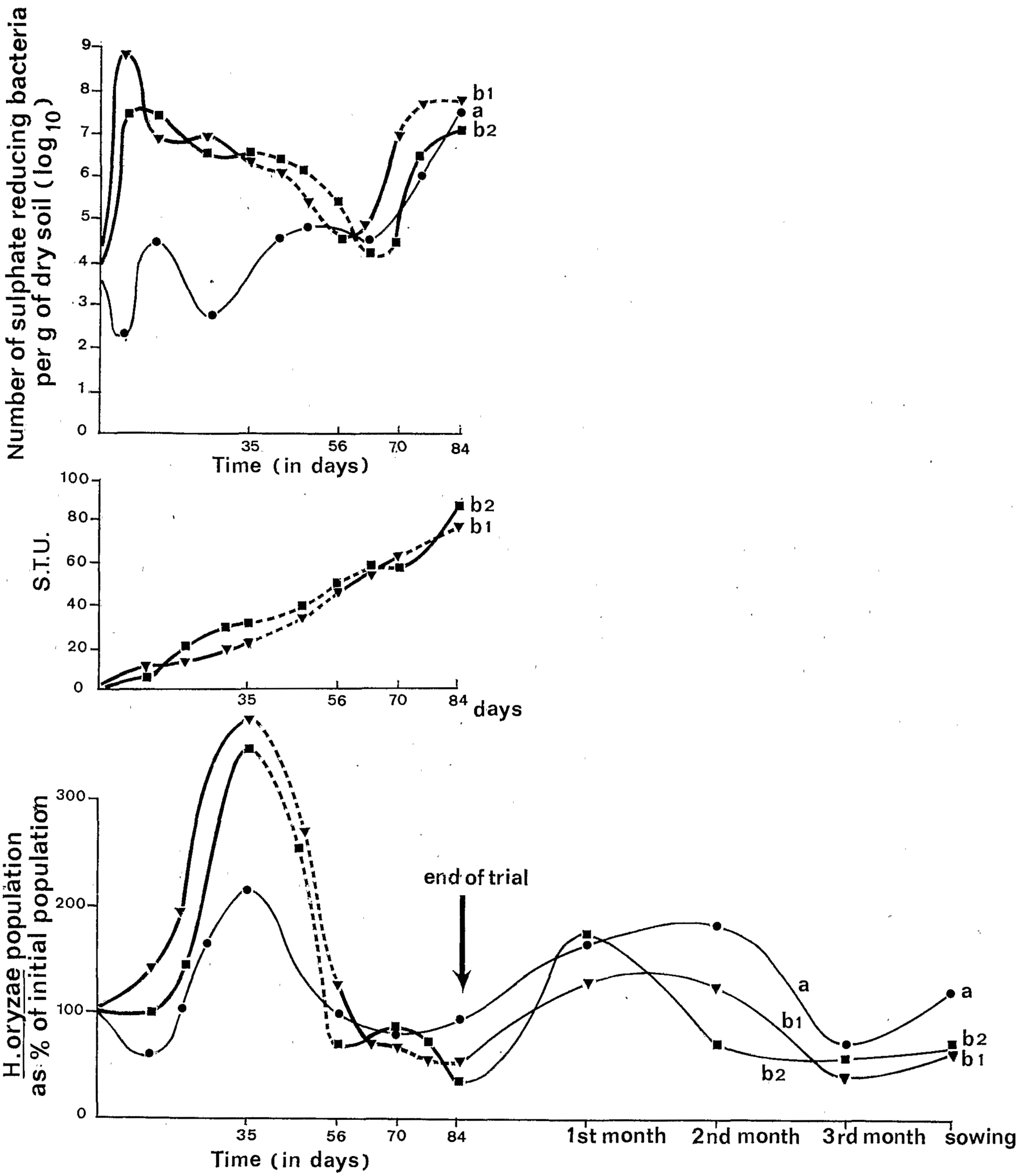

Fig. 3 : Second experiment : sulphate reducing bacteria populations, soluble sulphide toxicity and *Hirschmanniella oryzae* populations. a = control plots (dry soil) ; b_1 = treated plots, waterlogged during days : 0-35 then 56-70 ; b_2 = treated plots, waterlogged during days : 0-35 then 70-85.

day and 35% in group b_2 plots at the 48th day. The population of nematodes underwent a certain increase after final removal of water, though less than during the first experiment.

To determine the after effects of the control method rice was sown in the plots during the rainy seasons following the first and second experiments. Conditions were favourable for high sulphate reduction activity with heavy rains flooding the soil. However only 5% mortality of rice seedlings occurred in experiment 1, and 8% in experiment 2, and no damage at all was observed in mature plants. Thus, despite an artificial increase of sulphate reducing activity during the experiments, subsequent crops exhibited no significant damage.

Table 4 summarizes levels of nematode infestation on two rice cultures made in the plots before and after treatment.

At planting times, nematode populations in treated plots were always lower than in control plots. The difference seems to be slight ; however, only nematodes in the soil were extracted. It is known (Merny, 1972 ; Fortuner, 1977) that nematodes inside old roots remaining from the previous culture leave those roots at the begining of the next culture, joining the soil population and doubling its size. This is probably what happened in control plots. In treated plots, sulphate reducing bacteria used organic matter during the active phase of our experiment, thus partly destroying old roots and causing endoparasitic nematodes to join the soil population. At the begining of the next culture only soil nematodes remain and no other source of contamination is left.

At harvest, the populations remained significantly lower in treated plots.

Table 4

Influence of treatment on *Hirschmanniella oryzae* populations

*Base = 100% of nematodes per liter of soil, at harvest * before treatment*	*Experiment, and treatment*	*Percentage of nematodes during first crop after treatment*	
		at sowing	*at harvest*
100% = 1,500	1, a : control	88	83
100% = 1,350	1, b : treated	50	32
100% = 890	2, a : control	111	78
100% = 1,200	2, b_1+b_2 : treated	71	47

* experiment 1 : treatment began 35 days after the harvest,
experiment 2 : treatment began 7 days after the harvest.

Table 5

Effect of treatment on yields (expressed as grams of rice per harvested plant)

		Before treatment	*1st year after treatment*	*2nd year after treatment*
Experiment 1	a : control	38.84	27.80	26.55
	b : treated	42.65	31.05	42.19
Experiment 2	a : control	26.06	16.93	11.62
	b_1+b_2 : treated	23.58	20.19	21.52

The yields of the successive rice cultures are given in Table 5. Despite the effect of auto-intoxication from decomposing rice residues in soils (Chou & Lin, 1976), beneficial effects of the treatment on yields were detectable on the first crop (30% yield increase in the second experiment) or on the second crop (60% yield increase in experiments 1 and 2).

Discussion

These observations and experiments established that :

1) During rice cultivation, a high sulphate reducing bacteria activity may result in control of nematode populations.

2) Sulphides may be produced after harvest, and the result is a marked decrease of nematodes populations.

3) Higher yields of rice are obtained in subsequent rice cultures.

However, our experiments did not elucidate the mechanisms governing nematode population decrease. Among problems imperfectly understood and needing further studies, three are of main importance :

1) The role of added sulphur.

2) The reason that the numbers of Sulphide Time Units necessary to kill the nematodes are not similar in soils and *in vitro* experiments.

3) The cause of significant build-up of nematodes observed after removal of water in treated plots.

The following explanations are suggested :

1) *Role of sulphur*

Sulphur was added to increase sulphate reducing bacterial activity, it is oxidized by *Thiobacillus*, both in aerobiosis *(T. thiooxidans)* and in anaerobiosis *(T. denitrificans)*: we have measured the increase of these two bacterial populations when soils were waterlogged. Sulphate produced in this manner is reduced only in anaerobiosis by sulphate reducing bacteria. Populations also increased very quickly (Fig. 2 & 3). Another, bacterium, *Desulfuromonas acetoxidans* was found which, in strict anaerobiosis, directly reduces sulphur to sulphides (Pfennig & Biebl, 1976). After our experiments were performed, evidence was found for the presence of such bacteria in the soils used (Traoré, Mouraret & Jacq, unpublished data). Thus, it is suggested that production of sulphides is a very complex process, involving at least four groups of bacteria.

The amount of sulphur added (28 kg S/ha) may appear negligible when compared to the total quantity of sulphur (600 to 3,000 kg S/ha) of tested soils, even though part of it may be in a polysulphides form, unavailable to bacteria. It is suggested that such addition of sulphur under metalloidic form have a starter effect, at least on S.R.B. activity and perhaps on other bacterial activities, as demonstrated elsewhere in rice rhizosphere (Jacq, 1978 *b*).

2) *Sulphide toxicity*

During *in vitro* experiments in liquid media (Fortuner & Jacq, 1976) nematode mortality, and Sulphide Time Units appeared to be very well correlated, and 50% mortality occured at 120-130 S.T.U. In experiments in soils, similar populations (expressed in number/l^{-1}) were killed by much lower STU's (20-25 in experiment 1, or 60 to 80 in experiment 2). These results suggest that an S.T.U. is not a precise unit in soil, or that other toxicities may occur. For example, aliphatic acids resulting from fermentation of added rice straw are toxic to nematodes (Banage & Visser, 1965), as acetate accumulated by sulphate reducing bacteria. In addition, a sharp decrease of pH, resulting from reoxidation of sulphides to sulfuric acid by *Thiobacillus* sp, may be harmful to nematodes, as observed in our *in vitro* experiments.

3) *Reinfestation.*

After removal of water, an increase of nematode populations, mainly juveniles, was observed. We believe that some eggs, laid inside roots, hatched when sulphides disappeared, but we failed to determine if eggs are more resistant than free-living individuals, or if they are protected by roots tissues.

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